A Model of Organization of Size Invariant Positional Information in Taxis of Physarum Plasmodium
— A Reaction-Diffusion System Regulated by Phase of Chemical Oscillation —

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It is assumed that positional information within a developing organism is represented by a morphogen gradient. However, it is not yet understood how positional information is organized in a size invariant manner. To achieve such size invariance, it is necessary for an organism to organize the polarity of positional information in advance. We focus on tactic behavior of the Physarum plasmodium and propose a model that describes the organization of size invariant positional information, in which the direction of the phase wave represents the polarity. We would like to point out that self-referential dynamics exist in our model, and their implementation is discussed. Our model may be applied to other developmental systems.

§1. Introduction

During the development of an organism, the state of each part of the organism must be regulated with respect to its position and role in the organism. Information regarding such regulation is called 'positional information'. It is generally assumed that positional information is represented by a position-dependent concentration pattern of a single molecular species, termed the 'morphogen', and many experiments support this assumption.

Positional information must be size invariant, since organisms normally exhibit size invariance during development, although there is always some size distribution among the individuals of any given species. It seems that each part of an organism "knows" its relative position within the organism. However, it is not yet understood how positional information is organized in a size invariant manner.

Some developing organisms, such as the fertilized sea urchin in early development, can be regarded as one-dimensional systems. In such systems, positional information is represented by a one-dimensional morphogen gradient, where the morphogen concentration is maximal at one end and monotonically decreases in moving toward the other end. In addition, in order to realize the size invariance of the positional information, the concentration at each end must be independent of the system size.

Since the morphogen concentration must correspond uniquely to the positional information, the above described system needs to determine at which end the morphogen concentration should be the highest. Of course when one end is determined
to be the highest, the other end must be the lowest. Information concerning such a determination is called the ‘polarity of the positional information’. This specifies direction within the developing organism. Thus it is necessary for the system to fix the polarity in advance, in order to determine the size invariant morphogen gradient.

Although several types of models have been proposed to explain how positional information is organized, there is no model that fully satisfies the above stated necessities. Cohen suggested that the size invariant morphogen gradient could be realized in a one-dimensional system in the following manner. The morphogen concentration is maintained constant at each end for any size of the system. Then, when the concentration at one end becomes higher than that at the other end and the morphogen diffuses, the morphogen concentration comes to decrease monotonically from the highest end to the lowest end. However, it is not made clear how the polarity is organized within this model. Organization of the polarity is necessary to determine which end is to be of higher concentration.

Gierer and Meinhardt attempted to explain how a morphogen gradient is organized using an activator-inhibitor model, which was first proposed by Turing. However, in this activator-inhibitor model, a periodic pattern emerges when the size of the system is larger than a certain constant, which is determined by the diffusion coefficient. In this model, a saturation term is introduced to deal with this difficulty, but size invariance is realized for only a limited range of sizes. In addition, the polarity is not determined within the model but initially given as a shallow gradient of source density.

Goodwin and Cohen proposed a phase-shift model, in which the polarity is represented by the direction of a propagating oscillation—a phase wave. This wave is organized through entrainment between oscillators and propagates from one end to the other. To organize the positional information, they introduced another phase wave possessing a slower propagation velocity. This slower phase wave is generated only at a pacemaker site, where the faster phase wave originates. The positional information is organized through the phase difference between these two waves. In order for the slower wave to be generated only at the pacemaker site, they assumed that the nature of each oscillator changes when it is entrained by neighbors. However, at this time, there is no known oscillator with such a property.

In the model of Goodwin and Cohen, it is not clear how the organization of the morphogen gradient according to the polarity is realized. Since the polarity is represented by the direction of the faster phase wave, each part of the organism must detect the phase difference of oscillation between itself and its neighbors. However, there is an obvious problem here: For one who observes the phase wave from outside the system, it is easy to detect this phase difference, since one can observe the oscillation at all parts simultaneously, but from the point of view of a given part within the system, it seems impossible to know the phase difference, since each part can observe only its own oscillation and has no way of knowing that of its neighbors.

In this paper, we propose a mechanism to explain how each part of the system detects the polarity and thereby organizes positional information in a size invariant manner. We focus on tactic behavior of the Physarum plasmodium, which is a good example for the study of organization of the positional information.
§2. Model

2.1. The Physarum plasmodium

The Physarum plasmodium is a giant unicellular organism. Although it has no nervous system for information processing, it exhibits highly coordinated tactic behavior to the "best" stimulus, even in complicated situations. For example, when a strong attractive stimulus is applied to one end of a linear-shaped plasmodium and a weak one is applied to the opposite end, the plasmodium chooses only the strong stimulus and migrates toward it as a coordinated, single body. To migrate in this manner, the plasmodium must be such that the state of each of its parts is regulated according to its position within the whole organism. Thus there must exist positional information. Since both small (1 cm) and large (50 cm) 6) plasmodium display migration as single unified bodies, this positional information must be size invariant.

That which drives the migration of the plasmodium as a single body is a constant gradient of the Ca$^{2+}$ concentration through the cell. 7) This indicates that in the plasmodium Ca$^{2+}$ is the morphogen, and its gradient represents the positional information. Here, the Ca$^{2+}$ level is high at the front and low at the rear. 7), 8) Other chemicals, ATP, ADP, cAMP and cGMP, also constitute similar gradients in the plasmodium. 6), 9) A unidirectionally migrating plasmodium has size invariant gradients of chemical concentration such as ATP 6) and Ca$^{2+}$. 10)

Since a plasmodium migrates toward the most favorable stimulus, the polarity of its positional information must indicate the corresponding direction. Thus the mechanism that organizes the polarity must have the ability to choose the most favorable stimulus among all local stimuli. There exist phase waves of intracellular oscillation in the plasmodium, and it has been suggested that an organizing mechanism of the phase wave has such an ability, and thus that the direction of the phase wave represents the polarity. Tension oscillations, with a period of about 2 min., are observed in these organisms, 11), 12) and their local oscillations are spatially synchronized with each other. 13), 14) Tension oscillation has a fixed phase relation with chemical oscillation such as Ca$^{2+}$, 15), 16) ATP, 17) and H+. 18) Attractive stimuli increase the frequency of the tension oscillation, while repulsive stimuli decrease it. 19), 20) By artificially modulating a local frequency, Matsumoto et al. 21) found that both the nature and strength of local stimuli are encoded into the frequency of the local oscillation. This implies that a more favorable stimulus induces a higher frequency. Such local frequency modulation generates a phase wave. 22)- 24) Miyake et al. 25) observed the phase wave induced in a linear-shaped plasmodium when a strong attractive stimulus and a weak attractive stimulus were applied to either end. Initially, two phase waves propagating from the two ends were observed. Later, however, only one phase wave, propagating through the cell from the end corresponding to the strong stimulus to the other end, was observed, and the plasmodium migrated toward the strong stimulus. This observation indicates that the plasmodium chooses the most favorable stimulus through competition between these phase waves, and the direction of the surviving phase wave propagating through the cell comes to rep-
resent the polarity. By artificially modulating a local frequency, it was found that the migratory direction is controlled by the direction of the phase wave.\(^{21}\) Miyake et al.\(^{25}\) found that local frequency modulation is not necessary for such migration control.

2.2. One-dimensional reaction-diffusion system

We adopt a reaction-diffusion system for our model of the plasmodium. This is justified by studies of the plasmodium. Intracellular dynamics of the plasmodium are not only chemical but also mechanical. However, regular Ca\(^{2+}\) oscillations of a period equal to the protoplasmic shuttle streaming (100 – 200 s) have been observed in a *Physarum* homogenate.\(^{26}\) This strongly suggests\(^9\) that protoplasmic shuttle streaming is driven by an autonomous field of chemical oscillators in the cytoplasm, which function independently of tension generation. The intracellular interactions existing in the plasmodium include not only diffusion but also protoplasmic shuttle streaming. We assume that the streaming only enhances the diffusion process through mixing and thus increases an effective diffusion constant. We thus adopt a model including only diffusion as the intracellular interaction.

Tactic behavior of the plasmodium has often been studied with a linear-shaped plasmodium for simplicity. We focus on such a plasmodium and regard it as a one-dimensional system.

2.3. Modeling polarity organization

Since we believe that only the phase aspect of chemical oscillations is necessary for describing the polarity in the plasmodium, we do not attempt to mimic the waveform or the baseline change of oscillation. We thus describe oscillation with a simple oscillator. As we noted above, the system is one-dimensional, and the only intracellular interaction is diffusion. We describe the dynamics of the oscillation with the system

\[
\frac{\partial x(r, t)}{\partial t} = \varepsilon \left(x - \frac{x^3}{3}\right) - y + D_x \frac{\partial^2 x}{\partial r^2},
\]

\[
\frac{\partial y(r, t)}{\partial t} = \alpha^2(r)x,
\]

where \(x\) and \(y\) represent chemical oscillations and \(r\) is the position, \(t\) is the time, \(\varepsilon\) is a small constant, and \(D_x\) is the diffusion coefficient for \(x\). These equations constitute the diffusion-coupled Van der Pol oscillator.\(^{27}\) With the exception of the coefficient \(\alpha(r)\) in Eq. (2), which determines the natural frequency at each point, this system is spatially uniform. The local modulation of \(\alpha(r)\) corresponds to a local frequency modulation with a local stimulus in the plasmodium. An oscillator with higher natural frequency corresponds to a more favorable local condition. In a one-dimensional system, local modulation can occur only at the two ends.

If the spatial variation of \(x\) is sufficiently slow, this oscillatory system can be regarded as weakly coupled, and \(x\) can be approximately described as

\[
x(r, t) \cong x_0(\phi) (= x_0(\phi + 2\pi)), \phi(r, t) = \omega_0 t + \psi(r, t),
\]
where \( x_0 \) is a \( 2\pi \)-periodic function, \( \psi \) is the phase of oscillation \( x_0 \), and \( \omega_0 \) is the angular frequency.

A weakly coupled oscillatory reaction-diffusion system has properties similar to those of the plasmodium with regard to local frequency modulation and the competition and survival of phase waves. A nonlinear phase diffusion equation can be derived from such a reaction-diffusion system.\(^{28)}\) - \(^{30)}\) This equation describes behavior in which a phase wave develops from a point where the oscillation frequency is higher than the bulk oscillation. When there are multiple such points, multiple phase waves initially coexist. However, eventually there remains only one phase wave, that generated from the point of highest frequency.\(^{29)}\) Thus the phase wave generated by the highest frequency oscillation survives the competition between phase waves. This is very similar to the behavior observed in the plasmodium. Our simulations reveal such behavior, as discussed in §3.

2.4. Modeling morphogen gradient organization

We now consider how the Ca\(^{2+}\) gradient, representing the positional information, can be determined according to the polarity, i.e., the direction of the phase wave. Since local modulation can occur only at the two ends, the surviving phase wave is generated at the end which has the highest natural frequency, and propagates to the other end. This implies that the source of the surviving phase wave always exists at one end, and the sink exists at the other end. Thus, if the Ca\(^{2+}\) concentration is maintained at a maximal constant value at the phase wave source and a minimal constant value at the phase wave sink, and if Ca\(^{2+}\) can diffuse, a size invariant Ca\(^{2+}\) gradient could be realized, as Cohen\(^2\) suggested.

To achieve this, a quantity is needed that assumes different values at the phase wave source, at the phase wave sink, and at other points. The second order spatial derivative of the phase of oscillation \( \psi \), \( \partial^2 \psi / \partial r^2 \), is negative at the source of the phase wave, positive at the sink, and zero at other points. Thus we adopt \( \partial^2 \psi / \partial r^2 \) for the dynamics of the Ca\(^{2+}\) concentration as

\[
\frac{\partial c(r,t)}{\partial t} = \beta p + \gamma \left[ \frac{c - c_0 + c_h}{2} \right]^3 + D c \frac{\partial^2 c}{\partial r^2},
\]

where \( c(r,t) \) represents the Ca\(^{2+}\) concentration, \( \beta \) and \( \gamma \) are positive constants, \( c_l < c_h \), and we assume the variable \( p \) satisfies the condition

\[
p(r,t) \cong - \frac{\partial^2 \psi}{\partial r^2} \times \text{const.}
\]

Due to the effect of the saturation term, the second term in Eq. (4), \( c \) remains between \( c_l \) and \( c_h \). If \( \beta \) is sufficiently large, \( c \) is near \( c_h \) when \( \partial^2 \psi / \partial r^2 \) assumes negative values in a certain range and near \( c_l \) when \( \partial^2 \psi / \partial r^2 \) assumes positive values in a certain range. Also, due to the effect of the diffusion term, \( c \) monotonically decreases from the high end to the low end. Since this saturation term is infinity at \( c_l \) and \( c_h \), we set the initial value of \( c \) to be between \( c_l \) and \( c_h \).
2.5. **Dynamics of \( p(r, t) \)**

The quantity \( \partial^2 \psi / \partial r^2 \) can be derived from \( x(r, t) \) when there exists a steady phase wave. In this case, we have

\[
\frac{\partial^2 \psi}{\partial r^2} \times C \equiv \frac{\omega_0}{2\pi} \int_{t-2\pi/\omega_0}^t \frac{\partial x}{\partial t} D_x \frac{\partial^2 x}{\partial r^2} dt,
\]

where \( C \) is a positive constant defined in the Appendix. (Details of this derivation are also given in the Appendix.) Thus the relation

\[
p(r, t) \equiv -\frac{\omega_0}{2\pi} \int_{t-2\pi/\omega_0}^t \frac{\partial x}{\partial t} D_x \frac{\partial^2 x}{\partial r^2} dt
\]

is consistent with condition (5).

Equation (7) implies that \( p \) must be the time average of \( -(\partial x / \partial t)D_x(\partial^2 x / \partial r^2) \) for one period of oscillation, \( 2\pi/\omega_0 \). This average can be approximately calculated using a low pass filter whose cutoff frequency is about \( \omega_0 \). Thus the equation of motion

\[
\tau \frac{\partial p(r, t)}{\partial t} = -\frac{\partial x}{\partial t} D_x \frac{\partial^2 x}{\partial r^2} - p
\]

is appropriate. This equation describes the dynamics resulting from use of the simplest low pass filter. Here, \( \tau \) is a time constant on the order of \( 2\pi/\omega_0 \). Since \( p \) follows \( -(\partial x / \partial t)D_x(\partial^2 x / \partial r^2) \) with relaxation time \( \tau \), the function \( p(x, t) \) described by Eq. (8) satisfies the condition (7).

2.6. **Substitution for \( D_x(\partial^2 x / \partial r^2) \) and \( \partial x / \partial t \)**

It is important to note that \( D_x(\partial^2 x / \partial r^2) \) and \( \partial x / \partial t \) on the right-hand side of Eq. (8) cannot appear in the reaction-diffusion system. Thus we must obtain an equation for \( p \) in which they do not appear. \( \partial x / \partial t \) can be removed by introducing the new variable

\[
\frac{\partial x_f(r, t)}{\partial t} = \frac{x - x_f}{\varepsilon_f},
\]

where \( \varepsilon_f \) is a small constant. By differentiating Eq. (9), we obtain

\[
\varepsilon_f \frac{\partial^2 x_f}{\partial t^2} = \frac{\partial x}{\partial t} - \frac{\partial x_f}{\partial t}.
\]

Since \( x_f \) follows \( x \) and \( x \) does not change very rapidly, \( \partial^2 x_f / \partial t^2 \) is not large, and \( \varepsilon_f \partial^2 x_f / \partial t^2 \) is small. Thus we obtain

\[
\frac{\partial x}{\partial t} \approx \frac{\partial x_f}{\partial t} = \frac{x - x_f}{\varepsilon_f},
\]

and thus we can substitute \( (x - x_f) / \varepsilon_f \) for \( \partial x / \partial t \). To remove \( D_x(\partial^2 x / \partial r^2) \), we need only reconsider Eq. (1),

\[
D_x \frac{\partial^2 x}{\partial r^2} = -\varepsilon \left( x - \frac{x^3}{3} \right) + y + \frac{\partial x}{\partial t}.
\]
From Eqs. (11) and (12), the equation of motion

\[
\tau \frac{\partial p(r,t)}{\partial t} = x - x_f \left[ \varepsilon \left( x - \frac{x^3}{3} \right) - y - \frac{x - x_f}{\varepsilon_f} \right] - p 
\]

(13)
can be substituted for Eq. (8). Our model is composed of Eqs. (1), (2), (4), (9), and (13).

§3. Results

3.1. Summarized tactic behavior of the plasmodium

When an attractive stimulus is applied to one end of a linear-shaped plasmodium, the organism migrates toward the stimulus as one unified body (Fig. 1(a)). As we noted in the previous section, when a strong attractive stimulus is applied to one end of the plasmodium and a weak attractive stimulus is applied to the other end, the plasmodium chooses only the strong stimulus and migrates toward it (Fig. 1(b)).

To this point, we have focused only on attractive behavior. But there have been some observations of how a plasmodium behaves in response to repulsive stimuli. When a repulsive stimulus is applied to one end of the plasmodium, the plasmodium again has been observed to respond as a single unified body (Fig. 1(c)), but Miyake et al. \cite{31} found that in the case of a long plasmodium, such a response is observed only in the neighborhood of the stimulus (Fig. 1(d)). These observations indicate that the behavior of a plasmodium in response to repulsive stimuli is only local, and thus positional information is not organized in this situation.

![Diagram](https://example.com/diagram.png)

Fig. 1. Schematic illustration of summarized tactic behavior of the plasmodium. (a) One attractive stimulus case. (b) Two attractive stimuli case. (c) Behavior of a short plasmodium in response to a repulsive stimulus. (d) Behavior of a long plasmodium in response to a repulsive stimulus. Arrows indicate the migratory direction, A and A1 represent attractive stimuli, A2 represents an attractive stimulus that is weaker than A1, and R represents a repulsive stimulus.
3.2. Simulation results

We numerically studied our model as a one-dimensional system for comparison with the above summarized tactic behavior of the plasmodium. In all simulations we used \( \varepsilon = 5.0, \varepsilon_f = 0.010, \tau = 10.0, D_x = 3.0, D_c = 12.0, c_l = 8.0, c_h = 12.0, \beta = 1.0, \gamma = 1.0, \) and the time step was 0.010. Only \( \alpha \), which determines the local natural frequency of each element, can vary at the two ends. Since there is no diffusive flow at the boundary, we set free-end conditions at both ends.

3.2.1. One attractive stimulus

We first studied the case of a single attractive stimulus. The system has 15 elements. A stimulus is represented by the value of the natural frequency of each element. A period 290 (\( \alpha = 2.60 \)) corresponds to the case of no stimulus, while a period 224 (\( \alpha = 3.20 \)) corresponds to a strong attractive stimulus. Initially, all oscillators have the same natural frequency, \( 2\pi/290 \). After 1000 iterations, the element at position 1 comes to assume a natural frequency of \( 2\pi/224 \). A phase wave then travels from this period 224 site to the opposite end, corresponding to element 15. In this situation, \( p \) is positive at position 1 and negative at position 15 (Fig. 2(a)). The time evolution of \( p \) is depicted in Fig. 2(c).

![Figure 2(a)](https://academic.oup.com/ptp/article-abstract/100/2/235/1852772)

![Figure 2(b)](https://academic.oup.com/ptp/article-abstract/100/2/235/1852772)

![Figure 2(c)](https://academic.oup.com/ptp/article-abstract/100/2/235/1852772)
Fig. 2. Simulation results in the case of a single strong attractive stimulus. After 1000 iterations, the natural frequency of the element at position 1 changes from $2\pi/290$ to $2\pi/224$. The spatial distributions of $p$ and $c$ at iteration 10000 are shown in (a) and (b), respectively. $p$ and $c$ are shown as functions of time in (c) and (d), respectively.

After the phase wave becomes organized, the gradient of $c$ is formed. Here, the concentration of $c$ is the highest at position 1 and monotonically decreases to position 15 (Fig. 2(b)). The time evolution of $c$ is shown in Fig. 2(d). Since $c$ corresponds to the Ca$^{2+}$ concentration of the plasmodium, and since such a concentration pattern enables our simulated organism to migrate as a single unified body, our model can explain how an actual plasmodium migrates when a strong attractive stimulus is applied to one end of the organism.

To verify whether our model satisfies the condition (5), we plotted the relation between $p$ and $-\frac{\partial^2 \psi}{\partial r^2}$ using data corresponding to all 15 elements for times after the steady phase wave has been organized. As shown in Fig. 3, there are no data that deviate significantly from proportionality relationship.

Fig. 3. Relationship between $p$ and $-\frac{\partial^2 \psi}{\partial r^2}$ in the case of a single strong attractive stimulus. Data were taken every 100 step from iteration 9000 to 10000.
We also simulated the case of a single weak attractive stimulus. In this case, the period of position 15 was found to become 264 ($\alpha = 2.80$) after the application of the stimulus. Although the positive and negative values of $p$ at the ends are not as large here as they are in the strong stimulus case (Fig. 4(a)), the morphogen gradient is again organized according to the direction of the phase wave, and its shape is almost the same as in the strong stimulus case (Fig. 4(b)).

### 3.2.2. Two attractive stimuli

Now we consider our simulation of the case with both strong and weak attractive stimuli. Here, the period of the element at position 1 becomes 224 and that of the element at position 15 becomes 264 after 1000 iterations. A phase wave travels from the period 224 site to the opposite end after the competition between phase waves generated at each end is completed, and in this steady state, $p$ is positive at position 1 and negative at position 15 (Fig. 5(a)). The time development of $p$ is shown in Fig. 5(b). At position 15, $p$ is initially positive, but it ultimately becomes negative through the influence of the surviving phase wave.

After the phase wave becomes organized, the gradient of $c$ is formed. In this
Fig. 5. Simulation results in the case of two attractive stimuli. The element at position 1 comes to assume a period 224 and position 15 comes to assume a period 264 after 1000 iterations. The spatial distributions of $p$ and $c$ at iteration 10000 are shown in (a) and (b), respectively. $p$ and $c$ as functions of time are shown in (c) and (d), respectively.

state, the concentration of $c$ is maximal at position 1 and monotonically decreases to position 15 (Fig. 5(b)). The functional form of $c$ has almost the same shape here as in the case of a single attractive stimulus, in spite of two stimuli application. The time dependence of $c$ is shown in Fig. 5(d). At position 15, $c$ initially increases, and then it decreases to the lowest concentration. Thus our model can explain how the plasmodium chooses only the most favorable stimulus and migrates toward it as a single unified body.

3.2.3. Size invariance of the gradient of $c$

To confirm the size invariance of the gradient of $c$, we performed simulations for the case of a single strong attractive stimulus with systems of 7, 9, 15 and 30 elements. As shown in Fig. 6, the organized gradients have the same maximum and minimum value for all sizes. This implies the size invariance of the morphogen gradient. Thus our model can explain how the plasmodium organizes positional
information in a size invariant manner.

3.2.4. Repulsive stimulus

We simulated the case of a repulsive stimulus using two different system sizes. First, we used a 7 element system. In this case, the element at position 1 was set so that its natural frequency changed to $2\pi/441$ ($\alpha = 1.90$). This corresponds to a repulsive stimulus. In this case, $p$ is positive at position 7 and negative at position 1 (Fig. 7(a)), and the concentration of $c$ is maximal at position 7 and monotonically decreases to position 1 (Fig. 7(b)). Such a form implies repulsion from the stimulus of the body as a whole. However, when $\alpha$ is set so that element 1 attains a period of 441 in a system with 15 elements, $p$ is almost zero at position 15 (Fig. 7(a)), and a non-zero gradient of $c$ is seen only in the neighborhood of the period 441 site.

![Diagram](https://example.com/diagram.png)

Fig. 7. Simulation results in the case of a repulsive stimulus with small system size (7 elements) and large system size (15 elements). After 1000 iterations, the natural frequency of the element at position 1 has changed from $2\pi/290$ to $2\pi/441$. The spatial distributions of $p$ and $c$ at iteration 10000 are shown in (a) and (b), respectively.
§4. Discussion

In this paper, we have proposed a model that explains how the *Physarum* plasmodium organizes positional information in a size invariant manner in its tactic behavior, and our results are consistent with observations of actual plasmodia. In our model, the direction of the phase wave represents the polarity of positional information in the plasmodium, and the size invariant morphogen gradient is organized according to this polarity.

4.1. Implementation of our model

There is one peculiar feature of our model. In the derivation of Eq. (13), Eq. (12) is used to describe the quantity $\frac{\partial^2 \psi}{\partial r^2}$ through only local quantities, not through $D_x(\frac{\partial^2 x}{\partial r^2})$. Thus it is not correct to say that the dynamics of $p$ depend only on the variables $x$, $y$, and $x_f$. These dynamics also depend on the form of the equation for $x$. That is, when $x$ is not Van der Pol oscillator but another type of limit cycle oscillator, the dynamics of $p$ must change accordingly.

Here we propose an equivalent model that could allow for such dependence as intracellular dynamics in the plasmodium. Suppose $x$ in Eq. (1) is coupled to another chemical, $z$, through a reaction of the form

$$x \rightarrow z. \tag{14}$$

We also suppose that $z$ hardly diffuses. In this case, the dynamics of $z$ is described by

$$\frac{\partial z(r, t)}{\partial t} = -\varepsilon \left( x - \frac{x^3}{3} \right) + y + D_z \frac{\partial^2 z}{\partial r^2}, \quad \tag{15}$$

and since $D_z$ is very small,

$$D_x \frac{\partial^2 x}{\partial r^2} \approx \frac{\partial z}{\partial t} + \frac{\partial x}{\partial t}. \quad \tag{16}$$

We further introduce a new variable

$$\frac{\partial z_f(r, t)}{\partial t} = \frac{z - z_f}{\varepsilon_f}, \quad \tag{17}$$

and it is same as $x_f$ that we can substitute $(z - z_f)/\varepsilon_f$ for $\frac{\partial z}{\partial t}$. Then, we can describe the equation for $p$ in another way,

$$\frac{\partial p(r, t)}{\partial t} = -\frac{x - x_f}{\varepsilon_f} \left( \frac{z - z_f}{\varepsilon_f} + \frac{x - x_f}{\varepsilon_f} \right) - p. \quad \tag{18}$$

The equivalent model is composed of Eqs. (1), (2), (4), (9), (15), (17) and (18).

According to a model of a cytoplasmic Ca$^{2+}$ oscillator of the *Physarum* plasmodium, calcium exists as free Ca$^{2+}$ or is bound to myosin or stored in internal
vacuoles. Of course, bound or stored calcium diffuses far less than free Ca^{2+}. Thus we speculate that if Ca^{2+} oscillation plays the role of \( x \), myosin or vacuole can play the role of \( z \). However, note that this idea ignores the shuttle streaming effect.

### 4.2. Implication of our model

The equivalent model described above provides an interesting view of our model. In this model, \( z \) works as a reference to observe \( \partial^2 \psi / \partial r^2 \), and this reference is a component of the system. Thus, the dynamics of \( p \) can be regarded as self-referential.

The essence of the obvious problem, which we noted in the Introduction, is that it seems impossible to observe the derivative of the phase by using only local quantities. This problem is solved in our model. Thus, we think such self-referential dynamics are also necessary in the model of Goodwin and Cohen.\(^5\) In their model, each part of the system must judge for itself whether or not its oscillation is entrained by its neighbors. To achieve this, it is necessary for each part to acquire a reference for this judgment by itself.

### 4.3. Qualitative differences between experiment and our model

There are some qualitative differences between observations of the plasmodium and the results of our model. One difference is with regard to frequency. When a plasmodium is stimulated with an attractive stimulus, the period of the Ca^{2+} oscillation at the stimulated site decreases, and this period change propagates immediately throughout the entire organism.\(^7\) In the case of a repulsive stimulus, the period of the Ca^{2+} oscillation at the stimulated site increases, and the period change is only local.\(^8\) The results of our simulations are consistent with this observation in the case of an attractive stimulus. However, for the repulsive case in our simulations, there is no prolonged local period change at the stimulated site. We ignore this difference, because we assume that only the phase wave and not the period change is important for the polarity organization. Thus the fact that the organized phase wave found in our model is consistent with observations is the most significant result.

Another qualitative difference between actual observations and our results concerns the phase gradient. According to Miyake et al.,\(^25\) the first order spatial derivative of the phase decreases linearly in proportion to the distance from the attractive stimulus. This implies that the relative phase decreases linearly in proportion to the square of the distance. In our simulations, the relative phase was found to decreases linearly in proportion to the distance. We consider this difference to be due to the cytoplasm distribution. In the experiment of Miyake et al.,\(^25\) the cytoplasm distribution is high at the front and low in other parts. We believe that the oscillator distribution is high where the cytoplasm distribution is high, and thus in this case the phase gradient should be large. We feel that this explains the most important difference between actual plasmodia and our model, in which the oscillator distribution is uniform.

The final difference is with regard to the Ca^{2+} gradient. In our model, the morphogen pattern disappears when the phase gradient disappears. However, in real plasmodia, the Ca^{2+} gradient remains\(^7\) after the phase gradient has disappeared when the cell begins to migrate.\(^24\) This suggests that there are two distinct mecha-
nisms, the mechanism to organize the Ca$^{2+}$ gradient and the mechanism to maintain the Ca$^{2+}$ gradient. Our concern is with the organization of positional information, not its maintenance. Thus we have focused on the former mechanism; the latter mechanism is beyond the scope of this paper. To realize such a mechanism, it is necessary to add some hysteresis property of the morphogen gradient to our model.

4.4. Application for development of other species

Our model may be applied not only to unicellular organisms, such as the *Physarum* plasmodium, but also to multicellular organisms. The most likely species is the cellular slime mold *Dictyostelium discoideum*, whose aggregation is controlled by an external periodic pulse of cAMP.$^{33}$

Since oscillation is observed in many biological systems, there is the possibility that our model can be applied to developing organisms of other species. We believe that elucidation of the mechanism which determines the polarity is a more difficult problem for these systems than for the plasmodium. In the plasmodium, the polarity depends only on present environmental conditions, and it is easy to control the polarity by experiment. In developing organisms, it depends not only on present environmental conditions, but also on the results of past development. This is one reason why this is a more difficult problem for developing systems.

Although the activator-inhibitor model has been popular as a model of morphogenesis, it has a fundamental limitation for size regulation, as we noted in the Introduction. Our model can apply to any size system, since a phase wave can travel throughout a system of any size. Further experimental and theoretical investigations are needed to examine the possibilities of our model.

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**Appendix A**

*Derivation of $\partial^2 \psi/\partial r^2$ from $x$*

In this derivation, we consider the special case in which the competition between phase waves has been completed and there exists a steady phase wave. In this case, the temporal derivative of $\psi$ can be ignored. When we substitute $x$ in Eq. (3) into $D_x(\partial^2 x/\partial r^2)$, we obtain

$$D_x \frac{\partial^2 x}{\partial r^2} = D_x \frac{\partial^2 x_0(\phi)}{\partial r^2} = D_x \frac{d x_0}{d \phi} \frac{\partial^2 \phi}{\partial r^2} + D_x \frac{d^2 x_0}{d \phi^2} \left( \frac{\partial \phi}{\partial r} \right)^2, \quad (A\cdot1)$$

and since $\partial^2 \phi/\partial r^2 = \partial^2 \psi/\partial r^2$ and $\partial \phi/\partial r = \partial \psi/\partial r$, Eq. (A\cdot1) becomes

$$D_x \frac{\partial^2 x}{\partial r^2} = D_x \frac{d x_0}{d \phi} \frac{\partial^2 \psi}{\partial r^2} + D_x \frac{d^2 x_0}{d \phi^2} \left( \frac{\partial \psi}{\partial r} \right)^2. \quad (A\cdot2)$$
Since our aim is to derive an expression for \( \frac{\partial^2 \psi}{\partial r^2} \), we would like to remove the second term on the right-hand side of Eq. (A·2). To achieve this, we multiply Eq. (A·2) by \( \frac{\partial x}{\partial t} \) and integrate the resulting expression over one period. Since the temporal derivative of \( \psi \) can be ignored, we have
\[
\frac{\partial x}{\partial t} = \frac{\partial x_0(\phi)}{\partial t} \approx \frac{dx_0}{d\phi} \omega_0, \tag{A·3}
\]
and \( \frac{\partial^2 \psi}{\partial r^2} \) and \( \frac{\partial \psi}{\partial r} \) can be regarded as constant over one period of oscillation. After performing the multiplication and integration, Eq. (A·2) becomes
\[
\int_{t-2\pi/\omega_0}^{t} \frac{\partial x}{\partial t} D_x \frac{\partial^2 x}{\partial r^2} dt \approx D_x \frac{\partial^2 \psi}{\partial r^2} \int_{0}^{2\pi} \left( \frac{dx_0}{d\phi} \right)^2 d\phi + D_x \left( \frac{\partial \psi}{\partial r} \right)^2 \int_{0}^{2\pi} \frac{dx_0}{d\phi} \frac{d^2 x_0}{d\phi^2} d\phi. \tag{A·4}
\]
Since \( x_0 \) is a \( 2\pi \)-periodic function, the second term on the right-hand side of Eq. (A·4) is
\[
D_x \left( \frac{\partial \psi}{\partial r} \right)^2 \int_{0}^{2\pi} \frac{dx_0}{d\phi} \frac{d^2 x_0}{d\phi^2} d\phi = D_x \left( \frac{\partial \psi}{\partial r} \right)^2 \left[ \frac{1}{2} \left( \frac{dx_0}{d\phi} \right)^2 \right]_{0}^{2\pi} = 0. \tag{A·5}
\]
Thus we obtain
\[
\int_{t-2\pi/\omega_0}^{t} \frac{\partial x}{\partial t} D_x \frac{\partial^2 x}{\partial r^2} dt \approx D_x \frac{\partial^2 \psi}{\partial r^2} \int_{0}^{2\pi} \left( \frac{dx_0}{d\phi} \right)^2 d\phi. \tag{A·6}
\]
Then, since \( \int_{0}^{2\pi} \left( \frac{dx_0}{d\phi} \right)^2 d\phi \) is a positive constant, Eq. (6) can be derived, with the constant
\[
C = \frac{\omega_0}{2\pi} D_x \int_{0}^{2\pi} \left( \frac{dx_0}{d\phi} \right)^2 d\phi. \tag{A·7}
\]

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