Mode selection mechanism in traveling and standing waves revealed by Min wave reconstituted in artificial cells

Sakura Takada1, Natsuhiyo Yoshinaga2,3*, Nobuhide Doi1, Kei Fujiwara1*

Reaction-diffusion coupling (RDC) generates spatiotemporal patterns, including two dynamic wave modes: traveling and standing waves. Although mode selection plays a substantial role in the spatiotemporal organization of living cell molecules, the mechanism for selecting each wave mode remains elusive. Here, we investigated a wave mode selection mechanism using Min waves reconstituted in artificial cells, emerged by the RDC of MinD and MinE. Our experiments and theoretical analysis revealed that the balance of membrane binding and dissociation from the membrane of MinD determines the mode selection of the Min wave. We successfully demonstrated that the transition of the wave modes can be regulated by controlling this balance and found hysteresis characteristics in the wave mode transition. These findings highlight a previously unidentified role of the balance between activators and inhibitors as a determinant of the mode selection of waves by RDC and depict an unexplored mechanism in intracellular spatiotemporal pattern formations.

INTRODUCTION

Spatiotemporal patterns of molecules in living cells are formed by the self-organization of molecules. A notable mechanism that induces this self-organization is reaction-diffusion coupling (RDC). RDC induces two types of self-assembly: static and dynamic patterns. Examples of RDC static patterns in living cells are PAR protein patterns, which determine the anterior and posterior poles in the eukaryotic embryo (1, 2), and Cdc42 polarization, which contributes to the determination of the budding position of budding yeast (3, 4). Although mechanical stress and molecular transport by cytoskeleton have been suggested to involve the formation of these static patterns, it has been indicated that RDC has substantial roles in the pattern formation and maintenance. Dynamic patterns due to RDC are observed as waves of molecular transport. For example, actin/phosphatidylinositol (3,4,5)-triphosphate (PIP3)/PTEN waves are related to the motility of amoeba cells (5–8), the Rho/F-actin wave is related to the cell division of animal cells (9, 10), and the Min wave is related to the determination of the cell division plane of bacteria (11, 12).

Dynamic patterns caused by RDC are divided into two modes of waves: traveling waves that propagate spatially, and standing waves that oscillate at a fixed point. Both wave modes have been observed in living cells. For example, the actin waves described above are traveling waves (9, 13), and the Min wave is considered as a standing wave in living cells (14, 15). The modes of the RDC wave are related to its intracellular function. Actin waves guide the direction of cell movement by wave propagation on the membrane (6, 16, 17), and the Min wave restricts the initiation of cell division at the cell division plane by enriching the cell division inhibitor at the cell poles through oscillation between the cell poles of the inhibitor (11, 14). Therefore, elucidating the mechanism of mode selection between standing and traveling waves is essential for understanding spatiotemporal patterning in cells. Physical picture of the mode selection has been studied in specific systems such as fluid convection of binary mixtures (18, 19) and CO oxidation on Pt (20). However, the underlying mechanism remains elusive.

Because RDC dynamic waves emerge under nonlinear and far-from-equilibrium conditions, experiments using defined factors are indispensable for elucidating the mechanism of their spatiotemporal pattern formation. In this regard, the Min system, which uses the Min wave, is a promising material for elucidating the mechanism of wave mode selection for the following two reasons: (i) Min waves show both wave modes under certain conditions, and (ii) the Min wave is the only intracellular RDC wave that can be reconstituted using defined factors in both in vitro (21–26) and artificial cells (27–30). The Min system consists of MinC, MinD, and MinE, and the RDC of MinDE generates Min waves (11, 21, 31). The molecular mechanism is well understood and is shown in Fig. 1A. MinD binds to the membrane in an adenosine 5′-triphosphate (ATP)–dependent manner (32, 33) and is recruited to the membrane as a positive feedback loop (34–36). MinE, which senses the membrane-binding MinD, binds to the membrane and stimulates the adenosine triphosphatase (ATPase) activity of MinD (37). Hydrolyzing ATP bound to MinD triggers the dissociation of MinD from the membrane (33). MinE is detached from the membrane after lingering on the membrane for a short time (25, 34). Although MinC, which inhibits the polymerization of FtsZ that forms a cytokinetic Z ring as a cell division initiator (38), is not involved in the formation of RDC waves, it colocalizes with MinD to prevent cell division at the cell poles (11, 14). Previous studies have shown that MinD, MinE, and ATP are sufficient for the reconstitution of traveling waves on two-dimensional (2D) phospholipid membranes (21).

The reconstituted system illuminated not only the molecular mechanism, including the effect of the conformational equilibrium of MinE on Min waves (39, 40), but also the importance of physicochemical environments, such as salt environments and lipid
compositions (41). Furthermore, the reconstitution of Min waves in artificial cells revealed that the cell-sized space works as a regulator that determines the emergent conditions and properties of the Min waves (29). These cell-sized space effects, derived from closed geometry, the finite-size effect, and the large ratio of the membrane surface to cytosolic bulk volume indicate that experiments using artificial cells are required to verify the mechanism of the mode selection of dynamic waves.

To date, both standing and traveling waves have been observed in artificial cells. For example, a previous study reported that the behaviors of Min proteins spontaneously transit between traveling and standing waves (28, 29). As a determinant of wave mode selection, studies using 2D planar membrane chambers that mimic rod-shaped bacteria have proposed that geometry is key to the emergence of standing waves (23, 24). However, even in the case of a typical 2D planar membrane, standing waves have also been observed in the case of a MinD mutant (42) or MinD depletion (25), and both standing waves and traveling waves have been observed in living cells regardless of the geometry (43, 44). This suggests that factors other than geometry are also involved in the mode selection of RDC waves.

Here, we investigated the mechanism of mode selection between standing and traveling waves using Min waves reconstituted in artificial cells. We found that the dominant Min wave mode changes depending on the concentration ratio of MinDE and reaction constants, such as the membrane binding affinity of MinE and ATPase activity of MinD. Furthermore, we showed that the transition of the wave mode can be regulated by changing these parameters and successfully recapitulated the results using theoretical analysis. Our results showed that the balance between membrane binding and the dissociation of MinD determines the mode selection of Min waves, implying that the balance between positive and negative feedbacks determines the mode selection of dynamic RDC waves.

Fig. 1. Two dynamic modes of Min waves. (A) Molecular mechanism of the Min system. (B) Two modes of Min waves emerged in artificial cells encapsulating 0.1 μM msfGFP-MinC, 1 μM MinD, 1 μM MinE, and 2.5 mM adenosine 5’-triphosphate (ATP) with bovine serum albumin (BSA) (100 mg/ml). Scale bars, 10 μm.

RESULTS
Dominant Min wave mode changes depending on MinE concentration

Our previous study indicated that the Min wave initially showed a pulsing localization between the membrane and cytosol, transited to the standing wave, and, lastly, settled in the traveling wave (29). This Min wave mode transition suggests that the traveling wave is the most stable mode in spherical cells. However, it has been reported that standing waves appear even in spherical cells shaped by the inhibition of the cytoskeleton (43). We first assumed that this inconsistency is a consequence of the difference between our reconstitution system and intracellular conditions, such as the concentration ratio of MinD and MinE and/or the fluorescent protein fused to MinDE. MinD and MinE had the same concentration in our reconstituted system (29), while the concentration of MinD was 1.4 times that of MinE in living cells (45). Fusion of the fluorescent protein to MinDE possibly affects RDC characteristics because of changes in the molecular diffusion rate. In particular, the fusion of fluorescent proteins to the C terminus of MinE has been suggested to inhibit its function (46). Therefore, in this study, we used MinDE without a fluorescent tag, and instead, the Min waves were tracked by a small amount of msfGFP-MinC, which binds to MinD, whose concentration was one-fifth or less of the MinD concentration.

Under the 1 μM MinDE condition, Min waves were found in approximately 90% of the artificial cells, and both standing and traveling waves were observed (Fig. 1B). Because almost all the Min waves observed were traveling waves in our previous study (29), the removal of the fluorescent tag was considered as the cause of the appearance of the standing waves. The period of the Min wave was 93 ± 9 s for the traveling wave and 54 ± 8 s for the standing wave, which was approximately half the period of the previous study (~2 min) (29) and closer to the standing wave period in living cells (14, 47).

Next, we investigated the effect of the MinDE concentration on the wave modes. MinE concentration was varied in the range of 0.7 to 2.5 μM, while fixing MinD concentration at 1 μM. In this case, MinD localization was tracked using msfGFP-MinC, as described above. Similar to previous studies (29, 30), we classified MinC localization into six patterns: cytosol localization (cytosol), homogeneous localization on the membrane (membrane), inhomogeneous patterning on the membrane (inhomogeneous), pulsing between the membrane and the cytosol (pulsing), traveling wave, and standing wave. The ratio of these patterns varied substantially depending on the MinE concentration (fig. S1). The probability of the appearance of Min waves (traveling wave or standing wave) decreased as the MinE concentration increased, and MinC was localized in the cytosol for almost all the artificial cells at the MinE concentration of 2.5 μM. However, among the artificial cells with Min waves, standing waves became dominant as the concentration of MinE increased. A plot of the proportion of standing waves in the artificial cells with Min waves (standing wave/Min wave ratio) showed that the proportion of standing waves was less than 30% at a MinE concentration of 1.5 μM or less, whereas it was more than 60% for MinE concentration over 1.7 μM, and almost all Min waves were standing waves at the MinE concentration of 2.2 μM (Fig. 2A and movie S1).

In our experimental system, the differences in protein concentration among artificial cells are small and negligible (SD per average was less than 5%) (fig. S2). The Min wave modes were weakly but notably dependent on sizes of artificial cells, and the standing wave was observed in larger artificial cells (fig. S3). However, despite
Shift in the MinE concentration changes the initial transition of the Min wave mode after wave emergence

Next, we tested whether the MinE concentration shift affects the transition of the Min wave mode. However, in our first trial, we failed to observe the wave emergence using the procedure from our previous study (29). We assumed that this was because the period of the Min wave was shorter than the preparation time of the samples for microscopy. Therefore, we used an ATP regeneration system to generate Min waves. In this experiment, adenosine 5′-diphosphate (ADP) was converted to ATP using a creatine kinase–creatine phosphate (CP–CK) system in artificial cells. In the regeneration system, ATP reaches a sufficient concentration for Min wave generation after sample preparation for microscopy. Using this system to observe the initial states under the MinE concentrations of 0.5, 1, and 1.5 μM showed that the transition of wave modes was different among the three conditions. Under the MinE concentration of 0.5 μM, the traveling wave appeared just after Min wave emergence in 13% of the artificial cells. In the other artificial cells, the standing wave appeared at the initial stage of the Min wave and quickly transformed into traveling waves (Fig. 2B and fig. S4A and movie S2). Under the MinE concentration of 1 μM, the standing wave appeared at the initial stage of the Min wave in all the artificial cells, and approximately 70% of the standing waves transformed into traveling waves (Fig. 2B, fig. S4B, and movie S3). Analysis of kymographs showed that the standing wave transitioned to the traveling wave after approximately four oscillations under the 1 μM MinE condition (Fig. 2B, bottom). Because the standing wave transitions to the traveling at early stage under normal ATP condition (2.5 mM ATP) (29), this transition is not derived from some artifact due to gradual ATP accumulation by the CP–CK system. The standing wave remained for more than 30 min without transitioning into traveling wave for the MinE concentration of 1.5 μM (Fig. 2B, fig. S4C, and movie S4). These results supported the notion that the MinE concentration is involved in the Min wave mode, including the wave mode transition.

Mode selection of Min wave also depends on MinD concentration

Because the RDc of MinD and MinE causes Min waves, it is plausible that the MinD and MinE concentrations affect the wave mode. Therefore, MinD concentration was varied in the range of 0.5 to 1.5 μM, while MinE concentration was fixed at 1 μM. The dynamics of MinD were tracked using msfGFP-MinC, and Min waves were classified into six patterns as indicated in the MinE experiments. Consequently, the probability of standing waves increased as the MinD concentration decreased in contrast to the MinE case (Fig. 2C and fig. S5). The proportion of standing waves was 40 to 60% of the total patterns at low MinD concentrations (0.5 and 0.7 μM MinD), and that of the traveling waves was more than 80% at MinD concentrations of 1.2 μM or more (fig. S5). When the MinD concentration was low, most of the artificial cells without the standing wave showed cytosolic localization (fig. S5), similar to the condition of high MinE concentration (fig. S1). Furthermore, the relationship between MinD concentration and the standing wave/Min wave ratio clearly showed that the standing wave was dominant at low MinD concentrations and that the traveling wave was dominant at high MinD concentrations (Fig. 2C and movie S5). In other words, the mode of the Min wave also depends on the MinD concentration and is not determined by the concentration of either MinD or MinE only but by both concentrations.

Regulation of the Min wave mode by the activity of Min proteins

Because MinDE concentrations are inversely associated with dominant mode selection, the above results suggest that the concentration ratio of MinDE, rather than their absolute concentration, is important for mode selection. To clarify this point, we focused on the RDc mechanism of the Min system (Fig. 1A). Previous studies have indicated that the membrane binding of MinD (42) and MinE (41), self-interaction of MinD (36), and MinDE dissociation from the membrane via the ATPase activity of the MinDE complex (48, 49) are related to concentrations of MinD and MinE. The rates of these reactions were determined by the MinDE concentrations and their reaction constants. Therefore, changes in the reaction constants also act as the concentration shift of MinDE. To verify this hypothesis, we focused on the salt concentration and temperature, which have been shown to affect the membrane binding affinity of MinE and ATPase activity of the MinDE complex, respectively.

First, we investigated the effect of salt concentration on the Min wave mode. The previous study reported that salt concentration changes the membrane binding affinity of MinE by adjusting the electrostatic interaction between the negative charge of the lipids and cationic region of MinE (41). Therefore, a high salt concentration was expected to have a similar effect as reducing the MinE concentration of the standing waves. However, the standing wave mode was not affected by high salt concentrations, suggesting that the transition of wave modes is not derived from the electrostatic interaction.
concentration (Fig. 3A, left). The concentration of GluK added as salt was varied from 50 to 500 mM, and the wave modes were investigated. Consequently, standing waves became dominant at low salt concentrations, and the percentage of traveling waves increased as the salt concentration increased (Fig. 3A, fig. S6, and movie S6). Almost all Min waves were traveling waves at GluK concentrations of 300 mM or more (Fig. 3A). These results clearly indicate that the mode selection of Min waves can be varied by altering the membrane binding affinity of MinE.

Second, we examined whether the ATPase activity of the MinDE complex affects the mode of the Min wave. Although temperature affects various parameters in the RDc of Min waves, previous studies have pointed out that the ATPase activity of the MinDE complex is the most sensitive reaction to temperature (48, 49). To verify this, the ATPase activity of the MinDE complex was measured at our typical observation temperature for Min waves (25°C), and at 29°C and 37°C. ATPase activity increased as the temperature increased (fig. S7), and the activation energy was calculated as 7.0 kcal/mol. To verify the effect of increasing the temperature on ATPase activity in the Min wave mode, we varied the Min wave observation temperatures (29°C, 34°C, and 37°C). The phase diagram depicted by the dynamics of msfGFP-MinC over the MinE concentration range of 0.3 to 2 μM showed that an increase in the observation temperature shifts the MinE concentration range that induces

---

**Fig. 3. Activities of Min proteins regulate Min wave modes.** (A) Schematic illustration of the effect of K⁺ concentration on the membrane binding affinity of MinE (left) and changes of the standing wave/Min wave ratio at various K⁺ concentrations (right). The data of the number of artificial cells where Min waves appear were obtained from observing more than 130 artificial cells (fig. S6). The fitting line is a sigmoidal curve. (B) Changes in the standing wave/Min wave ratio at various temperatures under a MinE concentration of 0.7 μM (left). Phase diagram of the dynamics of msfGFP-MinC against MinE concentration and the temperature (right). Each square represents the most abundant pattern under each condition. Description of “traveling/standing” shows the case of that the probability of each wave mode is nearly half (0.35 to 0.65 standing wave/Min wave ratio). The detailed data of all the artificial cells observed are shown in fig. S8. The color code of the standing wave/Min wave ratio is shown on the right. (C) The transition of the frequencies of each pattern induced by temperature shifts. Spatiotemporal patterns of msfGFP-MinC in artificial cells encapsulating 0.2 μM msfGFP-MinC, 1 μM MinD, 0.7 μM MinE, and 2.5 mM ATP with BSA (100 mg/ml) are shown on the left (150 artificial cells were counted from three independent experiments). Only artificial cells where traveling wave emerged at the first temperature (29°C) were counted. The probabilities of each type of spatiotemporal pattern transition in the same artificial cells are shown in the middle (calculated from the same dataset as the bar graph; 50 artificial cells, N = 3). Time-lapse images and kymographs of representative pattern transitions are shown on the right. Standing waves are highlighted with white arrows. Time intervals for the time-lapse images are 20 s (traveling waves) and 10 s (standing waves). Scale bar, 20 μm.
the same spatiotemporal pattern (Fig. 3B and fig. S8). Furthermore, as a result of the calculation of the standing wave/Min wave ratio, the ratio of standing waves increased as the temperature increased at the same MinE concentration (Fig. 3B). In particular, more than 90% of all artificial cells showed standing waves for a MinE concentration of 1 µM at 29°C (Fig. S8). These results demonstrate that the relationship between the MinE concentration and mode of the Min wave shifts depending on the temperature. Even at low MinE concentrations, the behaviors of Min waves under high-temperature conditions were similar to those at high MinE concentrations under low temperature conditions, suggesting that the ATPase activity of the MinDE complex also contributes to the mode selection of the Min wave.

The changes in the dominant mode of the Min wave with temperature indicate that the wave mode can be controlled by shifting the temperature. To verify this point, we observed the effect of the temperature shift on Min wave selection for a MinE concentration of 0.7 µM, where traveling and standing waves are dominant at 29° and 37°C, respectively (Fig. 3B). As expected, traveling waves were mainly observed at 29°C, and increasing the temperature increased the number of standing waves. We successfully observed the transition by raising the temperature in the same artificial cells (movie S7). Raising the temperature from 37° to 29°C transformed 61% of the traveling waves into standing waves (Fig. 3C, left, and movie S8). It should be noted that this transition is in the opposite direction of the spontaneous transition of the Min wave mode shown in Fig. 2B and observed in our previous study (29). Cooling the same artificial cells to 29°C returned the dominant wave mode to the traveling wave mode (Fig. 3C and movie S8). However, this wave mode return was observed in approximately half of the artificial cells that had standing waves (Fig. 3C, middle), suggesting that the wave mode selection has hysteresis characteristics.

**Hysteresis of Min wave mode analyzed by a protein expression system**

Last, we investigated whether the Min wave mode transition has hysteresis characteristics by MinE concentration shift using a protein expression system. As shown in Fig. 2A, the MinE concentration is a determining factor of the dominant Min wave mode. Therefore, an increase in the MinE concentration in artificial cells was expected to change the dominant wave mode for a Min wave mode transition if the Min waves do not have hysteresis characteristics. To test this hypothesis, MinE was synthesized in artificial cells by coencapsulating a reconstituted transcription-translation system, the PURE system, and DNA encoding MinE with Min proteins in artificial cells (Fig. 4A).

Because our previous study demonstrated that Min waves reconstituted in artificial cells disappear when an excess amount of MinE is synthesized (30), adjusting the MinE synthesis level was necessary. To control protein synthesis levels, we decreased the amount of amino acids required for protein expression. To confirm the reliability of this strategy, NanoLuc, which shows high sensitivity and specificity for quantification, was used as a reporter gene. Consequently, we observed that the concentration of amino acids in the reaction mixture regulated the limited levels of protein synthesis (fig. S9). Then, we replaced the NanoLuc gene with the MinE gene and analyzed the effect of the increase in the MinE concentration on the Min wave mode. The concentration of the Min proteins contained was set to 1 µM MinD and 0.7 µM MinE, where the traveling wave is dominant. When using amino acid mixtures at 1.25 or 1.5 µM each (corresponding to the final MinE concentration synthesized by the protein expression), the traveling wave continued and did not transition to a standing wave, although the wave width gradually shortened with the synthesis of MinE (Fig. 4B and movie S9). In contrast, increasing the amino acid concentration to 2 µM each also decreased the wave width in a shorter time, and, simultaneously, the traveling wave disappeared after 20 min of the reaction, and the localization of msfGFP-MinC transited to the cytosol (Fig. 4B and movie S10). The traveling waves did not transition to a standing wave during this process. The results of the experiments varying MinE concentration (Fig. 2A and fig. S1) indicate that increasing MinE concentration makes the traveling wave transit to the standing wave, and the Min wave should disappear (that is, transition to cytosol localization) by a further increase in MinE concentration. However, we found that the traveling wave directly transits to the cytosol localization in the case of MinE synthesis in artificial cells. These pieces of evidence indicate that the Min wave mode is affected by the reaction path and/or the previous wave mode and suggest that the mode of the Min wave exhibits hysteresis characteristics.

**Theoretical analysis to capture the relationships between the Min wave mode and parameters**

To clarify the mechanism of mode selection between standing and traveling waves, we analyzed the reaction-diffusion equations for the Min system. We used the model that was studied in our previous study (see Materials and Methods) (29). The model includes MinD binding on the membrane from the cytosol $\omega_{M}$ and recruitment of MinD $\omega_{MD}$ and MinE $\omega_{ME}$ from the cytosol by membrane-bound MinD. In the model, the ADP/ATP exchange rate $\lambda$ of the MinD in the cytosol after the ATP hydrolysis of MinD (50) and the persistent binding of MinE on the membrane (44) generate the wave. The latter effect is supplemented by the binding of MinD and MinE $\omega_{MD}$ on the membrane. We also included spontaneous MinE binding on the membrane from the cytosol. This effect is described by a different term $\omega_{ME}$ from the previous study because we are interested in the dependence of wave selection on the concentration of MinE. Spontaneous MinE binding did not change the qualitative features of the phase diagram of the wave states. Without spontaneous MinE
binding, the standing waves occur at higher concentrations of MinE, lower concentrations of MinD, and a larger recruitment rate of MinE from the cytosol. Nevertheless, spontaneous MinE binding prevents wave generation at higher concentrations of MinE, resulting in a uniform cytosol state.

Figure 5 (A and B) shows the state diagram in the parameter spaces of the MinD and MinE concentrations and the recruitment of MinE(0_b)−MinD(0_de,m), respectively. When the concentration of MinE was low, MinD accumulated at the membrane and MinE was mainly in the cytosol (Fig. 5C). On the other hand, at higher MinE concentrations, MinD was present in the cytosol. At intermediate concentrations of MinE, both traveling and standing waves appeared depending on the MinDE concentration ratio. When the recruitment of MinE and MinD was varied, the state diagram was qualitatively identical to that of the MinE-MinD concentrations. Standing waves appeared for higher recruitment of MinE and lower recruitment of MinD. In the current model, we added the spontaneous binding of MinE to the membrane. Therefore, waves appear even at 0_b = 0. In both state diagrams, standing waves appeared when MinE frequently attached to the membrane and MinD detached from the membrane, as suggested by the experiments. Conversely, traveling waves dominated when MinD remained attached to the membrane. We also found a region of stationary inhomogeneous states at the lower concentrations of MinE, and smaller ω_de and ω_de,m (Fig. 5, A and B, gray regions). In this region, the speed of the wave propagation becomes zero. The stationary inhomogeneous states resemble the inhomogeneous localization of MinD observed in experiments (30). However, the inhomogeneous localization is not a dominant mode under all conditions we have experimentally tested so far, and the reason why the stationary inhomogeneous states appeared in the theoretical analysis is still elusive.

To model the effect of ATPase activity, we used different rates of MinD dissociation from the membrane ω_de,m (49). Following the experimental measurements of ATPase activity at 25° and 37°C (fig. S7), we set ω_de,m = 1 and ω_de,m = 1.6, respectively. In both state diagrams of the MinE-MinD concentrations and recruitment, the region where the standing waves occurred broadened with larger ATPase activity. In Fig. 5A, the traveling wave appears at MinD = 0.8 and MinE = 0.6 when ω_de,m = 1 (Fig. 5C). At stronger ATPase activity, ω_de,m = 1.6, the standing wave occurred at the same concentrations of MinD and MinE (Fig. 5D). Thus, the model reproduced the same tendency for the selection of the traveling and standing waves.

We also studied the wave transitions under dynamic parameter changes. Following the temperature change and MinE synthesis, we dynamically varied ω_de,m and the concentration of MinE, respectively. We applied a stepwise change in ω_de,m from t = 500 to t = 1000. After the initial relaxation, the traveling wave switched to the standing wave and returned to the traveling wave at ω_de,m = 5.0 (Fig. 5E). Even after ω_de,m was set to the original value, the standing wave remained for some time. This result demonstrates the hysteresis of the transition between the traveling and standing waves. At ω_de,m = 1.6, the transition did not occur, although the width of the wave became narrower. Next, we varied the MinE concentration. From t = 500, MinE was added to the cytosol at a constant rate (see Materials and Methods). When the final concentration of MinE was 1.0, the system remained in the traveling wave mode (Fig. 5F). On the other hand, when the final concentration was increased to 1.5 (>1.4), the transition to the standing waves occurred (Fig. 5F). Note that in the state diagram in Fig. 5A, a standing wave should appear at MinE > 0.65. Even at a concentration larger than the threshold, the system remained in the traveling wave mode. Together with the experimental result that showed that the traveling wave appears despite a dynamic increase in MinE concentration (Fig. 4B), the results of the theoretical analysis also demonstrate hysteresis of wave mode selection.

Comparison between experimental and theoretical phase diagram of Min wave mode determined by MinDE ratio

Since the theoretical analysis captures the qualitative relationships between the Min wave mode and parameters, we compared the theoretical and experimental results in more detail. For the aim, the phase diagram of the Min wave modes with respect to the MinDE concentration was experimentally obtained (Fig. 6A). In this experiment, concentrations of MinD and MinE were varied in the concentration ranges of 0.4 to 2.0 (MinD) or 0.4 to 2.2 μM (MinE), respectively. The resultant phase diagram showed that a higher MinE concentration is required at a higher MinD concentration for that the standing wave is dominant. This result clearly shows that the ratio of the MinD to MinE concentration is an important factor in determining the mode selection of the Min wave. Next, the theoretical analysis using the 2D model in Fig. 5 was extended to 3D with units (see Materials and Methods for translation from nondimensionalized parameters used in the simulations to the parameters with units). The theoretical analysis using the 3D model showed a similar dependence on the concentration ratio of MinD and MinE to the 2D model under the same parameters (Fig. 6B). The standing waves occur at the higher concentrations of MinE and lower concentrations of MinD. Similar to the 2D model shown in Fig. 5A, at the larger dissociation rate of MinD from the membrane ω_de,m = 0.8/s (ω_de,m = 1.6 under nondimensionalization), the region of the standing waves becomes wider (fig. S10). Compared with the 2D model under the same parameters, the area of the standing wave was relatively reduced, and the areas of stationary inhomogeneous states become wider at the lower concentrations of MinD and MinE. Although not a perfect match, the phase diagram obtained by the theoretical analysis using the 3D model was semiquantitatively similar to the experimental results with regard to Min wave mode selection. These results indicated that the reaction-diffusion model used in this study can explain the fundamental mechanism to determine the mode of Min wave.

**DISCUSSION**

Nonlinear waves generated by RDc exhibit two dynamic wave modes: standing and traveling waves. However, the mechanism underlying mode selection has not yet been clarified. Furthermore, the regulation and elucidation mode selection have not been achieved even with Min waves, which show both modes in living cells (14, 43, 44) and reconstitution systems (21, 25, 28, 29). In our study, using Min waves reconstituted in artificial cells and theoretical analysis, we found that traveling waves were dominant under the conditions of (i) a weak inhibitory effect of MinE [shown by MinE (Figs. 2A, 5A, and 6)] and salt concentration dependence (Figs. 3A and 5B) and (ii) an increase in the membrane binding rate of MinD [shown by MinD concentration (Figs. 2C, 5A, and 6)] (Fig. 7). In contrast, standing waves were dominant under the conditions of (i) a strong MinE inhibitory effect [shown by MinE and salt concentration dependence (Figs. 3A and 5B)] and (ii) facilitating the
Fig. 5. Selection of standing and traveling waves in our theoretical model. (A and B) State diagrams of the generated waves under varying MinE-MinD concentrations (A) and recruitment of MinE-MinD (B). The spatial distribution of each protein bound to the membrane under the state indicated by dashed circles numbered from "i" to "x" was shown in (C and D). The regions of stationary inhomogeneous states are marked in gray. (C and D) Spatial distribution of MinD (green), MinE (red), and MinDE (blue) on the membrane at the selected points in the state diagram (A) at (C) and (D). Each distribution from left to right corresponds to the dashed circle in (A) from lower to higher concentrations of MinE. (E and F) Dynamic change of the waves under varying concentrations of MinE.
even if the temperature is changed, although the period of the wave varies depending on the temperature (48). On the other hand, it has been shown also that the traveling waves appear in elongated living cells by inhibition of cell division (44). The space-size dependence of wave mode was also observed in our system (fig. S3). Therefore, space size is also a factor for the wave mode selection. Geometry of the space is another important factor as shown by reconstitution studies using supported bilayer membrane in microchambers (23, 24). A recent study on another reaction-diffusion system suggested that membrane binding strength is not uniform in nonsphere cells (51), implying that the geometry also adjusts the activator-inhibitor balance to be beneficial for standing wave selection. Therefore, geometry might be the reason the Min wave mode is not sensitive to parameters in living cells, unlike the reconstitution system, as previously proposed (23, 24). Min waves reconstituted in nonspherical artificial cells will provide an answer to this point in the future.

In this study, we also elucidated that the Min wave mode is controllable, and, on the basis of the findings, we achieved wave mode switching in artificial cells (movies S7 and S8). Although the Min wave mode selection finding indicates that the process is deterministic, both experimental and theoretical results of cooling to the original temperature after increasing the temperature (Figs. 3C and 5E) and the dynamic increase in MinE concentration (Figs. 4B and 5F) suggest that the Min wave mode has hysteretic characteristics. The hysteresis is due to the nonlinearity of the RDc systems, and its underlying mechanism is not clarified in the present study. A possible explanation of the origin of hysteresis is the multistability of the Min wave mode, as reported in previous studies (26, 52, 53). However, further analysis is needed to reveal the physical mechanisms underlying the phenomenon. Elucidation of the physical mechanism will provide a novel paradigm of the pattern formation theory for RDc and develop methods for further precise control of the wave modes.

Controlling Min wave mode selection can be used to expand the use and varieties of artificial cells. The time-averaged distribution was different between the two wave modes. The traveling wave generates a homogeneous time-averaged distribution of MinD on the membrane, while the standing wave generates a minimum time-dependent dynamic wave.
average concentration of MinD at the center of living cells. In living cells, the cell division plane is determined by the difference in the time-averaged distribution. This is because MinC, which inhibits FtsZ polymerization for cell division initiation, does not localize at the minimum, and cell division starts at the center of the cell (11). Therefore, finding stable generation conditions for the standing wave will contribute to the reconstitution of bacterial cell division machinery in artificial cells. Moreover, because these two RDC wave modes are linked to cellular functions, such as motility (6) and cell division (9, 10), the transition between the traveling and standing wave modes of Min waves could be used as a cue to the switching dynamics of artificial cells in response to environmental signals. Hence, the findings of this study pave the way for the construction of molecular robots with flexible function switches using the mode transition of RDC waves.

Last, we discuss the physiological importance of the elucidation of the mechanism Min wave mode selection in artificial cells. We showed not only the geometry but also the parameters involved in the mode selection of Min waves. This finding explains why both standing and traveling waves have been observed in rod-shaped and spherical cells. Furthermore, the findings will lead to an understanding of the mechanism of spatiotemporal pattern formation using RDCs other than Min waves. For example, in mast cells, the traveling wave of actin is the dominant wave mode, but the standing oscillation of actin also appears because of the oscillation of phosphatidylinositol 4,5-bisphosphate, which accompanies the oscillation of cytosolic calcium (54). Changes in parameters may influence the generation of this standing oscillation of actin waves, similar to the Min waves. Moreover, in the case of the PIP3/actin waves in amoeba cells, both the traveling wave and the standing wave emerge in the latrunculin-treated cells, and the theoretical analysis suggested that the reaction balance of PIP3 is involved in the mode selection (55). Therefore, the conclusion of our study, that is, the activator-inhibitor balance determines the Min wave mode, is expected to hold for other intracellular reaction-diffusion waves. As another example, our findings predict that standing waves can appear in the traveling wave of Rho and actin waves, in which no standing waves have been observed so far. To address these points in the future, we will illuminate novel roles for RDC waves in living cells.

MATERIALS AND METHODS
Expression and purification of Min proteins
Escherichia coli BL21-CodonPlus(DE3)-RIPL cells (Agilent Technologies, Santa Clara, CA, USA) were transformed with either pET15-MinD, pET29-MinE, or pET15-msfGFP-MinC and cultivated in LB medium with ampicillin (MinD and msfGFP-MinC) or kanamycin (MinE) at 37°C. The proteins were expressed by 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at OD600 (optical density at 600 nm) = 0.8 (MinD) or at OD600 = 0.2 (MinE and msfGFP-MinC), and the cells were further cultivated for 1.5 hours (MinD) or for 3 to 4 hours (MinE and msfGFP-MinC), respectively. The cells were collected by centrifugation at 8000g for 2 min at 4°C, resuspended in the lysis buffer [50 mM tris-HCl (pH7.6), 300 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM imidazole, and 1 mM DTT], and sonicated using Sonifier250 (Branson, Danbury, CT, USA). In the case of His-MinD, imidazole concentration of the lysis buffer was 20 mM, and 0.1 mM ADP was added to the lysis buffer. The soluble fraction in the lysate was fractionated by centrifugation at 20,000g for 30 min at 4°C. The supernatant was filtered by HPF Millex HV (Merck Millipore, Billerica, MA, USA) and mixed with 1 ml of Ni Sepharose 6 Fast Flow (Cytiva, Tokyo Japan) (MinD) or cComplete His-Tag Purification Resin (Roche, Basel, Switzerland) (MinE and msfGFP-MinC). After shaking for 30 min at 4°C, the mixture was loaded to a Poly-Prep Chromatography Column (Bio-Rad, Hercules, CA, USA) and washed by adding 25 ml of wash buffer [50 mM tris-HCl (pH7.6), 300 mM NaCl, 1 mM PMSF, 0.1 mM EDTA, 20 or 25 mM imidazole, and 10% glycerol]. Then, His-tagged proteins were eluted with 2 ml of elution buffer [50 mM tris-HCl (pH7.6), 300 mM NaCl, 1 mM PMSF, 0.1 mM EDTA, 250 or 500 mM imidazole, and 10% glycerol]. The elution buffer was exchanged to the storage buffer [50 mM Heps-KOH (pH7.6), 150 mM GluK, 0.1 mM EDTA, 10% glycerol, and 0.1 mM ADP in the case of MinD] by repeating the ultrafiltration using an AmoniUltra-4 3K (MinE) or 10K (MinD and msfGFP-MinC) (Merck Millipore) and the addition of the storage buffer. The proteins were separated by SDS–polyacrylamide gel electrophoresis, and the concentration was estimated by quantifying the band intensity after Coomassie Brilliant Blue (CBB) staining using Fiji software (National Institutes of Health, Bethesda, MD, USA).

Expression and purification of msfGFP and mCherry
For msfGFP expression, E. coli BL21-CodonPlus(DE3)-RIPL cells were transformed with pSUMO-msfGFP (msfGFP was cloned into homemade pSUMO vector (30)) and cultivated in LB medium with ampicillin at 37°C. The protein was expressed by 0.1 mM IPTG at OD600 = 0.4, and the cells were further cultivated for 3 hours. Cell collection, cell disruption, and purification by Ni column were the same as for msfGFP-MinC purification except the NaCl in buffers was 500 mM. After elution, SUMO-tag was digested by homemade Ulp1 (30) and was removed by using Ni column after 1:20 dilution with 20 mM Heps-KOH (pH 7.6). msfGFP in the flowthrough of the Ni column was further purified by using Hitrap Q Sepharose (Cytiva) by elution using KCl [20 mM Heps-KOH (pH 7.6) and 125 mM KCl]. The purified msfGFP was concentrated by ultrafiltration using an AmiconUltra-4 10K to 300 μM. The concentration of msfGFP was quantified by 280 nm. mCherry protein used in this study was further purified using HiTrap Q Sepharose (Cytiva) by elution using KCl [20 mM Heps-KOH (pH 7.6) and 125 mM KCl]. The purified msfGFP was concentrated by ultrafiltration using an AmiconUltra-4 10K to 300 μM. Both proteins were stored at −30°C.

Self-organization assay inside microdroplets covered with lipids
E. coli polar lipid in chloroform (25 mg/ml) (Avanti, Alabaster, AL, USA) was dried by gentle argon gas flow and dissolved in mineral oil (Nacalai Tesque, Kyoto, Japan) to a lipid concentration of 1 mg/ml in glass tubes. The lipid mixture was sonicated for 90 min at 60°C using Bransonic (Branson) and mixed by vortexing for 1 min. In the case of the standard condition, the inner solution consisted of 0.1 μM His-msfGFP-MinC, 1 μM His-MinD, 1 μM MinE-His, 2.5 mM ATP, and bovine serum albumin (BSA) (100 mg/ml) in the reaction buffer [25 mM tris-HCl (pH 7.6), 150 mM GluK, and 5 mM GluMg]. Before preparation of the mixture, BSA (A6003, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in water and washed with the reaction buffer by using AmiconUltra-0.5 50K (Merck Millipore), and the concentration was quantified by Pierce BCA.
Protein Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). The concentrations of each component were varied as indicated. One microliter of the inner solution was added to the 50-μl lipid mixture in microtubes, and the microdroplets were obtained by tapping microtubes. Twenty microliters of the droplet mixture was placed into two glass coverslip slits. The self-organization of Min proteins inside the droplets was observed using a fluorescence microscope (AxioObserver Z1; Carl Zeiss, Jena, Germany). The time-lapse images obtained were processed by using Fiji software.

In the case of the investigation of the effect of temperature, a glass-heating stage (Tpi- SQFTX, Tokai Hit, Shizuoka, Japan) was set on the microscope, and the prepared slide was placed on the glass-heating stage immediately after sample preparation.

In the case of the observation of the early stage of Min wave emergence, 10 mM creatine phosphate, 0.1 mM creatine kinase, and 2 mM ADP were added to the inner solution instead of ATP. After making the prepared slide of microdroplet mixture, the self-organization of Min proteins was observed immediately.

**Protein synthesis by the PURE system in microdroplets and in test tubes**

PUREfx ver. 1.0 (Gene Frontier, Chiba, Japan) was used to synthesize proteins. For the investigation of the regulation of the amount of protein synthesized, the mixture of small molecules [20 mM Hepes-KOH (pH 7.6), tRNA (30 ng/μl), 10-formyltetrahydrofolate (10 μg/ml), 20 mM creatine phosphate, 2 mM dithiothreitol (DTT), 180 mM potassium glutamate, 2 mM spermidine, and 14 mM magnesium acetate at final], 20 amino acid mixture, nucleoside triphosphate (NTP) mixture [2 mM ATP, 2 mM GTP, 1 mM CTP, and 1 mM UTP at final], and 1 mM DNA encoding NanoLuc at final were added to the solution II and III of PUREfx ver. 1.0 in the test tubes. The concentrations of 20 amino acids were varied as indicated. After the 4-hour reaction at room temperature, the concentration of NanoLuc was quantified by Nano-Glo Luciferase Assay System (Promega, Madison, WI, USA). In the case of the self-organization assay with MinE synthesis inside microdroplets, the mixture of 0.1 μM His-msfGFP-MinC, 1 μM His-MinD, 0.7 μM MinE-His, BSA (100 mg/ml), small molecules described above, 20–amino acid mixture, NTP mixture, 1 nM DNA encoding MinE, and the solution II and III of PUREfx ver. 1.0 was encapsulated in microdroplets. The microdroplets encapsulating the inner solution were obtained as described above and placed into two glass coverslip slits. The change of the dynamics of self-organized Min proteins during incubation was observed using a fluorescence microscope.

**ATPase assay**

For the investigation of the temperature dependence of ATPase activity of MinD, ATPase activity of MinD at various temperatures was tracked by measuring the increase in inorganic phosphate (Pi) concentration. To prepare small unilamellar vesicles (SUVs) used as lipids, *E. coli* polar lipid (25 mg/ml) in chloroform was dried under gentle argon gas flow. The lipid film was further dried in a desiccator for 3 hours at room temperature. The reaction buffer for the Min wave observation was added to the lipid film to adjust the lipid concentration of 4 mg/ml and incubated at room temperature overnight. The lipid solution was vortexed for 1 min and extruded by using Avanti Mini Extruder (Avanti). In the extrusion process, the lipid solution was passed through polycarbonate membranes with pore sizes 1.0, 0.4, and 0.05 μm, in this order, to obtain the SUVs. The reaction mixture for the ATPase assay consisted of 1 μM His-MinD, 1 μM MinE-His, 2.5 mM ATP, and SUV solution (1 mg/ml) in the reaction buffer. The mixture was incubated at 25°C, 29°C, and 37°C for 0 to 30 min after 5-min incubation at 25°C. The concentration of Pi of each reaction mixture incubated under three temperature conditions from 0 to 30 min was quantified by using BIOMOL Green (Enzo Life Science, Farmingdale, NY, USA). ATPase rates were calculated from the slope of the increase in Pi with time.

**Theoretical model**

We performed numerical simulations of the model based on the previous works (29, 44, 50). ATP-MinD, ADP-MinD, and MinE concentrations inside a spherical membrane with its radius, R, were denoted by cDT, cDD, and cE, respectively. Concentrations of MinD, MinE, and their complex (MinDE) bound to a membrane were denoted by cD, cDE, and cE, respectively. Each reaction shows a rate, ω, specified by its subscript. Recruitment of MinD onto the membrane from the cytosol is given by ωDT and ωDD, where in the latter reaction, membrane-bound MinD recruits MinD in the cytosol. Recruitment of MinE in the cytosol by membrane-bound MinD is described by ωE. The binding rate ωed of MinD and MinE on the membrane is assumed to be fast. Attachment and detachment of MinE from/to the cytosol are described by ωE and ωe, respectively. The rate of MinD dissociation from the membrane is expressed by ωdd,m. We assumed the same diffusion constants D in proteins bound to the membrane and set them to be unity without loss of generality. We also assumed that the bulk diffusion constants of unbound proteins are the same and are denoted by D. The time scale was normalized by 1/ωe. The model is given by the following equations:

\[ \frac{\partial c_{DT}}{\partial t} + \frac{c_{DD}}{D} = D \left( \frac{\Delta - 1}{\xi^2} \right) c_{DD} \]  
\[ \frac{\partial c_{DD}}{\partial t} = D \Delta c_E \]  
\[ \frac{\partial c_D}{\partial t} = \Delta c_D + \omega_{DT} c_E c_D - \omega_{DD} c_D \]  
\[ \frac{\partial c_{DE}}{\partial t} = \Delta c_{DE} + \omega_{DE} c_D c_E - \omega_{ee} c_E c_D \]  
\[ \frac{\partial c_E}{\partial t} = \Delta c_E + \omega_{ed} c_D c_E - \omega_{dd,m} c_E c_D - \omega_{ed} c_E c_D \]  

Here, Δ and D are the Laplacian operator in d-dimensional bulk space and the Laplace-Beltrami operator on the (d-1)-dimensional surface, respectively, where d is dimension of the system. The boundary conditions of Eqs. 1 to 3 are

\[ -D\nabla_n (c_{DT} + c_{DD}) = c_{DT} (\omega_{DT} + \omega_{DD} c_D) - c_{DE} \]  
\[ -D\nabla_n c_{DD} = -c_{DE} \]  
\[ -D\nabla_n c_E = \omega_{EE} c_E c_D - \omega_{ee} c_E c_D \]  

The length scale associated with ATP hydrolyzation is denoted by \( \xi = \sqrt{D/\lambda} \), where λ is the rate of ADP/ATP exchange rate. We set λ = 1. Equations 1 to 5 are solved using the software of the finite elements.
element method, COMSOL. To compute wave generation in a broad range of parameters, we mainly used a 2D system in which the cytosol is inside the disk, and the membrane is on a circle. Nevertheless, we also performed simulations in 3D systems in Fig. 6 and fig. S10. We mainly chose the parameters as $D = 100$, $\omega_D = 0.1$, $\omega_{oDi} = 5.0$, $\omega_p = 0.1$, $\omega_c = 100$, $\omega_{de,m} = 1.0$, and $\omega_{de,E} = 0.04$ (29), and varied $\omega_{oDi}$, $\omega_p$, and $\omega_{de,m}$ in the text. In the parameter space of $\omega_p$ or $\omega_{oDi}$, each parameter was discretized in 21 mesh points. We also used the same data points in the parameter space of MinE-MinD. For each parameter set, we perform the simulations for the time $T = 1000$. The initial condition was chosen to be a small gradient in one direction on the membrane with the Gaussian random distribution. We have also tested the initial condition where all proteins are in cytosol, but it is sometimes trapped by metastable states. The wave generation occurred around $t = 100$ as a standing wave, and then the wave switched to the traveling wave when the parameters are at its state. After the relaxation time $t \geq 1000$, the wave states were estimated. For the 3D model, we performed the simulations for the time $T = 1000$, and their data are averaged for $t \geq 300$.

Inhomogeneity of the concentration field was expressed by the amplitude of each mode in the expansion of the concentration $c_d$ by the Fourier expansion in the polar coordinates $(\rho, \sigma)$ as

$$c_d(\rho, \sigma, t) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} c_{d,l}^{(m)}(t) e^{i(l\rho - m\sigma)}$$

(10)

For example, the uniform distribution of MinD on a membrane was expressed by the $l = 0$ mode and its norm, $\|c_{d,0}\|$, whereas the first mode ($l = 1$) corresponds to the inhomogeneous concentration field of a single wave, which is characterized by the norm $\|c_{d,1}\| = \sqrt{c_{d,1,0}^2 + c_{d,1,-1}^2}$. The state diagram was drawn by the following criteria: The wave state is at $(\|c_{d,1}(t)\|) \geq 0.2$ and $\min Re c_{d,l}(t) - \min Re c_{d,l}(t) \geq 0.2$, where $(\cdot)$ denotes the average over time. The traveling and standing waves were detected by the dynamics of the phase $\phi(t) = \tan^{-1}c_{d,1,0}/c_{d,1,-1}$ of MinD on the membrane. When $\max(\theta(t)) - \min(\theta(t)) \geq 3\pi/2$, the wave is the traveling wave, whereas the standing wave is at $\max(\theta(t)) \sim \min(\theta(t)) < 3\pi/2$.

When we varied the parameter $\omega_{de,m}$, we applied the stepwise change from $t = 500$ to $t = 1000$, that is, $\omega_{de,m}$ is larger at $500 \leq t \leq 1000$, whereas $\omega_{de,m} = 1$ otherwise. The parameter change was performed continuously with a time width of 1. When we varied the concentration of MinE, we added the source term in the right-hand side of Eq. 3. From $t = 500$, we added 0.001 at each time until the total MinE became the target value (either MinE = 1.0 or MinE = 1.5). This procedure implies that the MinE concentration in the cytosol changes linearly in time.

The analysis of the 3D systems was performed similar to the 2D systems. The amplitude of each mode of the concentration $c_d$ is expressed by the spherical harmonics expansion in the spherical coordinates $(\rho, \theta, \phi)$ as

$$c_d(\rho, \theta, \phi, t) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} c_{d,l}^{(m)}(t) Y_l^m(\theta, \phi)$$

(11)

where $\theta$ and $\phi$ are the polar and azimuthal angles, respectively, and $Y_l^m(\theta, \phi)$ is the spherical harmonics of degree $l$ and order $m$. The inhomogeneous concentration field of a single wave is characterized by the norm $\|c_{d,l}\| = \sqrt{c_{d,l,0}^2 + c_{d,l,1}^2 + c_{d,l,-1}^2}$. We used the same threshold to identify the region of wave generation. The phase was defined after rotating the wave on spherical surface so that the high concentration of MinDE is located on the equator.

Parameters with unit in the theoretical model
We nondimensionalized the parameters in the theoretical model. The unit length and time are chosen as $\sqrt{D_e/m}$ and $1/\omega_e$, respectively. We have chosen these units as $\sqrt{0.3/0.5} \approx 0.77\mu m$ and $1/0.5 \approx 2.0$ s. We also used the unit of concentration in bulk as $10^3/\mu m^3$.

In Fig. 6B, we have translated the concentration using the unit discussed here.

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abm4860

REFERENCES AND NOTES
1. B. Goldstein, I. G. Macara, The PAR proteins: Fundamental players in animal cell polarization. Dev. Cell 13, 609–622 (2007).
2. C. Hoege, A. A. Hyman, Principles of PAR polarity in Caenorhabditis elegans embryos. Nat. Rev. Mol. Cell Biol. 14, 315–322 (2013).
3. A. B. Goryachev, A. V. Pokhilko, Dynamics of Cdc42 network embodies a Turing-type mechanism of yeast cell polarity. FEBS Lett. 582, 1437–1443 (2008).
4. E. B. H. O. Park, Cell polarization and cytokinesis in budding yeast. Genetics 191, 347–387 (2012).
5. T. Killich, P. J. Plath, X. Wei, H. Bultmann, L. Rensing, M. G. Vicker, The locomotion, shape and pseudopodial dynamics of unstimulated Dictyostelium cells are not random. J. Cell Sci. 106, 1005–1013 (1993).
6. M. G. Vicker, Eukaryotic cell locomotion depends on the propagation of self-organized reaction-diffusion waves and oscillations of actin filament assembly. Exp. Cell Res. 275, 54–66 (2002).
7. G. Gerisch, M. Ecke, B. Schroth-Diez, S. Gerwig, U. Engel, L. Madderda, M. Clarke, Self-organizing actin waves as planar phagocytic cup structures. Cell Adh. Migr. 3, 373–382 (2009).
8. Y. Arau, T. Shibata, S. Matsuoka, M. J. Sato, T. Yanagida, M. Ueda, Self-organization of the phosphatidylinositol lipids signaling system for random cell movement. Proc. Natl. Acad. Sci. U.S.A. 107, 12399–12404 (2010).
9. W. M. Bement, M. Leda, A. M. Moe, A. M. Kita, E. M. Larson, A. E. Goldberg, C. Pfeuti, K.-C. Su, A. L. Miller, A. B. Goryachev, G. von Dassow, Activator-inhibitor coupling between Rho and actin assembly makes the cell cortex an excitable medium. Nat. Cell Biol. 17, 1471–1483 (2015).
10. A. B. Goryachev, M. Leda, A. L. Miller, G. von Dassow, W. M. Bement, How to make a static cytokinetic furrow out of traveling excitable waves. Small GTPases 7, 65–70 (2016).
11. V. W. Rowlett, W. Margolin, The bacterial Min system. Curr. Biol. 23, R553–R556 (2013).
12. B. Ramn, T. Heeram, P. Schiwie, The E. coli MinCDE system in the regulation of protein patterns and gradients. Cell. Mol. Life Sci. 76, 4245–4273 (2019).
13. T. Bretschneider, S. Diez, K. Anderson, J. Heuser, M. Clarke, A. Müller-Taubenberger, J. Kühler, G. Gerisch, Dynamic actin patterns and Arp2/3 assembly at the substrate-attached surface of matole cells. Curr. Biol. 14, 1–10 (2004).
14. D. M. Raskin, P. A. J. De Boer, Rapid pole-to-pole oscillation of a protein required for directing division to the middle of Escherichia coli. Proc. Nat. Acad. Sci. U.S.A. 96, 4971–4976 (1999).
15. Z. Hu, J. Lutkenhaus, Topological regulation of cell division in Escherichia coli involves rapid pole to pole oscillation of the division inhibitor MinC under the control of MinD and MinE. Mol. Microbiol. 34, 82–90 (1999).
16. G. Gerisch, T. Bretschneider, A. Müller-Taubenberger, E. Simmeth, M. Ecke, S. Diez, K. Anderson, Mobile actin clusters and traveling waves in cells recovering from actin depolymerization. Biophys. J. 87, 2943–3050 (2004).
17. O. D. Weiner, A. M. Marganski, L. F. Wu, S. J. Altschuler, M. W. Kirschner, An actin-based wave generator organizes cell motility. PLoS Biol. 5, e221 (2007).
18. E. Knobloch, Oscillatory convection in binary mixtures. Phys. Rev. A 34, 1538–1549 (1986).
19. W. Schopf, W. Zimmermann, Convection in binary fluids: Amplitude equations, codimension-2 bifurcation, and thermal fluctuations. Phys. Rev. E 47, 1739–1764 (1993).
20. A. M. Zhbotosinsky, M. Dolnik, L. R. Epstein, Pattern formation arising from wave instability in a simple reaction-diffusion system. J. Chem. Phys. 103, 10306–10314 (1995).
21. M. Loose, F. Fischer-friedrich, J. Ries, K. Kruse, P. Schwille, Spatial regulators for bacterial cell division self-organize into surface waves in vitro. Science 320, 789–792 (2008).
22. J. Schweizer, M. Loose, M. Bonny, K. Kruse, I. Mönch, P. Schwille, Geometry sensing by self-organized protein patterns. Proc. Natl. Acad. Sci. U.S.A. 109, 15283–15288 (2012).

23. K. Zieske, P. Schwille, Reconstitution of pole-to-pole oscillations of Min proteins in microengineered polydimethylsiloxane compartments. Angew. Chem. Int. Ed. 52, 459–462 (2013).

24. K. Zieske, P. Schwille, Reconstitution of self-organizing protein gradients as spatial cues in cell-free systems. eLife 3, e03949 (2014).

25. A. G. Vecchiarelli, M. Li, M. Mizuuchi, K. Hwang, Y. Seol, K. C. Neuman, K. Mizuuchi, Membrane-bound MinDE complex acts as a toggle switch that drives Min oscillation coupled to cytoplasmic depletion of MinD. Proc. Natl. Acad. Sci. U.S.A. 113, E1479–E1488 (2016).

26. J. Schweizer, M. Reiter, E. Kingma, E. Frey, C. Dekker, Multistability and dynamic transitions of intracellular Min protein patterns. Mol. Syst. Biol. 12, 873–873 (2016).

27. K. Zieske, G. Chwastek, P. Schwille, Protein patterns and oscillations on lipid monolayers and in microdroplets. Angew. Chem. Int. Ed. 55, 13455–13459 (2016).

28. T. Litschel, B. Ramm, R. Maas, M. Heymann, P. Schwille, Beating vesicles: Encapsulated protein oscillations cause dynamic membrane deformations. Angew. Chem. Int. Ed. 57, 16286–16290 (2018).

29. S. Koyama, N. Yoshinaga, M. Yanagisawa, K. Fujisawa, N. Doi, Cell-sized confinement controls generation and stability of a protein wave for spatiotemporal regulation in cells. eLife 8, e44591 (2019).

30. A. Yoshida, S. Koyama, K. Fujisawa, S. Nishikawa, N. Doi, Regulation of spatiotemporal patterning in artificial cells by a defined protein expression system. Chem. Sci. 10, 11064–11072 (2019).

31. P. A. J. de Boer, R. E. Crossley, L. I. Rothfield, A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of Min proteins and the phospholipid membrane in vitro. J. Cell Biol. 185, 735–749 (2003).

32. M. Loose, E. Fischer-Friedrich, C. Herold, K. Kruse, P. Schwille, Min protein patterns emerge from rapid rebinding and membrane interaction of MinE. Nat. Struct. Mol. Biol. 10, 367–373 (2003).

33. Z. Hu, J. Lutkenhaus, A conserved sequence at the C-terminus of MinD is required for binding to the membrane and targeting MinC to the septum. Mol. Microbiol. 47, 345–355 (2003).

34. L. L. Lackner, D. M. Raskin, P. A. J. De Boer, ATP-dependent interactions between Escherichia coli Min proteins and the phospholipid membrane in vitro. J. Bacteriol. 185, 1096–1107 (2003).

35. M. K. Huang, Y. Meir, N. S. Wingreen, Dynamic structures in Escherichia coli: Spontaneous formation of MinE rings and MinD polar zones. Proc. Natl. Acad. Sci. U.S.A. 100, 12724–12728 (2003).

36. R. Gessesse, J. Halatek, L. Wurthner, E. Frey, Geometric cues stabilise long-axis polarisation of PAR protein patterns in C. elegans. Nat. Commun. 11, 539 (2020).

37. P. Gock, B. Ramm, T. Heermann, S. Kretschmer, J. Schweizer, J. Mücksch, G. Slagel, P. Schwille, Stationary patterns in a two-protein reaction-diffusion system. ACS Synth. Biol. 8, 148–157 (2019).

38. F. Braun, G. Pavlik, J. Halatek, J. Kerssemakers, E. Frey, C. Dekker, Bulk-surface coupling identifies the mechanistic connection between Min-protein patterns in vivo and in vitro. Nat. Commun. 12, 3312 (2021).

39. M. Wu, X. Wu, P. De Camilli, Calcium oscillations-coupled conversion of actin travelling waves to standing oscillations. Proc. Natl. Acad. Sci. U.S.A. 110, 1339–1344 (2013).

40. D. Taniuchi, S. Ishihara, T. Oonuki, M. Honda-Kitahara, K. Kaneko, S. Sawai, Phase geometries of two-dimensional excitable waves govern self-organized morphodynamics of amoeboid cells. Proc. Natl. Acad. Sci. U.S.A. 110, 5016–5021 (2013).

41. T. Akiu, K. Fujisawa, G. Sato, M. Takinoue, S.-i. M. Nomura, N. Doi, System concentration shift as a regulator of transcription-translation system within liposomes. iScience 24, 102859 (2021).

Acknowledgments: We thank M. Yanagisawa (The University of Tokyo) for the helpful discussion and an advice on the manuscript. Funding: This work was funded by JSPS KAKENHI grant number JP20H01875 (to N.Y. and K.F.), JSPS KAKENHI grant number JP19K04717 (to K.F.), and JSPS KAKENHI grant number JP20K03874 (to N.Y.). Author contributions: Conceptualization: K.F. Methodology: S.T., N.Y., and K.F. Formal analysis: S.T., N.Y., and K.F. Funding acquisition: N.Y. and K.F. Investigation: S.T., N.Y., and K.F. Visualization: S.T. and N.Y. Project administration: K.F. Supervision: N.Y. and N.D., and K.F. Writing—original draft: S.T., N.Y., and K.F. Writing—review and editing: S.T., N.Y., and K.F. Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Submitted 4 November 2021
Accepted 22 April 2022
Published 8 June 2022