Vol. 21, e210022 (2024)
doi: 10.2142/biophysico.bppb-v21.0022

Biophysics and Physicobiology
https://www.jstage.jst.go.jp/browse/biophysico/

Review Article (Invited)

Artificial cell system as a tool for investigating pattern formation mechanisms of intracellular reaction-diffusion waves

Sakura Takada, Kei Fujiwara

Department of Biosciences and Informatics, Keio University, Yokohama, Kanagawa 223-8522, Japan

Received September 5, 2024; Accepted October 8, 2024;
Released online in J-STAGE as advance publication October 10, 2024
Edited by Yasushi Sako

Intracellular positional information is crucial for the precise control of biological phenomena, including cell division, polarity, and motility. Intracellular reaction-diffusion (iRD) waves are responsible for regulating positional information within cells as morphogens in multicellular tissues. However, iRD waves are explained by the coupling of biochemical reactions and molecular diffusion which indicates nonlinear systems under far from equilibrium conditions. Because of this complexity, experiments using defined elements rather than living cells containing endogenous factors are necessary to elucidate their pattern formation mechanisms. In this review, we summarize the effectiveness of artificial cell systems for investigating iRD waves derived from their high controllability and ability to emulate cell-size space effects. We describe how artificial cell systems reveal the characteristics of iRD waves, including the mechanisms of wave generation, mode selection, and period regulation. Furthermore, we introduce remaining open questions and discuss future challenges even in Min waves and in applying artificial cell systems to various iRD waves.

Key words: bottom-up synthetic biology, synthetic cell, reconstitution, spatiotemporal pattern, cell polarity

Significance

Intracellular reaction-diffusion (iRD) waves determine positional information within cells. The generation mechanism of iRD waves is not fully understood due to the complexity of living cells and iRD waves. Artificial cell systems composed of defined factors can overcome this problem. This review introduces previous studies that have elucidated the formation and regulatory mechanisms of iRD waves and the effects of cell-size space on iRD waves. Applying an artificial cell system to various iRD waves can become a powerful tool for elucidating the underlying principles of shaping positional information within cell-size spaces.

Introduction

Positional information such as the placement of the head, limbs, and dorsal-ventral axis is crucial for multicellular organisms. In 1952, Turing proposed that pattern formation caused by reaction-diffusion (RD) systems was the basis of morphogenesis [1], which has been demonstrated experimentally and theoretically over the past several decades [2–4], leading biologists and theorists to attract attention to the roles of RD systems in biological processes. In recent years, it has been shown that RD systems regulate intracellular positional information. For example, the cell division site [5] and cell motility [6–8] are regulated by RD systems that form periodic structures in space and time, even within micrometer-sized cells. This periodic localization of molecules generated by RD systems, similar to the gradients of morphogens, is

Corresponding author: Sakura Takada, Department of Biosciences and Informatics, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan. ORCID iD: https://orcid.org/0000-0003-3431-2996, e-mail: sakura.taka0639@keio.jp

called intracellular RD (iRD) waves and transduces their positional information to downstream elements. As the generation mechanism of iRD waves is based on physics, simple investigation of interactions and reactions among molecules is not sufficient; therefore, physical analysis is necessary.

To date, studies of RD systems have been conducted through experiments using chemical model systems, such as the two- or three-dimensional chemical waves in Belousov–Zhabotinsky reactions [9–12] and theoretical analyses based on RD equations [13]. Reaction networks including feedback loops cause local chemical reactions against molecules that spread throughout space by diffusion. These local reactions at different molecular diffusion speeds generate wave structures with periodicities (waves). Although iRD waves have been found in various cell types, understanding their formation mechanisms is challenging because knowledge of RD waves in open systems is not necessarily applicable to mesoscopic closed spaces, such as cells whose small volume and spatial closure affect the behavior of RD waves [14].

In this review, we describe the dynamics of iRD waves and their roles in regulating biological phenomena. Although this knowledge has been revealed by using living cells and theoretical models, there are problems, including the complexity of cells and the correspondence between experiments and theoretical models. As a tool to overcome these problems, we introduce artificial cell systems in which biological phenomena are reconstituted using defined factors. We summarize the characteristics of artificial cells and introduce recent achievements to reveal the regulatory mechanism of iRD waves using the artificial cell system of Min waves, the only iRD wave reconstituted in artificial cells. Finally, we discuss the unsolved questions regarding Min waves and other iRD waves, and the outlook for how they can be addressed using artificial cell systems.

Regulation of Biological Phenomena by iRD Waves

In cells that possess the unique geometric properties of micro-size confinement (cell-size space), positional information, including the position of cell division [5], direction of movement [6,7], and cell polarity [15] are precisely controlled. iRD waves are responsible for spatial control and are utilized in various species from eukaryotic to prokaryotic cells. Depending on the reaction network and physicochemical parameters of each RD system, the iRD waves exhibit different dynamics, ranging from dynamic to stationary patterns.

Actin waves are an example of iRD waves. In eukaryotic cells, although actin is known to form dynamic structures through its assembly and disassembly, it also self-organizes into iRD waves with periodic structures [16,17]. Actin waves are generated on the membrane by actin polymerization promoted by Hem1 and Rho, coupled with F-actin-dependent negative feedback [6,18,19] (Figure 1a). Although their biological significance is not completely understood, they are involved in controlling cell motility, cell shape deformation, and cytokinesis. Furthermore, in *Dictyostelium* cells, iRD waves of the lipid molecule PIP_3 and PIP_3 phosphatase (PTEN) are formed through amplification of PIP_3 by positive feedback and mutual inhibition of PIP_3 and PTEN [8,20–22]. PIP_3 induces actin polymerization, and actin pushes the cell membrane, causing cell deformation and locomotion [7]. Additionally, some bacterial cells determine the cell division site at the cell center using the Min wave oscillating between the cell poles [23,24] (Figure 1b). The Min wave is generated by a reaction cycle of ATP-dependent membrane binding of the ATPase MinD and its membrane dissociation induced by MinE [5,25]. In any iRD wave, individual molecules diffuse freely, and as a population of molecules, behave like macroscopic waves (Figure 1b).

RD systems also form macroscopic stationary patterns, such as Turing patterns. Examples of stationary structures in cells include PAR polarity in *Caenorhabditis elegans* early embryo [15] and cluster formation of Cdc42 in budding yeast [26]. In the PAR system, aPAR and pPAR proteins form polarized domains by mutual inhibition and recruitment to the cell cortex via positive feedback, which determines cell polarity [15,27–29] (Figure 1c). In budding yeast, a cycle of GTPase Cdc42 activation by GEF, recruitment to the membrane by positive feedback, and inactivation by GAP results in the formation of an active Cdc42 cluster that recruits the factors necessary for budding [26,30–32] (Figure 1d). It has been suggested that these stationary patterns are formed by a Turing-type mechanism consisting of local activation and global depletion, which has the same effect as lateral inhibition required for the formation of Turing patterns [31,33]. However, as they are affected by advective flow or molecular transport by the cytoskeleton [34,35], a complete scenario as a pure RD system remains elusive.

Although the detailed reaction networks are different in each RD system, iRD waves are formed by nonlinear reactions of activators and inhibitors under far-from-equilibrium conditions in various cells. Their inhomogeneous but well-organized localization and complicated dynamics regulate the positional information for proper biological functions, thereby contributing to the maintenance of cell homeostasis.

Investigation of Formation Mechanisms of iRD Waves by Cell Analyses and Theoretical Models

Cellular observation is the most commonly used method to clarify the molecular mechanisms and characteristics of iRD waves. Observations of iRD waves in genetically modified cells have revealed a relationship between the wave behavior

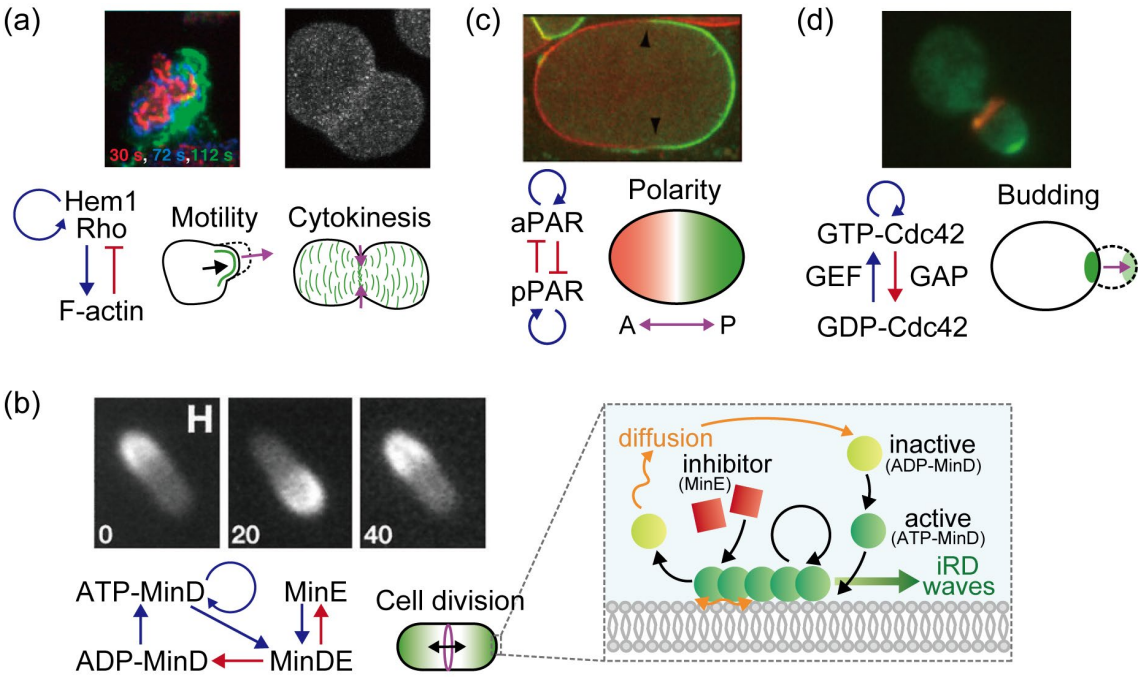

Figure 1 Various intracellular reaction-diffusion (iRD) waves. (a)-(d) iRD waves *in vivo* and schematic representation of their reaction network and function as a regulator of biological phenomena are shown. Blue arrows indicate activation, recruitment, or positive feedback, and red arrows indicate inhibition or negative feedback. (a) Hem1 waves, which promote actin polymerization in human cell (left) [6], and actin waves generated by interaction with Rho in a starfish oocyte (right) [19]. The human cell is 10–20 μm in length, and the starfish oocyte is about 100 μm. (b) Time-lapse images of Min wave in *Escherichia coli* (2–3 μm in length) oscillating between cell poles (left) [23]. Times are indicated in sec for the pictures. Schematic of the mechanism of iRD wave generation using Min wave as a model are shown (right). (c) Polarized localization of mCherry::PAR-6 (one of aPAR proteins) and GFP::PAR-2 (one of pPAR proteins) in *Caenorhabditis elegans* zygotes (~50 μm in length) [29]. aPAR (pPAR) domain determines anterior (posterior) of zygotes. (d) Localization of GTP-Cdc42 (green) and septin ring (red) in budding yeast (5–10 μm in length) [32]. A microscopic image of Hem1waves in (a) is reproduced from Ref. [6] (open access article) under CC BY license. A microscopic image of actin waves in (a) is reproduced from Ref. [19] (open access article) under CC BY license. Microscopic images of Min waves in (b) are reproduced from Ref. [23]. Copyright (1999) National Academy of Sciences. A microscopic images of a *C. elegans* zygote is reproduced with permission from Ref. [29]. Copyright (2010) The Company of Biologists. A microscopic image of budding yeast is reproduced from Ref. [32] (open access article) under CC BY-NC-SA license.

and molecules of interest. In addition, the measurement of diffusion rates by FRAP [36–38] and *in vitro* biochemical experiments [25,39,40] have also contributed to our understanding of the molecular interactions, reaction rates, and diffusion rates involved in the formation of iRD waves. However, it is difficult to interpret the results obtained from *in vivo* analyses accurately because unconsidered factors can affect the results. This black box property makes it challenging to identify the minimum factors required for iRD wave formation (Figure 2a).

One approach to resolve this problem is to reconstitute iRD waves in cells where iRD waves do not normally emerge. For example, excitable waves of Rho and F-actin appear in immature *Xenopus* oocytes that do not naturally show excitability when Ect2 and RGA-3/4 are exogenously expressed [19]. This reconstitution of actin waves in cells experimentally demonstrates that the expressed factors are sufficient for wave generation. In addition, PAR polarity in cells have been reconstituted by expressing PAR proteins in unpolarized human [41] or yeast cells [42] and by controlling the localization of PAR proteins using cap formation by designed clustering proteins [43]. These studies have led to the elucidation of the role of molecules of interest in pattern formation and how molecules interact each other for self-organization.

In parallel with the cell observations, theoretical analyses have been conducted to reveal the iRD wave mechanisms (Figure 2b). These studies were based on the fact that spatiotemporal patterns can be described using RD equations. Using RD equations based on the molecular mechanisms revealed by *in vivo* cell analysis, which are composed of activation, inhibition, positive feedback, and negative feedback, it has been investigated whether intracellular patterns could be explained by the RD model [18,31,44–48]. Moreover, structure of reaction network and reaction kinetics were adjusted

to correspond to *in vivo* experiments, and it has been examined whether the same behavior of patterns as *in vivo* can be recapitulated by theoretical models [19,34,49–51]. Through these analyses, the theoretical validity of the reaction network revealed by cell observations was verified.

As described above, studies on iRD waves have been conducted by combining *in vivo* and theoretical analyses that consider iRD waves as biological and physical phenomena, respectively. However, it is difficult to isolate RD systems from other regulatory systems, such as molecular transport by the cytoskeleton and cytoplasmic flows even in the case of the reconstitution of iRD waves in cells, because the influence of endogenous factors cannot be eliminated and because the effects of downstream effectors cannot be distinguished from the molecules of interest (Figure 2a). Moreover, mechanical stimuli due to cytoplasmic flow [52] and geometric changes [53,54] can affect waves. Competition for common resources, such as ATP and cell membranes, also affects the dynamics of biological reaction networks, which may vary wave behaviors that are sensitive to parameter changes [55–57]. Thus, the results of the cell experiments do not directly correlate with the theoretical analysis, and they are not sufficient to elucidate the mechanism of iRD waves.

Understanding the Characteristics of Biological Phenomena Using Artificial Cell Systems

The characteristics and mechanisms of biological phenomena can be interpreted more clearly using artificial cells that encapsulate biomolecules within cell-like structures covered by lipid membranes, such as W/O emulsions and liposomes [58] (Figure 2c). W/O emulsions, water microdroplets covered with a lipid monolayer, are very stable and easy to prepare. Therefore, they are a good material for investigating the cell-size space effect discussed below. Liposomes, compartmentalized vesicles formed by lipid bilayers, are closer to those of living organisms than W/O emulsions. However, due to the preparation method and their stability, the contents and lipids cannot always be freely adjusted, and there is considerable variation between liposomes, making systematic physical analysis challenging. Because artificial cells enable to encapsulate elements at precise concentrations under controlled physicochemical environments, roles of the molecule of interest and effects of physicochemical parameters can be verified directly [59]. Although there are also

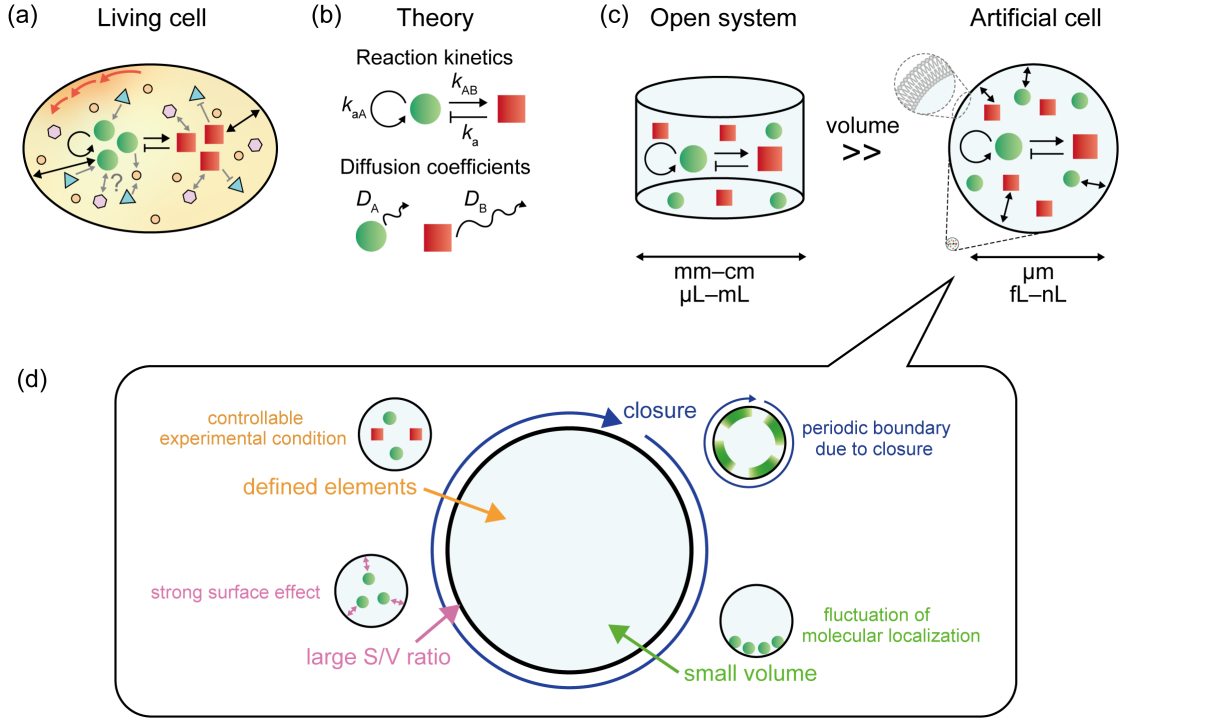

Figure 2 Schematic illustrating comparison of methods of investigating intracellular reaction-diffusion (iRD) waves. (a) *In vivo* analyses include influences of interactions with endogenous factors (grey arrows) and physical stimuli such as cytoplasmic flow (red arrows). (b) iRD waves can be described by RD equations characterized by network structure, reaction kinetics, and diffusion coefficients. (c) *In vitro* reconstitution of iRD waves is classified into open systems and artificial cell systems. While both systems consist of defined factors, there are differences in system size and whether they are closed space or not. (d) Characteristics of artificial cells and their effects on iRD waves. Spatial closure, small volume, and large surface-area-to-volume (S/V) ratio of cell-size space affect periodicity of iRD waves, fluctuation of molecule concentration, and surface effect on molecules, respectively. Due to defined components, effects of element concentrations and parameters on iRD waves can be investigated.

open systems, such as tube reactions and planar lipid membranes with bulk, as reconstitution systems, the phenomena exhibited in artificial cells reflect effects specific to cell-size spaces, such as confinement in micro-size spaces, finite element characteristics, and strong surface effects due to the large surface-area-to-volume ratio [60] (Figure 2d). Therefore, this simplified experimental system can clarify the mechanisms, even if the system that belongs to nonlinear physics in cell-size spaces that are too complicated to understand by *in vivo* and theoretical analyses.

A notable example of a biophysical study using artificial cells is the analysis of self-organized actin structures. Artificial cells encapsulating purified proteins such as actin, Arp2/3, and myosin, with or without cell extracts, have revealed events spontaneously driven by actin, such as the formation of actin ring structures [61], induction of droplet motility by membrane localization of actin and adhesion of droplets to the substrate [62], regulation of actomyosin structure by balancing its contractile force and surface interaction [63], and spontaneous symmetry breaking of actomyosin in the cell-size space [64]. Previous studies using artificial cells have also elucidated phenomena specific to cell-size spaces, such as the generation of a vortex flow of microtubules and kinesin owing to the mechanical action of the surface [65], the importance of molecular crowding on the membrane for the deformation of artificial cells owing to the polymerization of the bacterial cytoskeleton MreB [66], and the mechanism of the scaling of spindle size in droplets containing cell extracts of *Xenopus* eggs [67,68].

These practical examples demonstrate the unique characteristics of artificial cells, such as the ability to observe the early stages of phenomena, make corresponding observed phenomena and element concentrations, and verify surface effects. These advantages enable the understanding of complex biological phenomena from a physical perspective. Artificial cell systems can also offer breakthroughs in elucidating the mechanism of iRD wave formation because RD systems are highly sensitive to parameters and are influenced by confinement effects and geometric shapes (Figure 2d). For example, a strong surface effect due to small space [60] is a critical factor that changes the behavior of iRD waves generated on the cell membrane. Because this small volume also limits the number of molecules, asymmetric molecular localization causes the depletion of molecules in some areas. Moreover, the periodicity of space itself may affect the periodicity of the iRD waves. The usefulness of artificial cells shown from these points is evident from previous studies of Min waves, which have been analyzed both *in vivo* and *in vitro*.

Characteristics of Min Waves Revealed by Artificial Cell Studies

The Min wave is the only iRD wave that has been successfully reconstituted in the artificial cells. The generation of Min waves requires two proteins (MinD and MinE), ATP, and a lipid membrane [69]. The core molecular mechanisms of MinDE were elucidated through cellular observations and biochemical analyses [5]. The oscillation of the Min wave between cell poles is generated by Min proteins repeating the ATP-dependent membrane binding of MinD, the formation of the MinDE complex on the membrane, the induction of MinD ATPase activity by MinE, the hydrolysis of ATP by MinD, and dissociation from the membrane as ADP-bound MinD [5] (Figure 1b). The addition of MinDE and ATP to a 2D planar lipid membrane generated Min waves, demonstrating that these elements are the minimum factors required for Min wave generation [69].

The reconstitution of Min waves on lipid planar membranes revealed significant differences in the properties of Min waves between *in vivo* and open systems, highlighting the necessity for *in vitro* experiments in cell-size spaces. For instance, on planar lipid membranes, Min waves exhibit traveling waves rather than the standing waves observed in living cells, with a wavelength approximately 10 times larger than those *in vivo* [69,70]. In addition, standing waves are generated only under specific conditions using a MinD mutant with enhanced membrane binding [71], indicating that the conditions required for the generation of standing waves differ between open systems and cell-size spaces.

Furthermore, cell-size spaces have different effects on RD systems from open systems, as evidenced by the following examples: standing waves appeared in chambers of similar size to living cells covered with planar lipid membranes [72], wave modes and wavelengths changed depending on the surface-area-to-volume ratio in open systems [73], and wave behaviors and axial direction were regulated by space size and shape in *E. coli* cells whose shapes were variously manipulated [54,74]. Therefore, it is crucial to obtain more detailed information using artificial cell systems with precise control over their physicochemical parameters and geometric characteristics.

Approximately 10 years after Min wave reconstitution on planar lipid membranes [69], it was reported that Min waves had also been reconstituted in artificial cells. By encapsulating purified MinD, MinE, and ATP in lipid-covered microdroplets or liposomes, a single Min wave similar to that in living cells, and not multiple waves, was observed [75–77]. Notably, the stable generation of Min waves in artificial cells formed by *E. coli* polar lipids requires the addition of a high concentration of BSA (10–100 mg/mL) to mimic molecular crowding in cells [77]. Min waves have also been found to be highly sensitive to parameter changes; therefore, they only appear under limited MinDE concentrations [78]. Min waves were generated by expressing MinDE using a cell-free protein expression system (PURE system) in artificial cells, and the generation and disappearance of Min waves were controlled by changing MinDE concentrations within the same artificial cell [79].

iRD waves are nonlinear and far from equilibrium in mesoscopic spaces, making it challenging to understand their principles. However, experiments using artificial cells with defined factors have elucidated the reason for the difference in wave generation conditions between cell-size spaces and open systems. In artificial cells, a high concentration of BSA is required for Min wave generation to suppress nonspecific adsorption of MinE to the membrane by competitive membrane binding (multimolecular competition) [56]. That is, BSA plays a role in modulating the surface effect and ensuring proper molecular placement, similar to that in living cells (Figure 3a). This difference was due to the large surface-area-to-volume ratio of the cell-size space. This highlights that in cell-size spaces, not only the essential elements for Min wave generation but also the exogenous elements that tune the surface effect are crucial for wave generation.

Moreover, the high controllability of artificial cells has contributed to the clarification of the formation and regulatory mechanisms of Min waves. For instance, although the MinE mutants which show a different conformational equilibrium from the wild-type cannot generate Min waves in living cells, they can generate Min waves in artificial cells, depending on their concentration [78] (Figure 3b). Thus, the reaction rates determined by the elemental concentrations and reaction constants are critical for wave generation. Such extensive verification of the mutant concentrations is difficult without the use of artificial cells.

The mechanism of the mode selection of Min waves, a long-standing issue in this field, has also been elucidated using an artificial cell system. In the case of equimolar MinDE, the Min waves mainly show a traveling wave, not a standing wave [77], although the standing wave is necessary to determine the cell division site in living cells. By controlling MinDE concentrations and reaction constants, it was found that standing waves increased when the membrane dissociation of MinD, or the effect of MinE, was relatively dominant over MinD membrane binding [80] (Figure 3c). Since no waves were generated when the effect of either MinD or MinE was extremely strong, an exquisite balance determined the mode. This mechanism can be generalized as the mode selection mechanism of iRD waves by the activator-inhibitor balance. Such precise parameter adjustments allow a clear understanding of nonlinear iRD waves under far from equilibrium conditions.

Artificial cells offer the advantage of eliminating factors that are essential for cell survival. In particular, ATP, an energy source for various iRD waves, including Min waves, is involved in many biological phenomena, and the effects of ATP can only be examined *in vitro*. It was found that Min waves could also be generated using deoxyATP (dATP) as an energy source and that Min waves generated by dATP were twice as fast as those generated by ATP (Figure 3d). Furthermore,

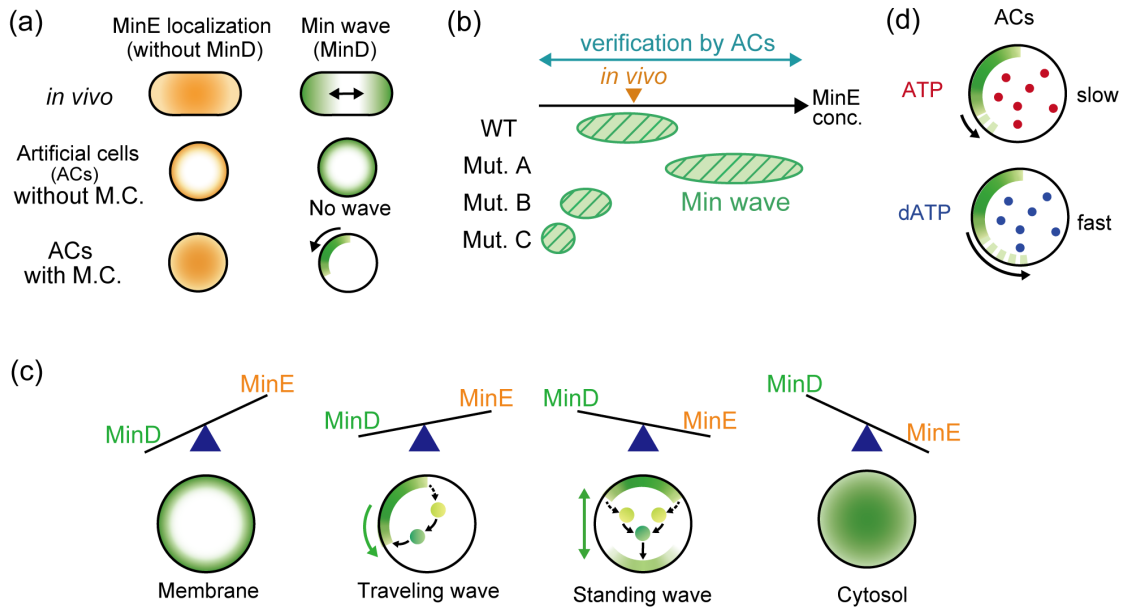

Figure 3 Formation and regulation mechanisms of Min waves revealed by artificial cell system. (a) Relationship between MinE localization in the absence of MinD and Min wave generation. Cytosolic localization of MinE by multimolecular competition (M. C.) is necessary to generate Min wave in cell-size space covered with physiological lipids. (b) Schematic representation of difference in concentration ranges for Min wave generation between wild-type and MinE mutants. Although Min wave is not generated by MinE mutants under physiological concentrations *in vivo*, artificial cells enable verification in a wide range of concentration. (c) The mode selection mechanism of Min waves. Balance between MinD (activator) and MinE (inhibitor) determines MinD dynamics: membrane localization (no wave), traveling wave, standing wave, and cytosolic localization (no wave). (d) Difference in Min wave dynamics between ATP and deoxyATP (dATP). Min wave generated by dATP is faster than that by ATP.

mixing ATP and dATP allows linear control of the wave period depending on the mixing ratio [81]. This phenomenon is impossible to observe in living cells, where ATP is 100 times more abundant than dATP. Altogether, these results illustrate that customizing the reconstitution conditions in artificial cells can clarify the formation and regulation mechanisms of Min waves, such as the influence of mutants, the wave mode selection mechanism, and the influence of energy molecules.

Perspectives for Future Challenges to Reveal Formation Mechanisms of iRD Waves

Previous studies have elucidated the mechanisms of wave formation, such as the mode selection and the influence of energy molecules on the dissipation process, using Min waves reconstituted in artificial cells [80,81]. However, some aspects remain unclear, including the mechanism of adaptation of the wave period, wavelength, and wave shape to spatial size changes, which is crucial for determining the cell division plane in living cells. Furthermore, the application of artificial cells to various iRD waves is required to understand iRD waves other than Min waves from a physical perspective.

To control the position of cell division, the Min wave must dwell at each cell pole for an appropriate amount of time. Although Min waves with a period of 40–120 s properly determine the cell division site [23,70], a Min wave with a longer period (230 s) has been reported to impede normal cell division [23], and a Min wave with a shorter period is assumed to be insufficient to prevent cell division. Although the period of the Min wave can be controlled by energy molecules [81], its precise mechanism remains unclear (Figure 4a). Therefore, it is necessary to elucidate the regulatory mechanism of the wave period by analyzing the effects of protein concentration, reaction parameters, and spatial size in future studies.

The mechanism for determining the wavelength of a Min wave is also a long-standing problem. The wavelength of Min waves is approximately 10 μm in filamentous living cells [23], and normal cells (2–5 μm in length) are smaller than this length. In contrast, on lipid membranes in open systems, the wavelength of Min waves (30–100 μm in length) is approximately 10 times larger than *in vivo* [69,72,82]. In addition, artificial cells with a single Min wave have a circumference of about 60 μm. This suggests that the wavelength of the *in vitro* Min wave is much larger than *in vivo* (Figure 4a). Previous studies with experiments in open systems have shown that the wavelength changes depending on molecular crowding [83], ionic conditions [84], and the surface-area-to-volume ratio [73]. However, it is unclear how a single wave with short wavelength (~10 μm) forms in living cells. Therefore, verification by using artificial cells is necessary to mimic the geometric properties of living cells.

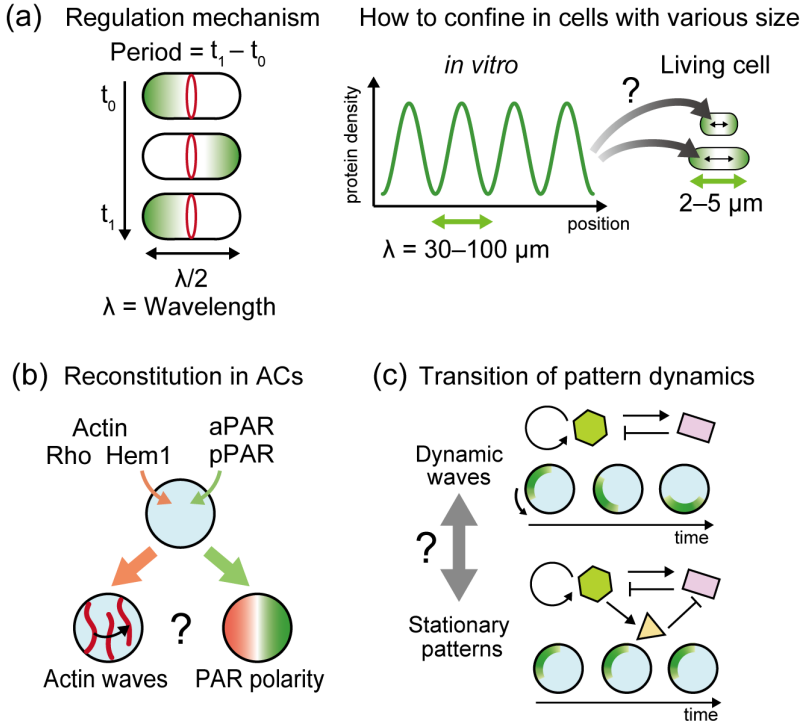

Figure 4 Major questions about intracellular reaction-diffusion (iRD) waves. (a) Regulation mechanism of Min wave period and wavelength is still elusive (left). Regarding wavelength, it is not clear how Min waves which show large wavelength (~100 μm) *in vitro* are confined in small living cells (2–5 μm) and adjust their properties to each cell size (right). (b) Reconstitution of iRD waves other than Min waves in artificial cells will reveal their formation mechanism. (c) Conditions for generation of Turing-type stationary patterns are still elusive. Using artificial cell systems, whether dynamic iRD waves can be converted to stationary patterns can be investigated.

Because the period, wavelength, and wave shape of iRD waves change nonlinearly with parameter changes, it is difficult to predict their behavior. Despite the nonlinearity, surprisingly, the period of the Min wave remains almost constant as the cell grows and changes in size [14]. However, the wave number does not change, and the coverage area of the waves on the membrane increases as the cells grow [14]. Therefore, the wavelength and wave shape are likely to vary depending on the cell size. These results suggest that iRD waves have some type of control system to adapt their properties to changes in spatial size. Because the wavelength of iRD waves is comparable to the spatial size, and the wave number is discretized, unlike RD systems in open systems, neither the mechanism of how waves adapt to spatial size changes nor the responses of waves have been examined. Verification of this wave adaptation is expected to be achieved by utilizing the easy preparation of artificial cells of various sizes.

Currently, only Min waves can be used to verify iRD waves in artificial cells. The knowledge obtained from artificial cell systems of Min waves can potentially be applied to other iRD waves, as a phenomenon similar to mode selection by the activator-inhibitor balance has been reported in PIP_3/PTEN waves in *Dictyostelium* cells [49]. However, to understand individual iRD waves, it is necessary to construct artificial cell systems for each RD system (Figure 4b). These achievements enable a wide range of developments in understanding the principles of iRD waves, such as the elucidation of more detailed molecular mechanisms, mechanical forces generated by iRD waves, and the dynamics of coupling with downstream events. This will also allow for the investigation of the differences and similarities between the formation mechanisms of each iRD wave. Furthermore, there are no examples of Turing-type stationary patterns, such as PAR systems and Cdc42 clusters, formed in artificial cells using biomolecules. Therefore, it has not been experimentally demonstrated whether these pattern formations are derived from the Turing-type mechanism and whether dynamic iRD waves can be converted to stationary iRD waves in cell-size spaces (Figure 4c). In the future, the reconstitution of iRD waves other than Min waves and the creation of Turing patterns in artificial cells will contribute to the general understanding of iRD waves and the spatiotemporal regulation of cells.

Conclusion

Various cells use iRD waves to determine positional information, and spatiotemporal patterns are crucial for understanding fundamental biological phenomena such as cell division and polarity formation. iRD waves exhibit various dynamics depending on the structure of the reaction network. Because iRD waves are nonlinear systems under far from equilibrium conditions, it is difficult to understand their formation mechanism. The artificial cell system of Min waves, which is the only iRD wave reconstituted using purified proteins, allows for experiments under specific conditions that cannot be tested in living cells. This system enables the precise adjustment of protein concentrations and regulation of reaction parameters. These advantages have contributed to the clarification of the influence of protein conformation on wave generation, the regulatory mechanism of wave modes, and the influence of energy molecules. Because the composition of the elements in artificial cells is completely defined, the wave behaviors exhibited in artificial cells can easily correspond to theoretical models, providing valuable insights from both experimental and theoretical analyses. In the future, artificial cell systems are expected to be used to characterize wave period, wavelength, and shape, which are important for Min waves to function properly in living cells. Furthermore, reconstitution of other iRD waves is necessary to understand the regulatory mechanisms of individual iRD waves. Artificial cell systems are expected to bridge the gap between the knowledge of RD systems in open systems and insights obtained from *in vivo* experiments, leading to highly accurate verification from a physical perspective in various iRD waves.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

S.T. wrote the original draft and prepared figures, and S.T. and K.F. reviewed and edited the manuscript.

Data Availability

The all data used in this Review article is available from corresponding references.

Acknowledgements

Projects supported by the Ishii-Ishibashi Fund (The Keio University Grant for Early Career Researchers).

References

[1] Turing, A. M. The chemical basis of morphogenesis. Philos. Trans. R. Soc. London B 237, 37–72 (1952). https://doi.org/10.1098/rstb.1952.0012

[2] Kondo, S., Miura, T. Reaction-diffusion model as a framework for understanding biological pattern formation. Science 329, 1616–1620 (2010). https://doi.org/10.1126/science.1179047

[3] Sheth, R., Marcon, L., Bastida, M. F., Junco, M., Quintana, L., Dahn, R., et al. *Hox* genes regulate digit patterning by controlling the wavelength of a Turing-type mechanism. Science 338, 1476–1480 (2012). https://doi.org/10.1126/science.1226804

[4] Glover, J. D., Sudderick, Z. R., Shih, B. B.-J., Batho-Samblas, C., Charlton, L., Krause, A. L., et al. The developmental basis of fingerprint pattern formation and variation. Cell 186, 940–956.e20 (2023). https://doi.org/10.1016/j.cell.2023.01.015

[5] Rowlett, V. W., Margolin, W. The bacterial Min system. Curr. Biol. 23, R553–R556 (2013). https://doi.org/10.1016/j.cub.2013.05.024

[6] Weiner, O. D., Marganski, W. A., Wu, L. F., Altschuler, S. J., Kirschner, M. W. An actin-based wave generator organizes cell motility. PLoS Biol. 5, e221 (2007). https://doi.org/10.1371/journal.pbio.0050221

[7] Vicker, M. G. 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). https://doi.org/10.1006/excr.2001.5466

[8] Devreotes, P. N., Bhattacharya, S., Edwards, M., Iglesias, P. A., Lampert, T., Miao, Y. Excitable signal transduction networks in directed cell migration. Annu. Rev. Cell Dev. Biol. 33, 103–125 (2017). https://doi.org/10.1146/annurev-cellbio-100616-060739

[9] Zaikin, A. N., Zhabotinsky, A. M. Concentration wave propagation in two-dimensional liquid-phase self-oscillating system. Nature 225, 535–537 (1970). https://doi.org/10.1038/225535b0

[10] Welsh, B. J., Gomatam, J., Burgess, A. E. Three-dimensional chemical waves in the Belousov–Zhabotinskii reaction. Nature 304, 611–614 (1983). https://doi.org/10.1038/304611a0

[11] Kuksenok, O., Yashin, V. V., Balazs, A. C. Spatial confinement controls self-oscillations in polymer gels undergoing the Belousov-Zhabotinsky reaction. Phys. Rev. E 80, 056208 (2009). https://doi.org/10.1103/PhysRevE.80.056208

[12] Bánsági, T., Vanag, V. K., Epstein, I. R. Tomography of reaction-diffusion microemulsions reveals three-dimensional Turing patterns. Science 331, 1309–1312 (2011). https://doi.org/10.1126/science.1200815

[13] Cross, M. C., Hohenberg, P. C. Pattern formation outside of equilibrium. Rev. Mod. Phys. 65, 851–1112 (1993). https://doi.org/10.1103/RevModPhys.65.851

[14] Fischer-Friedrich, E., Meacci, G., Lutkenhaus, J., Chaté, H., Kruse, K. Intra- and intercellular fluctuations in Min-protein dynamics decrease with cell length. Proc. Natl. Acad. Sci. U.S.A. 107, 6134–6139 (2010). https://doi.org/10.1073/pnas.0911708107

[15] Motegi, F., Seydoux, G. The PAR network: Redundancy and robustness in a symmetry-breaking system. Philos. Trans. R. Soc. B Biol. Sci. 368, 20130010 (2013). https://doi.org/10.1098/rstb.2013.0010

[16] Allard, J., Mogilner, A. Traveling waves in actin dynamics and cell motility. Curr. Opin. Cell Biol. 25, 107–115 (2013). https://doi.org/10.1016/j.ceb.2012.08.012

[17] Beta, C., Edelstein-Keshet, L., Gov, N., Yochelis, A. From actin waves to mechanism and back: How theory aids biological understanding. eLife 12, e87181 (2023). https://doi.org/10.7554/eLife.87181

[18] Bement, W. M., Leda, M., Moe, A. M., Kita, A. M., Larson, M. E., Golding, A. E., et al. Activator-inhibitor coupling between Rho signalling and actin assembly makes the cell cortex an excitable medium. Nat. Cell Biol. 17, 1471–1483 (2015). https://doi.org/10.1038/ncb3251

[19] Michaud, A., Leda, M., Swider, Z. T., Kim, S., He, J., Landino, J., et al. A versatile cortical pattern-forming circuit based on Rho, F-actin, Ect2, and RGA-3/4. J. Cell Biol. 221, e202203017 (2022). https://doi.org/10.1083/jcb.202203017

[20] Gerisch, G., Schroth-Diez, B., Müller-Taubenberger, A., Ecke, M. PIP3 waves and PTEN dynamics in the emergence of cell polarity. Biophys. J. 103, 1170–1178 (2012). https://doi.org/10.1016/j.bpj.2012.08.004

[21] Matsuoka, S., Ueda, M. Mutual inhibition between PTEN and PIP3 generates bistability for polarity in motile cells. Nat. Commun. 9, 4481 (2018). https://doi.org/10.1038/s41467-018-06856-0

[22] Arai, Y., Shibata, T., Matsuoka, S., Sato, M. J., Yanagida, T., Ueda, M. Self-organization of the phosphatidylinositol lipids signaling system for random cell migration. Proc. Natl. Acad. Sci. U.S.A. 107, 12399–12404 (2010). https://doi.org/10.1073/pnas.0908278107

[23] Raskin, D. M., De Boer, P. A. J. Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 96, 4971–4976 (1999).

https://doi.org/10.1073/pnas.96.9.4971
[24] Hu, Z., Mukherjee, A., Pichoff, S., Lutkenhaus, J. The MinC component of the division site selection system in *Escherichia coli* interacts with FtsZ to prevent polymerization. Proc. Natl. Acad. Sci. U.S.A. 96, 14819–14824 (1999). https://doi.org/10.1073/pnas.96.26.14819
[25] Lackner, L. L., Raskin, D. M., De Boer, P. A. J. ATP-dependent interactions between *Escherichia coli* Min proteins and the phospholipid membrane in vitro. J. Bacteriol. 185, 735–749 (2003). https://doi.org/10.1128/JB.185.3.735-749.2003
[26] Bi, E., Park, H. O. Cell polarization and cytokinesis in budding yeast. Genetics 191, 347–387 (2012). https://doi.org/10.1534/genetics.111.132886
[27] Cuenca, A. A., Schetter, A., Aceto, D., Kemphues, K., Seydoux, G. Polarization of the *C. elegans* zygote proceeds via distinct establishment and maintenance phases. Development 130, 1255–1265 (2003). https://doi.org/10.1242/dev.00284
[28] Hoege, C., Hyman, A. A. Principles of PAR polarity in *Caenorhabditis elegans* embryos. Nat. Rev. Mol. Cell Biol. 14, 315–322 (2013). https://doi.org/10.1038/nrm3558
[29] Zonies, S., Motegi, F., Hao, Y., Seydoux, G. Symmetry breaking and polarization of the *C. elegans* zygote by the polarity protein PAR-2. Development 137, 1669–1677 (2010). https://doi.org/10.1242/dev.045823
[30] Johnson, J. M., Jin, M., Lew, D. J. Symmetry breaking and the establishment of cell polarity in budding yeast. Curr. Opin. Genet. Dev. 21, 740–746 (2011). https://doi.org/10.1016/j.gde.2011.09.007
[31] Goryachev, A. B., Pokhilko, A. V. Dynamics of Cdc42 network embodies a Turing-type mechanism of yeast cell polarity. FEBS Lett. 582, 1437–1443 (2008). https://doi.org/10.1016/j.febslet.2008.03.029
[32] Iwase, M., Luo, J., Nagaraj, S., Longtine, M., Kim, H. B., Haarer, B. K., et al. Role of a Cdc42p effector pathway in recruitment of the yeast septins to the presumptive bud site. Mol. Biol. Cell 17, 1110–1125 (2006). https://doi.org/10.1091/mbc.e05-08-0793
[33] Goehring, N. W. PAR polarity: From complexity to design principles. Exp. Cell Res. 328, 258–266 (2014). https://doi.org/10.1016/j.yexcr.2014.08.009
[34] Goehring, N. W., Trong, P. K., Bois, J. S., Chowdhury, D., Nicola, E. M., Hyman, A. A., et al. Polarization of PAR proteins by advective triggering of a pattern-forming system. Science 334, 1137–1141 (2011). https://doi.org/10.1126/science.1208619
[35] Vendel, K. J. A., Tschirpke, S., Shamsi, F., Dogterom, M., Laan, L. Minimal *in vitro* systems shed light on cell polarity. J. Cell Sci. 132, jcs217554 (2019). https://doi.org/10.1242/jcs.217554
[36] Bretschneider, T., Anderson, K., Ecke, M., Müller-Taubenberger, A., Schroth-Diez, B., Ishikawa-Ankerhold, H. C., et al. The three-dimensional dynamics of actin waves, a model of cytoskeletal self-organization. Biophys. J. 96, 2888–2900 (2009). https://doi.org/10.1016/j.bpj.2008.12.3942
[37] Meacci, G., Ries, J., Fischer-Friedrich, E., Kahya, N., Schwille, P., Kruse, K. Mobility of Min-proteins in *Escherichia coli* measured by fluorescence correlation spectroscopy. Phys. Biol. 3, 255–263 (2006). https://doi.org/10.1088/1478-3975/3/4/003
[38] Goehring, N. W., Hoege, C., Grill, S. W., Hyman, A. A. PAR proteins diffuse freely across the anterior-posterior boundary in polarized *C. elegans* embryos. J. Cell Biol. 193, 583–594 (2011). https://doi.org/10.1083/jcb.201011094
[39] Jaffe, A. B., Hall, A. Rho GTPases: Biochemistry and biology. Annu. Rev. Cell Dev. Biol. 21, 247–269 (2005). https://doi.org/10.1146/annurev.cellbio.21.020604.150721
[40] Motegi, F., Zonies, S., Hao, Y., Cuenca, A. A., Griffin, E., Seydoux, G. Microtubules induce self-organization of polarized PAR domains in *Caenorhabditis elegans* zygotes. Nat. Cell Biol. 13, 1361–1367 (2011). https://doi.org/10.1038/ncb2354
[41] Kono, K., Yoshiura, S., Fujita, I., Okada, Y., Shitamukai, A., Shibata, T., et al. Reconstruction of Par-dependent polarity in apolar cells reveals a dynamic process of cortical polarization. eLife 8, e45559 (2019). https://doi.org/10.7554/eLife.45559
[42] Ramanujam, R., Han, Z., Zhang, Z., Kanchanawong, P., Motegi, F. Establishment of the PAR-1 cortical gradient by the aPKC-PRBH circuit. Nat. Chem. Biol. 14, 917–927 (2018). https://doi.org/10.1038/s41589-018-0117-1
[43] Watson, J. L., Krüger, L. K., Ben-Sasson, A. J., Bittleston, A., Shahbazi, M. N., Planelles-Herrero, V. J., et al. Synthetic Par polarity induces cytoskeleton asymmetry in unpolarized mammalian cells. Cell 186, 4710–4727.e35 (2023). https://doi.org/10.1016/j.cell.2023.08.034
[44] Knoch, F., Tarantola, M., Bodenschatz, E., Rappel, W.-J. Modeling self-organized spatio-temporal patterns of PIP_3 and PTEN during spontaneous cell polarization. Phys. Biol. 11, 046002 (2014). https://doi.org/10.1088/1478-3975/11/4/046002
[45] Meinhardt, H., De Boer, P. A. J. Pattern formation in *Escherichia coil*: A model for the pole-to-pole oscillations of Min proteins and the localization of the division site. Proc. Natl. Acad. Sci. U.S.A. 98, 14202–14207 (2001).

https://doi.org/10.1073/pnas.251216598
[46] Howard, M., Rutenberg, A. D., de Vet, S. Dynamic compartmentalization of bacteria: Accurate division in *E. coli*. Phys. Rev. Lett. 87, 278102 (2001). https://doi.org/10.1103/PhysRevLett.87.278102
[47] Bonny, M., Fischer-Friedrich, E., Loose, M., Schwille, P., Kruse, K. Membrane binding of MinE allows for a comprehensive description of Min-protein pattern formation. PLoS Comput. Biol. 9, e1003347 (2013). https://doi.org/10.1371/journal.pcbi.1003347
[48] Geßele, R., Halatek, J., Würthner, L., Frey, E. Geometric cues stabilise long-axis polarisation of PAR protein patterns in *C. elegans*. Nat. Commun. 11, 539 (2020). https://doi.org/10.1038/s41467-020-14317-w
[49] Taniguchi, D., Ishihara, S., Oonuki, T., Honda-Kitahara, M., Kaneko, K., Sawai, S. 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). https://doi.org/10.1073/pnas.1218025110
[50] Wigbers, M. C., Tan, T. H., Brauns, F., Liu, J., Swartz, S. Z., Frey, E., et al. A hierarchy of protein patterns robustly decodes cell shape information. Nat. Phys. 17, 578–584 (2021). https://doi.org/10.1038/s41567-021-01164-9
[51] Lee, M. E., Lo, W. C., Miller, K. E., Chou, C. S., Park, H. O. Regulation of Cdc42 polarization by the Rsr1 GTPase and Rga1, a Cdc42 GTPase-activating protein, in budding yeast. J. Cell Sci. 128, 2106–2117 (2015). https://doi.org/10.1242/jcs.166538
[52] Illukkumbura, R., Bland, T., Goehring, N. W. Patterning and polarization of cells by intracellular flows. Curr. Opin. Cell Biol. 62, 123–134 (2020). https://doi.org/10.1016/j.ceb.2019.10.005
[53] Ross, J., Müller, S. C., Vidal, C. Chemical waves. Science 240, 460–465 (1988). https://doi.org/10.1126/science.240.4851.460
[54] Wu, F., van Schie, B. G, Keymer, J. E., Dekker, C. Symmetry and scale orient Min protein patterns in shaped bacterial sculptures. Nat. Nanotechnol. 10, 719–726 (2015). https://doi.org/10.1038/nnano.2015.126
[55] Rondelez, Y. Competition for catalytic resources alters biological network dynamics. Phys. Rev. Lett. 108, 018102 (2012). https://doi.org/10.1103/PhysRevLett.108.018102
[56] Nishikawa, S., Sato, G., Takada, S., Kohyama, S., Honda, G., Yanagisawa, M., et al. Multimolecular competition effect as a modulator of protein localization and biochemical networks in cell-size space. Adv. Sci. 11, 2308030 (2024). https://doi.org/10.1002/advs.202308030
[57] Sato, G., Kinoshita, S., Yamada, T. G., Arai, S., Kitaguchi, T., Funahashi, A., et al. Metabolic tug-of-war between glycolysis and translation revealed by biochemical reconstitution. ACS Synth. Biol. 13, 1572–1581 (2024). https://doi.org/10.1021/acssynbio.4c00209
[58] Salehi-Reyhani, A., Ces, O., Elani, Y. Artificial cell mimics as simplified models for the study of cell biology. Exp. Biol. Med. 242, 1309–1317 (2017). https://doi.org/10.1177/1535370217711441
[59] Théry, M., Blanchoin, L. Reconstituting the dynamic steady states of actin networks in vitro. Nat. Cell Biol. 26, 494–497 (2024). https://doi.org/10.1038/s41556-024-01379-x
[60] Yanagisawa, M., Watanabe, C., Yoshinaga, N., Fujiwara, K. Cell-size space regulates the behavior of confined polymers: From nano- and micromaterials science to biology. Langmuir 38, 11811–11827 (2022). https://doi.org/10.1021/acs.langmuir.2c01397
[61] Miyazaki, M., Chiba, M., Eguchi, H., Ohki, T., Ishiwata, S. Cell-sized spherical confinement induces the spontaneous formation of contractile actomyosin rings *in vitro*. Nat. Cell Biol. 17, 480–489 (2015). https://doi.org/10.1038/ncb3142
[62] Sakamoto, R., Izri, Z., Shimamoto, Y., Miyazaki, M., Maeda, Y. T. Geometric trade-off between contractile force and viscous drag determines the actomyosin-based motility of a cell-sized droplet. Proc. Natl. Acad. Sci. U.S.A. 119, e2121147119 (2022). https://doi.org/10.1073/pnas.2121147119
[63] Sakamoto, R., Tanabe, M., Hiraiwa, T., Suzuki, K., Ishiwata, S., Maeda, Y. T., et al. Tug-of-war between actomyosin-driven antagonistic forces determines the positioning symmetry in cell-sized confinement. Nat. Commun. 11, 3063 (2020). https://doi.org/10.1038/s41467-020-16677-9
[64] Pinot, M., Steiner, V., Dehapiot, B., Yoo, B. K., Chesnel, F., Blanchoin, L., et al. Confinement induces actin flow in a meiotic cytoplasm. Proc. Natl. Acad. Sci. U.S.A. 109, 11705–11710 (2012). https://doi.org/10.1073/pnas.1121583109
[65] Sakuta, H., Nakatani, N., Torisawa, T., Sumino, Y., Tsumoto, K., Oiwa, K., et al. Self-emergent vortex flow of microtubule and kinesin in cell-sized droplets under water/water phase separation. Commun. Chem. 6, 80 (2023). https://doi.org/10.1038/s42004-023-00879-5
[66] Garenne, D., Libchaber, A., Noireaux, V. Membrane molecular crowding enhances MreB polymerization to shape synthetic cells from spheres to rods. Proc. Natl. Acad. Sci. U.S.A. 117, 1902–1909 (2020). https://doi.org/10.1073/pnas.1914656117
[67] Good, M. C., Vahey, M. D., Skandarajah, A., Fletcher, D. A., Heald, R. Cytoplasmic volume modulates spindle size during embryogenesis. Science 342, 856–860 (2013). https://doi.org/10.1126/science.1243147

[68] Hazel, J., Krutkramelis, K., Mooney, P., Tomschik, M., Gerow, K., Oakey, J., et al. Changes in cytoplasmic volume are sufficient to drive spindle scaling. Science 342, 853–856 (2013). https://doi.org/10.1126/science.1243110
[69] Loose, M., Fischer-friedrich, E., Ries, J., Kruse, K., Schwille, P. Spatial regulators for bacterial cell division self-organize into surface waves in vitro. Science 320, 789–792 (2008). https://doi.org/10.1126/science.1154413
[70] Ramm, B., Heermann, T., Schwille, P. The *E. coli* MinCDE system in the regulation of protein patterns and gradients. Cell. Mol. Life Sci. 76, 4245–4273 (2019). https://doi.org/10.1007/s00018-019-03218-x
[71] Kretschmer, S., Heermann, T., Tassinari, A., Glock, P., Schwille, P. Increasing MinD's membrane affinity yields standing wave oscillations and functional gradients on flat membranes. ACS Synth. Biol. 10, 939–949 (2021). https://doi.org/10.1021/acssynbio.0c00604
[72] Zieske, K., Schwille, P. Reconstitution of self-organizing protein gradients as spatial cues in cell-free systems. eLife 3, e03949 (2014). https://doi.org/10.7554/eLife.03949
[73] Brauns, F., Pawlik, G., Halatek, J., Kerssemakers, J., Frey, E., Dekker, C. Bulk-surface coupling identifies the mechanistic connection between Min-protein patterns in vivo and in vitro. Nat. Commun. 12, 3312 (2021). https://doi.org/10.1038/s41467-021-23412-5
[74] Wu, F., Halatek, J., Reiter, M., Kingma, E., Frey, E., Dekker, C. Multistability and dynamic transitions of intracellular Min protein patterns. Mol. Syst. Biol. 12, 873 (2016). https://doi.org/10.15252/msb.20156724
[75] Zieske, K., Chwastek, G., Schwille, P. Protein patterns and oscillations on lipid monolayers and in microdroplets. Angew. Chem. Int. Ed. Engl. 55, 13455–13459 (2016). https://doi.org/10.1002/anie.201606069
[76] Litschel, T., Ramm, B., Maas, R., Heymann, M., Schwille, P. Beating vesicles: Encapsulated protein oscillations cause dynamic membrane deformations. Angew. Chem. Int. Ed. Engl. 57, 16286–16290 (2018). https://doi.org/10.1002/anie.201808750
[77] Kohyama, S., Yoshinaga, N., Yanagisawa, M., Fujiwara, K., Doi, N. Cell-sized confinement controls generation and stability of a protein wave for spatiotemporal regulation in cells. eLife 8, e44591 (2019). https://doi.org/10.7554/eLife.44591
[78] Kohyama, S., Fujiwara, K., Yoshinaga, N., Doi, N. Conformational equilibrium of MinE regulates allowable concentration ranges of a protein wave for cell division. Nanoscale 12, 11960–11970 (2020). https://doi.org/10.1039/d0nr00242a
[79] Yoshida, A., Kohyama, S., Fujiwara, K., Nishikawa, S., Doi, N. Regulation of spatiotemporal patterning in artificial cells by a defined protein expression system. Chem. Sci. 10, 11064–11072 (2019). https://doi.org/10.1039/c9sc02441g
[80] Takada, S., Yoshinaga, N., Doi, N., Fujiwara, K. Mode selection mechanism in traveling and standing waves revealed by Min wave reconstituted in artificial cells. Sci. Adv. 8, eabm8460 (2022). https://doi.org/10.1126/sciadv.abm8460
[81] Takada, S., Yoshinaga, N., Doi, N., Fujiwara, K. Controlling the periodicity of a reaction–diffusion wave in artificial cells by a two-way energy supplier. ACS Nano 16, 16853–16861 (2022). https://doi.org/10.1021/acsnano.2c06756
[82] Martos, A., Petrasek, Z., Schwille, P. Propagation of MinCDE waves on free-standing membranes. Environ. Microbiol. 15, 3319–3326 (2013). https://doi.org/10.1111/1462-2920.12295
[83] Martos, A., Raso, A., Jiménez, M., Petrášek, Z., Rivas, G., Schwille, P. FtsZ polymers tethered to the membrane by ZipA are susceptible to spatial regulation by Min waves. Biophys. J. 108, 2371–2383 (2015). https://doi.org/10.1016/j.bpj.2015.03.031
[84] Vecchiarelli, A. G., Li, M., Mizuuchi, M., Mizuuchi, K. Differential affinities of MinD and MinE to anionic phospholipid influence Min patterning dynamics *in vitro*. Mol. Microbiol. 93, 453–463 (2014). https://doi.org/10.1111/mmi.12669