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Robust patterning of gene expression based on internal coordinate system of cells

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ABSTRACT

Cell-to-cell communication in multicellular organisms is established through the transmission of various kinds of chemical substances such as proteins. It is well known that gene expression triggered by a chemical substance in individuals has stable spatial patterns despite the individual differences in concentration patterns of the chemical substance. This fact reveals an important property of multicellular organisms called "robustness", which allows the organisms to generate their forms while maintaining proportion. Robustness has been conventionally accounted for by the stability of solutions of dynamical equations that represent a specific interaction network of chemical substances. However, any biological system is composed of autonomous elements. In general, an autonomous element does not merely accept information on the chemical substance from the environment; instead, it accepts the information based on its own criteria for reaction. Therefore, this phenomenon needs to be considered from the viewpoint of cells. Such a viewpoint is expected to allow the consideration of the autonomy of cells in multicellular organisms. This study aims to explain theoretically the robust patterning of gene expression from the viewpoint of cells. For this purpose, we introduced a new operator for transforming a state variable of a chemical substance from an external coordinate system to an internal coordinate system of each cell, which describes the observation of the chemical substance by cells. We then applied this operator to the simplest reaction–diffusion model of the chemical substance to investigate observation effects by cells. Our mathematical analysis of this extended model indicates that the robust patterning of gene expression against individual differences in concentration pattern of the chemical substance can be explained from the viewpoint of cells if there is a regulation field that compensates for the difference between cells seen in the observation results. This result provides a new insight into the investigation of the mechanism of robust patterning in biological systems composed of individual elements.

1. Introduction

In the field of theoretical biology, many researchers have devoted their efforts to investigate how a biological system can generate various kinds of well-ordered patterns, even though each element has only limited information on the environment, including other elements (Goodwin and Cohen, 1969; Wolpert, 1969). To help explain this problem, physics provides an answer in which the driving mechanism is various local interactions between elements. Based on this idea, many theoretical studies have provided mathematical models of interesting biological phenomena such as the development of multicellular organisms. Specifically, cell-to-cell interactions of a biological system are modeled based on the reaction–diffusion mechanism of chemical substances intermediating cells. Reaction–diffusion models are commonly applied to the concentration of chemical substances in the environment of a biological system, and many interesting solutions have been studied in such models (Geirier and Meinhardt, 1972; Haken, 1983; Murray, 2003; Prigogine, 1981; Turing, 1952).

However, a problem still exists from a biological perspective: how does each cell of a multicellular organism receive the chemical substances that intermediate interactions with the other cells? Any biological system is composed of autonomous elements. In general, an autonomous element does not merely accept information on the chemical substance from the environment; instead, it accepts the information based on its own criteria for reaction. Therefore, investigating what chemical substances intermediating interactions look like from an autonomous element making up a biological system and how the observation of the chemical substances by the element affects the interactions are

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important to understand the autonomy of the element. In this study, our aim is to answer theoretically these questions in terms of robust patterning of gene expression in a biological system because biological robustness is essentially a cognitive feature dependent on the viewpoint of an observer.

2. Background

It is known that many patterns formed by biological systems are invariant in response to environmental fluctuation. This property is called "robustness" (Barkai and Shilo, 2009; Wolpert, 1969). A typical phenomenon can be found in anterior–posterior axis formation of *Drosophila melanogaster*. During this process, the spatial boundary of hunchback gene expression is robust for an individual (early embryo) difference in the concentration pattern of the bicoid protein. The bicoid protein is a trigger factor of the hunchback expression (Houchmandzadeh et al., 2002). Robustness is an important property for the formation and maintenance of the body proportion of a multicellular organism. In general, this property is essential for multicellular organisms to retain their identities while responding flexibly to unknown environments (Kitano, 2007). Against this background, many theoretical studies have investigated the mechanism of robust patterning of the concentration of chemical substances during the development of multicellular organisms.

There are two approaches to investigating the mechanism. The first focuses on the autonomy of a biological system from the outside of the system; the second focuses on the autonomy of each element from the inside of the system. System biology is a typical example of the first approach. In system theory, a biological system is regarded as a network composed of elements and their interactions (Alon, 2006). From the viewpoint of system biology, many models have been proposed to explain the robust patterning of hunchback expression (Aegerter-Wilmsen et al., 2005; Howard and ten Wolde, 2005; McHale et al., 2006). Specifically, a phenomenological model based on the cross-regulation mechanism of gap genes has been proposed and is thought of as being highly consistent with experimental insights (Manu et al., 2009a, b). According to this model, four gap genes (*Hb*, *Kni*, *Kr*, *Gt*) mutually regulate the activation and inhibition of other gap genes, thereby reducing the effect of individual differences in the concentration pattern of the bicoid protein on hunchback expression. This property can be mathematically understood from the stability of solutions of the model equation. This represents the situation where the concentration pattern is robust for fluctuation of an initial state of a system. This approach to understanding the behaviors of a biological system from the outside of the system is useful in grasping a whole picture of the system.

The second approach includes relational biology as an important research field to understand the behaviors of biological systems by focusing on their functions (Rosen, 1991). Relational biology emphasizes functional closure as the basis of biological autonomy. This insight relates to the concept of autopoiesis, which puts weight on the autonomy of elements and in particular observation by the elements (Varela, 1979). According to autopoiesis, biological systems consist entirely of communications between autonomous elements through their observations of each other. The interaction of elements from an external viewpoint of the system is equivalent to an interobservation (communication) between the elements from an internal viewpoint of the system. Therefore, from the perspective of autopoiesis, robustness in multicellular organisms can be understood as invariance for observation by elements. From this standpoint, a mathematical model has been proposed such that each cell of a biological system observes the concentration of the bicoid protein at each position to transform the concentration value to a positional value (Ogawa and Miyake, 2011). This model explains the robust patterning of hunchback gene expression as the robustness of spatial pattern of the positional value. This hypothetical model provides a new insight for considering the autonomy of elements in multicellular organisms. This insight is useful for understanding biological systems from the inside of those systems, and for grasping the behaviors of their elements.

The two approaches are mutually complementary, and a combination of these approaches is thus crucial to develop a deeper understanding of biological systems. However, there is an important problem to be considered: how can we deal with the individuality of autonomous elements in the second approach? The term "individuality" is defined here as the difference between the autonomous elements belonging to the same species. During the development of multicellular organisms, cell-to-cell communication is frequently realized via transmission of chemical substances. A typical example of such chemical substances is various kinds of proteins connecting with cell membrane receptors. When a cell receives a kind of protein, a second messenger is transmitted to the cell nucleus, thereby generating gene expression. Seen in that light, receptors serve as an observer that connects the inside with the outside of a cell. Conventional models of such cell-to-cell communication through a kind of protein have assumed that the receptors on the cells of the same species have a common threshold for protein concentration and gene expression is generated by comparison of the threshold with the concentration at the position at which each cell is placed (Wolpert, 1969). Therefore, under this assumption, many studies have focused on how to form the concentration patterns of various kinds of proteins in the extracellular environment.

However, actual receptors on the cells of the same species have individual differences in shape (three-dimensional (3D) conformation), and much the same is true for many kinds of proteins (Wolpert et al., 2007). Such a difference in the cells or proteins of the same species has been conventionally handled with random fluctuation from an external viewpoint of the system (the first approach). However, the difference has to be captured as individuality from an internal viewpoint of the system (the second approach). In fact, the cells of the same species have a different response to the same concentration depending on the history and position (Alberts et al., 1994). Similarly, regarding the proteins of the same species, a slight difference in 3D configuration makes a change in function (James and Tawfik, 2003; Wang et al., 2004). Thus, robustness in the second approach can be regarded as invariance for the individuality of cells. Therefore, it is important for the second approach to investigate the communication between individual cells belonging to the same species through individual proteins belonging to the same species. Additionally, because genes in the cell nucleus have 3D configurations, much the same is true for the relationship between genes and proteins.

In this study, we assume a biological system composed of individual cells, and consider the dynamics of a chemical substance intermediating cell-to-cell communication from the perspective of individual cells. Specifically, this study aims to answer mathematically the following two questions about such a biological system.

(Q1) What are the conditions for the dynamics of the chemical substance to realize the consistent behavior of the biological system as a whole, even if each cell receives the chemical substance individually?

(Q2) What are the conditions for the robust patterning of gene expression in this biological system?

This study proposes a new perspective on the mechanisms of robust patterning in distributed autonomous systems composed of individual elements, such as biological systems.

3. Fundamental mechanism of the concentration pattern formation of proteins in multicellular organisms

During the development of a multicellular organism, many kinds of proteins are involved in gene expression. For instance, in the development of *D. melanogaster*, which is a typical model organism, the bicoid protein serves as a trigger for the anterior–posterior axis formation. Previous studies have reported that the steady concentration pattern of the bicoid protein approximately fits an exponential function (Houchmandzadeh et al., 2005). This suggests that the dynamics of the bicoid protein are described in one spatial dimension along the anterior–posterior axis as a simple reaction–diffusion equation:

$$\partial_t A(x,t) = \alpha \partial_x{}^2 A(x,t) - \gamma A(x,t). \tag{1}$$

Here, $A(x,t)$, α, and γ are the concentration, diffusion velocity, and degradation rate of the bicoid protein, respectively. ∂_t and ∂_x denote the partial differential operators in the temporal and spatial directions, respectively. The bicoid protein is a protein for regulating the expression of the hunchback gene involved in the formation of thoracic regions. Specifically, the bicoid protein is a homeodomain-containing transcription factor that regulates the transcription of the hunchback gene by connecting the homeodomain with an upstream sequence of the hunchback gene in each cell nucleus of the early embryo. That is, the bicoid protein plays a role in intermediating cell-to-cell communication to the anterior–posterior axis formation in the early embryo of *D. melanogaster*.

Eq. (1) represents the phenomenological mechanism such that the bicoid protein, which is produced from the bicoid mRNA localized at the anterior pole of an early embryo during the early development of *D. melanogaster*, gradually diffuses from the anterior pole to the posterior pole with degradation. The concentration pattern of the bicoid protein is then represented by

$$\begin{aligned} A(x) &= \frac{A_0}{1-\exp(-2L/\lambda)}\exp(-x/\lambda) \\ &\quad + \frac{A_0}{1-\exp(2L/\lambda)}\exp(x/\lambda), \\ \lambda &\equiv \sqrt{\alpha/\gamma}, \end{aligned} \tag{2}$$

as a steady solution of Eq. (1) under the Dirichlet boundary conditions $A(0) = A_0$ and $A(L) = 0$. Here, A_0 is a constant denoting the concentration at the anterior pole, and λ is a constant denoting the diffusion length of the bicoid protein. Furthermore, given that $\lambda = 0.26L$ in the literature (Houchmandzadeh et al., 2005). The solution (2) can be approximated as

$$A(x) \approx A_0 \exp(-x/\lambda). \tag{3}$$

It is known that steady concentration patterns of Dpp protein involved in the central–peripheral axis formation of limbs and wingless protein involved in the formation of wings, as well as the bicoid protein, fit exponential functions (Ibans and Belmonte, 2008). This shows that Eq. (1) represents the fundamental dynamics of proteins involved in the morphogenesis of multicellular organisms.

4. Mathematical model of the concentration pattern formation of proteins on an internal coordinate system of each cell

The mathematical model, Eq. (1), shows the mechanism of transmitting proteins as a chemical mediator of cell-to-cell communication. This dynamics strongly affects the behaviors of multicellular organisms. Because the proteins belonging to the same species have different 3D configurations in reality as explained in Section 2, we consider that many kinds of proteins have a plurality of different states in the 3D configuration in a biological system. This study supposes that a kind of protein involved in gene expression, hereinafter referred to as "protein A", has n different states and the concentration of protein A is written as

$$\vec{A}(x,t) = (A_1(x,t), \cdots, A_n(x,t))^T. \tag{4}$$

Here, $A_i(x,t) \geq 0 (i = 1, 2, \ldots, n)$, and T denotes the transposition operator. Eq. (1) is then extended as

$$\hat{\partial}_t \vec{A}(\mathrm{x},\mathrm{t}) = \alpha \hat{\partial}_x{}^2 \vec{A}(\mathrm{x},\mathrm{t}) - \hat{\gamma}\vec{A}(\mathrm{x},\mathrm{t}), \tag{5}$$

where $\hat{\partial}_t \equiv \partial_t \hat{I}$, $\hat{\partial}_x \equiv \partial_x \hat{I}$, $\hat{\gamma} \equiv \gamma \hat{I}$ and $\hat{I}$ is the $n \times n$ unit matrix. Eq. (5) shows that the n different states $A_i(x,t)$ of protein A diffuse with the same diffusion velocity α and decompose with the same degradation rate γ.

Protein A acts on cells with a combination of the n different states $A_i(x,t)$. However, as well as the states of protein A, the states of the cells that are a target of protein A, such as the 3D configuration of receptors and gene, are also involved in gene expression as explained in Section 1. In general, cells have receptors with different sensitivities for the same kind of chemical substance. We therefore introduce a transformation operator

$$\begin{aligned} \hat{U}(x,t) : \vec{A}(x,t) \mapsto \vec{A}'(x,t) &= \hat{U}(x,t)\vec{A}(x,t) \\ &= (u_{1i}(x,t)A_i(x,t), \ \cdots, u_{ni}(x,t)A_i(x,t))^T \end{aligned} \tag{6}$$

to take into account the individuality of the cells. $\hat{U}(x,t)$ is an $n \times n$ matrix in which the n^2 elements $u_{ij}(x,t)$ are functions of space and time. Here, the Einstein convention is used for the components of the vector $\vec{A}'(x,t)$. $\vec{A}'(x,t)$ represents the n states of protein A in terms of cells. That is, $\hat{U}(x,t)$ plays a role in transforming the state $\vec{A}(x,t)$ in the extracellular environment to another state $\vec{A}'(x,t)$ from the viewpoint of the cells. Therefore, the concentration $A'_i(x,t)$ $(i = 1, \ldots, n)$ after transformation takes values in an internal coordinate system that is associated with each cell parameterized in a space–time coordinate system (x,t). Such a function of $\hat{U}(x,t)$ is hereinafter called "observation by cells".

Then, if $\hat{U}(x,t)$ does not depend on space and time, $\vec{A}'(x,t)$ satisfies Eq. (5). In this case, if all cells have the same internal coordinate system, the dynamics of protein A having n different states $A'_i(x,t)$ $(i = 1, \ldots, n)$ are the same as the original dynamics.

On the other hand, if $\hat{U}(x,t)$ depends on space and time, $\vec{A}'(x,t)$ does not satisfy Eq. (5). That is, if each cell has a different internal coordinate system, the difference in observational results by cells is reflected in the dynamics of protein A. Thus, the dynamics of protein A having the n different states $A'_i(x,t)$ $(i = 1, \ldots, n)$ are different from the original dynamics described as Eq. (5). However, even though the observational results of the concentration are different for each cell, we can consider the invariance of the dynamics described as Eq. (5) for the local transformation (6). This is the necessary condition for the dynamics of protein A as a chemical mediator of cell-to-cell communication to realize the consistent behavior of the biological system composed of individual cells as a whole. To satisfy this condition, the partial differential operators $\hat{\partial}_t$ and $\hat{\partial}_x$ have to be replaced with

$$\hat{D}_t \equiv \hat{\partial}_t - g\hat{T}(x,t), \ \hat{D}_x \equiv \hat{\partial}_x - g\hat{X}(x,t). \tag{7}$$

Here, $D^t \equiv D_t I$ and $D^x \equiv D_x I$ are new differential operators used in an internal coordinate system associated with each cell.

Furthermore, the newly introduced $n \times n$ matrices $\hat{T}(x,t) \equiv T(x,t)\hat{I}$ and $\hat{X}(x,t) \equiv X(x,t)\hat{I}$ require a transformation depending on $\hat{U}(x,t)$:

$$\hat{U}(x,t) : \hat{T}(x,t) \mapsto \hat{T}'(x,t) = \hat{U}\hat{T}\hat{U}^{-1} + g^{-1}(\partial_t \hat{U})\hat{U}^{-1}, \tag{8}$$

$$\hat{U}(x,t) : \hat{X}(x,t) \mapsto \hat{X}'(x,t) = \hat{U}\hat{X}\hat{U}^{-1} + g^{-1}(\partial_x \hat{U})\hat{U}^{-1}. \tag{9}$$

The new differential operators $\hat{D}_t$ and $\hat{D}_x$ then fulfill the commutative condition

$$\hat{D}'_t\left(\hat{U}\vec{A}\right) = \hat{U}\left(\hat{D}_t\vec{A}\right),\ \hat{D}'_x(\hat{U}\vec{A}) = \hat{U}(\hat{D}_x\vec{A}), \tag{10}$$

where

$$\hat{D}'_t = \hat{\partial}_t - g\hat{T}',\ \hat{D}'_x = \hat{\partial}_x - g\hat{X}'. \tag{11}$$

From the above analysis, we can confirm the invariance of the dynamical equation

$$\hat{D}_t\vec{A}(x,t) = \alpha\hat{D}_x{}^2\vec{A}(x,t) - \hat{\gamma}\vec{A}(x,t) \tag{12}$$

for the local transformation (6). Eq. (12) represents the dynamics of protein A from the perspective of the cells located at each position (x,t) of a space–time coordinate system.

Fig. 1 shows the relationship between internal coordinate systems of cells and an external coordinate system in this model. This figure, for simplicity, illustrates two internal coordinate systems (I_1 and I_2), each of which is associated with a cell located in the extracellular environment. Each cell is identified by the value (x,t) of the space–time coordinate system. In this figure, each cell observes the state vector $\vec{A}(x,t)$ of protein A in an external coordinate system associated with the extracellular environment to transform it to the state vector $\vec{A}'(x,t)(=\hat{U}(x,t)\vec{A}(x,t))$ in each internal coordinate system. Suppose here that protein A has two different states $\vec{A}(x,t) = (A_1(x,t), A_2(x,t))^T$. The cell at the position x_1 accepts protein A by transforming the state vector $\vec{A}(x_1,t_1)$ to $\vec{A}'(x_1,t_1)$ at the time t_1, while the cell at the position x_2 also accepts

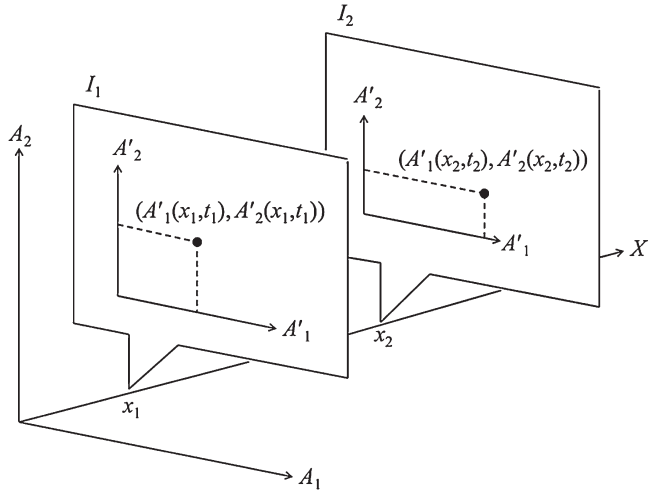

Fig. 1. Relationship between two kinds of coordinate systems specifying the concentration of protein A. $\vec{A}(x,t)$ is the concentration of protein A in an external coordinate system (A_1,A_2) at position (x,t) of a space–time coordinate system. $\vec{A}'(x,t)(=\hat{U}(x,t)\vec{A}(x,t))$ is the concentration of protein A in an internal coordinate system (A'_1,A'_2) at the position (x,t).

protein A by transforming the state vector $\vec{A}(x_2,t_2)$ to $\vec{A}'(x_2,t_2)$ at the time t_2.

By substituting Eq. (7) into Eq. (12), we obtain

$$\hat{\partial}_t\vec{A}(x,t) = \alpha\hat{\partial}_x{}^2\vec{A}(x,t) - \hat{\gamma}\vec{A}(x,t) + \hat{T}\vec{A} - 2\alpha g\hat{X}(\hat{\partial}_x\vec{A}) - \alpha g(\hat{\partial}_x\hat{X})\vec{A} + \alpha g^2\hat{X}^2\vec{A}. \tag{13}$$

This equation uses the external coordinate system and describes an extended model of the simplest reaction–diffusion equation. The third to sixth terms on the right-hand side denote the interactions between protein A and new fields described by matrices $\hat{T}(x,t)$ and $\hat{X}(x,t)$. The new fields serve as compensation fields for coordinating the dynamics of protein A in the external coordinate system with those in the internal coordinate system. Note here that these compensation fields become obvious only in the external coordinate system.

5. Robust patterning of gene expression

In this section, we focus on the expression pattern of the hunchback gene in considering the robustness of biological systems. It is reported that even though the concentration patterns of the bicoid protein have huge individual differences for every early embryo of *D. melanogaster*, there are only slight differences between the early embryos in the spatial boundary of hunchback expression (Houchmandzadeh et al., 2002). This means that the expression pattern of the hunchback gene is robust against individual differences in the concentration pattern of the bicoid protein.

The present model provides an explanation for this phenomenon as follows. Here, we suppose that the bicoid protein has only one state as the simplest example. The state vector $\vec{A}(x,t)$ of the bicoid protein is then replaced with a real-valued function $A(x,t)$ representing the concentration, the matrices $\hat{T}(x,t)$, $\hat{X}(x,t)$, and $\hat{U}(x,t)$ in the local transformations (8) and (9) are replaced with real-valued functions $T(x,t)$, $X(x,t)$, and $U(x,t)$, and the matrix-formed differential operators $\hat{D}_t$, $\hat{D}_x$, $\hat{\partial}_t$, and $\hat{\partial}_x$ are replaced with ordinary partial differential operators D_t, D_x, ∂_t, and ∂_x. Additionally, to focus on a steady concentration pattern of the bicoid protein, we impose the conditions $\partial_t A = 0$ and $D_t A = 0$ and therefore suppose that $T(x,t) = 0$. The local transformation (6) then shows that the concentration pattern $A(x)$ of the bicoid protein in an external coordinate system associated with the extracellular environment (early embryo) and the concentration pattern $A'(x)$ of protein A in an internal coordinate system associated with each cell (cell nucleus) are related by the transformation

$$U(x) : A(x) \to A'(x) = U(x)A(x). \tag{14}$$

If there exists a compensation field $X(x)$ with which to establish the commutative condition (11), the concentration pattern $A'(x)$ of the bicoid protein obeys

$$\alpha D'_x{}^2 A'(x) - \gamma A'(x) = 0 \tag{15}$$

according to Eq. (12). In other words, the concentration pattern $A(x)$ of the bicoid protein from the perspective of cells obeys

$$\alpha D_x{}^2 A(x) - \gamma A(x) = 0 \tag{16}$$

in an internal coordinate system associated with each cell. According to Eq. (13), this equation can be rewritten as

$$\alpha\partial_x{}^2 A(x) - \gamma A(x) - 2\alpha g X(\partial_x A) - \alpha g(\partial_x X)A + \alpha g^2 X^2 A = 0 \tag{17}$$

in the external coordinate system associated with the early embryo. The solution $A(x)$ of Eq. (17) depends on the compensation

field $X(x)$. Because each individual (early embryo) is characterized by the compensation field $X(x)$ in this model, the steady concentration pattern of the bicoid protein depends on the compensation field $X(x)$.

This model explains the robust patterning of hunchback expression against individual differences in the concentration pattern of the bicoid protein as follows. We now suppose that there are M early embryos having the same body length ($x=0$: anterior pole, $x=L$: posterior pole). The steady concentration patterns $A_j(x)$ ($j=1, \ldots, M$) of the bicoid protein then obey

$$\alpha_j \partial_x{}^2 A_j(x) - \gamma_j A_j(x) = 0 \tag{18}$$

in the external coordinate system associated with each early embryo j. This equation is a well-known model that takes no account of observation by cells. The steady solution of Eq. (18) that satisfies the Dirichlet boundary conditions, $A_j(0)=A_0$ and $A_j(\infty)=0$, is

$$A_j(x) = A_0 \exp(-x/\lambda_j),\ \lambda_i \equiv \sqrt{\alpha_i/\gamma_i}, \tag{19}$$

where A_0 is a constant representing the concentration at the anterior pole and is assumed to take the same value in all early embryos. Although the boundary condition at the posterior pole should be given at the position $x=L$ in a precise sense, here it is given at the position $x=\infty$ to simplify the function form of the steady solution. However, this does not affect the essence of the following discussion. In addition, λ_j ($j=1, \ldots, M$) is a constant denoting the diffusion length. Here, we assume that λ_j is different from one early embryo to another. In other words, it is assumed that the diffusion velocity α_j ($j=1, \ldots, M$) or the degradation rate γ_j ($j=1, \ldots, M$) have different values for each early embryo. Fig. 2(a) illustrates such a situation. In the figure, the red line (early embryo 1) is the concentration pattern of the bicoid protein obtained as the steady solution of Eq. (18) with diffusion length λ_1, the blue line (early embryo 2) is the concentration pattern of the bicoid protein obtained as the steady solution of Eq. (18) with diffusion length λ_2, and the green line (early embryo 3) is the concentration pattern of the bicoid protein obtained as the steady solution of Eq. (18) with diffusion length λ_3. This situation shows that when the diffusion length λ_j of the bicoid protein is different from one early embryo to another, there are individual differences in the concentration pattern $A_j(x)$ of the bicoid protein.

However, even though the concentration pattern $A_j(x)$ of the bicoid protein in the external coordinate system of each early embryo j has such individual differences, the solution $A_j(x)$ of Eq. (17) represents the same pattern for all early embryos. That is, in this model, the concentration pattern $A_j(x)$ of the bicoid protein in the internal coordinate system of each cell is robust. The details are as follows. Here, we set the condition $\partial_t X(x)=0$ to consider the simplest case. This condition means that the compensation field $X(x)$ is not dependent on the position x. Eq. (17) then becomes

$$\alpha_j \partial_x{}^2 A_j(x) - \gamma_j A_j(x) - 2\alpha_j g X_j(\partial_x A_j) + \alpha_j g^2 X_j{}^2 A_j = 0, \tag{20}$$

where X_j ($j=1, \ldots, M$) is the compensation field characterizing each early embryo j. Eq. (20) has the steady solution

$$A_j(x) = A_0 \exp\{-(1/\lambda_j - gX_j)x\},\ \lambda_j \equiv \sqrt{\alpha_j/\gamma_j}, \tag{21}$$

under the Dirichlet boundary conditions $A_j(0)=A_0$ and $A_j(\infty)=0$. If the compensation field X_j meets the condition

$$1/\lambda_j - gX_j = 1/\lambda \tag{22}$$

for each early embryo, then the same concentration pattern $A_j(x)$ of the bicoid protein is established as

$$A_j^{eff}(x) = A_0 \exp(-x/\lambda) \tag{23}$$

in all early embryos. A^{eff} represents the concentration of the bicoid protein effective for expressing hunchback gene from the viewpoint of cells. Furthermore, if we regard the function (23) as the solution of Eq. (17),

$$\begin{aligned}\alpha_j \partial_x{}^2 A_j(x) - \gamma_j A_j(x) - 2\alpha_j g X_j(\partial_x A_j) - \alpha_j g(\partial_x X_j) A_j \\ + \alpha_j g^2 X_j{}^2 A_j = 0,\end{aligned} \tag{24}$$

then the compensation field $X_j(x)$ has to satisfy the condition

$$X_j(x) = 1/\{C\exp(-a_j x) - g/a_j\} + b_j, \tag{25}$$

where

$$a_j = 2gb_j + 2/\lambda. \tag{26}$$

Here, b_j is a solution of the equation

$$b_j{}^2 + (2/g\lambda)b_j + (1/\lambda^2 - 1/\lambda_j{}^2)/g^2 = 0, \tag{27}$$

and C is a constant to be determined by boundary conditions.

Then, if each cell has a threshold for response to the concentration pattern $A_j^{eff}(x)$ represented by the solution (23), the robust boundary of hunchback expression can be explained. Fig. 2(b) illustrates the robust concentration pattern $A_j^{eff}(x)$ of the bicoid protein in this model and, in particular, shows that the concentration patterns $A_j^{eff}(x)$ have the same shape in all three early embryos. In this way, this model indicates that, if we consider individual observation by cells, the concentration pattern $A_j(x)$ of the bicoid protein has the same robust shape in all early embryos.

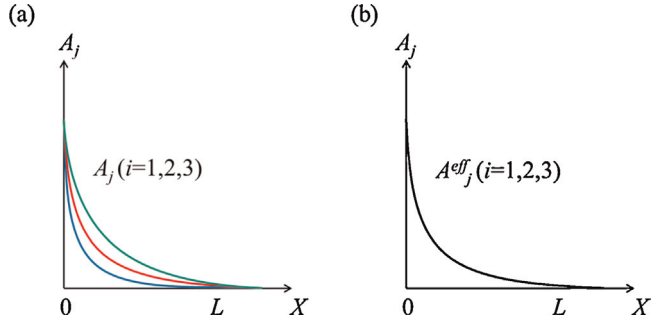

Fig. 2. Concentration patterns of the bicoid protein in three early embryos of *Drosophila melanogaster*. Fig. 2(a) shows the concentration patterns of the bicoid protein without the observation effects of cells. Fig. 2(b) shows the robust concentration patterns of the bicoid protein with their observation effects.

6. Discussion and conclusion

The present model suggests answers to the two questions (Q1) and (Q2) asked in Section 2. Regarding (Q1), this study supposes that each cell has an internal coordinate system for observing the concentration of a chemical substance that intermediates cell-to-cell communication. Then, even if each cell responds to the concentration of the chemical substance observed in a different internal coordinate system, the cells can exhibit consistent behavior as a whole according to the concentration of the chemical substance observed in each internal coordinate system in the presence of a regulation field. This field compensates for the difference between cells in the observation results. In particular, this compensation field plays a role in regulating the dynamics of the chemical substance by affecting the spatial rate of change in the concentration of the chemical substance.

Regarding (Q2), under a condition to be satisfied by the compensation field, the robust patterning of gene expression is

realized in the concentration pattern of a chemical substance as a trigger factor of gene expression from the perspective of cells. The condition is determined by the type and dynamics of the chemical substance (see for example Eq. (22)). From such a perspective, the compensation field plays an important role in giving cells a frame for accepting a chemical substance according to the type and dynamics of the chemical substance. Therefore, in this model the cells can consistently respond to the concentration of the chemical substance observed in each internal coordinate system. Although such a mechanism of robust patterning proposed in this study is provided as a hypothesis, it is reasonable to consider that in the process of receiving a chemical substance each cell responds to a concentration meaningful to itself. However, if the concentration A_0 at the anterior pole (the solution (19)) has a different value in each early embryo, it is difficult to explain the robust patterning of gene expression with high precision using this theoretical framework alone. This problem remains to be investigated as future work.

The robustness considered in this study is based on the invariance of the dynamics (Eq. (16)) of a chemical substance for individual observation $\hat{U}(x,t)$ by cells. Therefore, this study indicates that the robust patterning of gene expression can be explained as the robustness of the concentration pattern of a chemical substance against a difference in observation results by cells. However, this does not mean that the individual observation by the cells can be regarded as a fluctuation, because the observation operator $\hat{U}(x,t)$ is allowed to obey some deterministic dynamical equation. We will refer to the details of this point in the near future.

Next, we consider the entity of the compensation field $X(x)$ in Eq. (17). The compensation field $X(x)$ has three features: Feature (F1) is to transport the bicoid protein in a constant direction depending on the concentration (the third term on the left side); Feature (F2) is to increase the bicoid protein nonlinearly depending on the concentration (the fifth term), and Feature (F3) is to increase or decrease the bicoid protein depending on the spatial change in the concentration (the fourth term).

Although it is considered normal that the bicoid protein diffuses in an early embryo, a previous study reported that the diffusion velocity of the bicoid protein is too slow to establish the stationary concentration gradient rapidly, and therefore its diffusion alone cannot account for the generation of the stationary concentration gradient over a long distance (Gregor et al., 2007). In this regard, another previous study proposed a mechanism such that the bicoid mRNA forms a complex with the staufen protein and is then transported via the microtubular network in an early embryo, thereby generating the concentration gradient of the bicoid mRNA (Spirov et al., 2009). However, we can consider another mechanism such that the bicoid protein itself is transported in a constant direction. The feature (F1) indicates that the compensation field $X(x)$ is regarded as a mechanical structure for the transport of the bicoid protein in a given direction. In fact, although the concentration gradient of the bicoid mRNA contributes to the generation of the concentration gradient of the bicoid protein, this mechanism alone cannot account for the concentration gradient of the bicoid protein. Therefore, another previous study demonstrated that movement of the bicoid protein, whether active or passive, is a necessary condition for generating the concentration gradient of the bicoid protein (Little et al., 2011). The feature (F1) of the compensation field $X(x)$ suggests this possibility.

Further, we can read from the feature (F2) that the compensation field $X(x)$ also reacts chemically with the bicoid protein. In contrast, it is a little difficult to interpret the feature (F3) from a phenomenological viewpoint. If the compensation field $X(x)$ obeys some kind of dynamics, we can understand from the feature (F3) that the compensation field $X(x)$ moves in a constant direction depending on the concentration $A(x)$ of the bicoid protein. This suggests that the compensation field $X(x)$ has a mechanical structure that includes chemical substances interacting with the bicoid protein. As such, we may consider a structure with a reactive transport mechanism based on a cytoskeleton. In addition, although it is considered that the compensation field $X(x)$ obeys various kinds of dynamics in general, if the dynamics have to be invariant for individual observation $\hat{U}(x,t)$ by cells, the possibility of such dynamics being present will be quite limited.

In this study, we conducted a mathematical analysis of only the simplest model equation (Eq. (12)) representing the dynamics of protein. We will look at more realistic mathematical models including nonlinear reaction–diffusion models in future work.

Finally, the mathematical framework in this study is known as gauge theory for fiber bundles (Isham, 2003). This theory is the guiding principle for finding the fundamental dynamics of quantum particles such as leptons and quarks in high-energy physics (O'Raifeartaigh, 1997). However, this theory can be applied to the modeling of the dynamics of classical mechanical systems with nonholonomic constraint conditions, called deformable bodies, such as a falling cat, a moving car, and N-body dynamics (Montgomery, 1993; Fecko, 1996; Littlejohn and Reinsch, 1997; Shapere and Wilczek, 1989). In this study, we used the theory to explain the robust patterning of concentrations formed by a chemical mediator of the communication between individual cells. The theory is expected to become a useful tool in studying the consistency between local and global behaviors of cells in multicellular organisms.

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