Cell Biology Modeling Development

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1 Overview

The fundamental problem of pattern formation for example, how to specify body axes, limbs, digits during development comes down to interpreting a common set of genetic instructions differently at different locations in space (Wolpert 1969). (See Figure 1.1 for an illustration). In all multicellular animals, this process is orchestrated by morphogens, molecules that are produced at discrete sites and disperse to form inspection ration gradients. Such gradients establish patterns because cells are preprogrammed to do very different things at different morphogen concentrations. Each cell responds to morphogens by reading their concentrations, and interprets them through intracellular machineries. A morphogen system usually consists of a region of morphogen-responsive cells, a region of morphogen producing cells, and a set of boundary conditions (Lander 2007). The objective of morphogen-responsive cells is to generate an intracellular signal, the amount of which reflects the level of moprhogen receptor occupancy, to instruct cells to perform their functions or to obtain their fates.

A morphogen system often contains: 1) regulated morphogen transport - some families of morphogens (Wnts, Hhs) undergo lipid modifications that presumably make them less diffusible and others may undergo active transport (via transcytosis, argosomes, cytonemes, etc); 2) multiple morphogen species - Several BMP gradients utilize multiple types of BMP monomers; 3) Multiple morphogen receptor type; 4) nonreceptor binding sites - polypeptide morphogens bind to cell surface proteins and/or proteoglycans other than receptors; 5) secreted competitive inhibitors; 6) co-receptors - cell surface molecules affect morphogen signaling by acting as co-receptors; 7) extracellular enzymes - enzymes cleave inhibitors and co-receptors; 8) feedback regulation - feedback regulation of morphogens, receptors, and nonreceptor binding sites; 9) complex feedback loops in intracellular signaling; and along with many other regulations and components (Lander 2007).

Here we present a set of basic modeling tools based on a continuum approach for a description of several fundamental biological processes during development. This approach has been successfully

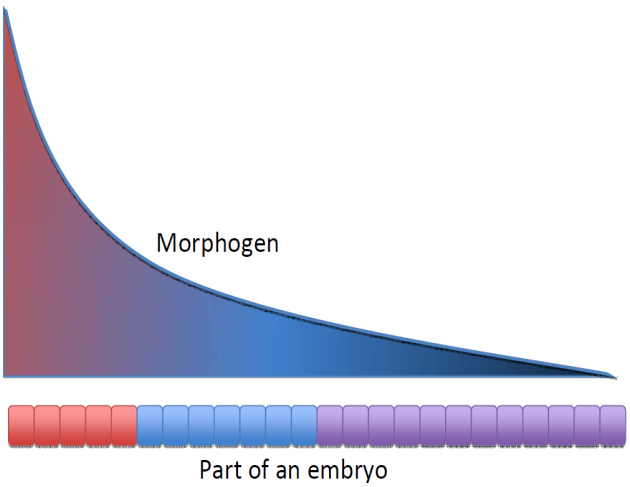

Figure 1.1: An illustration for a morphogen system at tissue scale: different color of cells representing different fate of the cell.

applied to studying many morphogen systems (Lander 2007).

2 Models

Bio-chemical reaction in a biological system is usually modeled through the rate equation which is derived through a mass balance of the reactants in terms of reaction rate and concentration of reactants. For a typical reaction that uses m molecules P and n molecules Q to produce one molecule R without intermediate steps during reaction, written as $mP + nQ \rightarrow R$, then the rate of such reaction based on the law of mass action is given by

$$r[P]^m[Q]^n \tag{2.1}$$

where [ ] stands for concentration of each species and r is called the rate coefficient or rate constant of reaction and its value depends on the properties of the reactants and environment of the reactions. Equation 2.1 will be used repeatedly for modeling bio-chemical reactions in this section.

2.1 ligand-ligand interactions

If the number of cells in a developmental system is large and the interest of study is at the tissue scale, the living tissue (e.g. part of an embryo) may be modeled as continuous media. The interactions among free diffusible morphogens A and B, often called ligands (see Figure 2.1), that undergo Brownian motions in extra-cellular space of the tissues may simply be described based on the rate equation 2.1 and principle of diffusions:

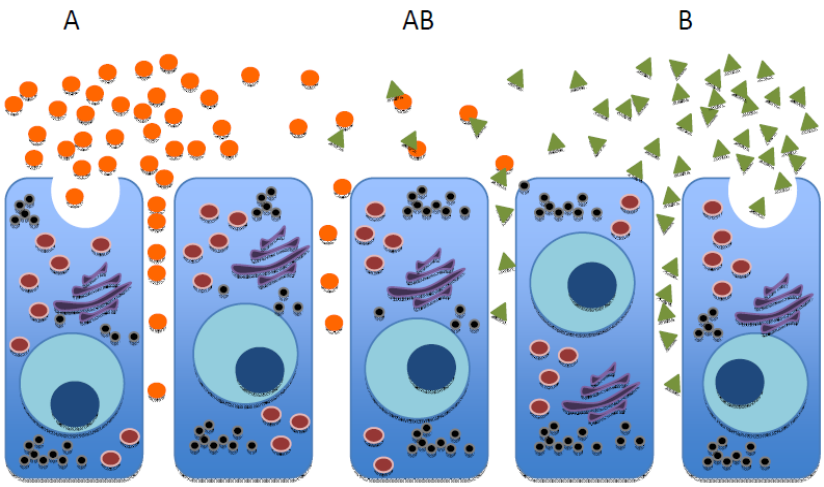

Figure 2.1: Ligand-ligand interactions in the extra-cellular space at multi-cellular scale: diffusion, local production of ligands, and binding and formation of new complex.

$$\frac{\partial[A]}{\partial t} = D_A\Delta[A] - i_{on}[A][B] + i_{off}[AB] - i_{deg}[A] + V_A, \tag{2.2}$$

$$\frac{\partial[B]}{\partial t} = D_B\Delta[B] - i_{on}[A][B] + i_{off}[AB] - j_{deg}[B] + V_B, \tag{2.3}$$

$$\frac{\partial[AB]}{\partial t} = D_{AB}\Delta[AB] + i_{on}[A][B] - i_{off}[AB] - w_{deg}[AB] \tag{2.4}$$

where AB is a new complex produced by binding between A and B, i_{on} is the reaction rate, i_{off} is the reaction dissociation rate, i_{deg}, j_{deg}, and w_{deg} are the degradation rates for each species, D_A, D_B, and D_{AB} are the diffusion coefficients, respectively, V_A and V_B are the synthesis rates of each morphogen that may be spatially localized, and Δ is the Laplacian operator.

2.2 Ligand-receptor interaction

The free morphogen communicates with cells usually through receptors of cells in plasma membrane. Ligands bind to receptors and dissociate from them according to the rate equation similar to the ligand-ligand interaction. This interaction may be described as

$$\frac{\partial[A]}{\partial t} = D_A\Delta[A] - k_{on}[A][R] + k_{off}[AR] - i_{deg}[A] + V_A, \tag{2.5}$$

$$\frac{d[R]}{dt} = -k_{on}[A][R] + k_{off}[AR] - k_{1deg}[R] + V_R, \tag{2.6}$$

$$\frac{d[AR]}{dt} = k_{on}[A][R] - k_{off}[AR] - k_{2deg}[AR] \tag{2.7}$$

where D_A is the diffusion coefficient of the ligand, k_{on} is the binding rate, k_{off} is the disassociation rate, i_{deg}, k_{1deg}, and k_{2deg} are the degradation rates, V_A is the synthesis rate of the ligand, and V_R is the synthesis rate of the receptor.

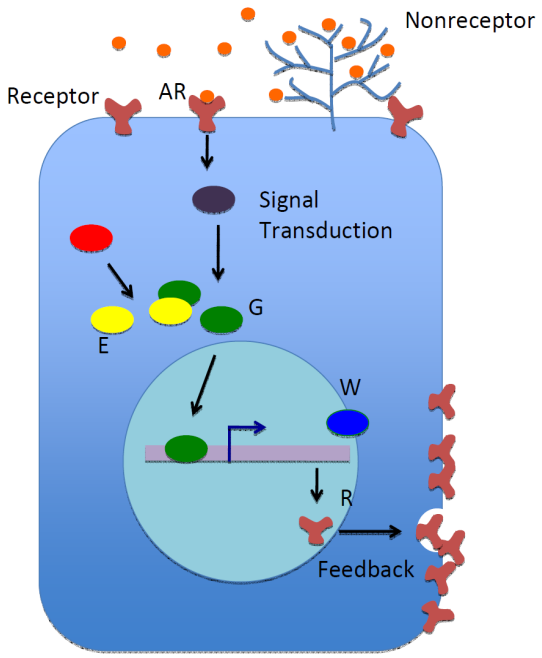

Figure 2.2: Ligand-cell interactions at single cell scale: ligand-receptor binding, ligand-nonreceptor binding.

2.3 Ligand-nonreceptor interaction

It is known that many morphogens bind to cell surface proteins and/or proteoglycans other than receptors (See Figure 2.2). Nonreceptors, referring to this class of cell surface proteins, usually take away the ligands from the extracellular space and prevent action of morphogens. The model for ligand-nonrecptor interaction is similar to the equations 2.5 - 2.7 except that the complex formed between the free morphogen and nonreceptor does not directly activate the patterning signal pathway within the cell.

2.4 Intracellular signal transduction

Binding of a ligand to a cell-surface receptor stimulates a series of events inside the cell, with different types of receptor stimulation of different intracellular responses. Through binding, the ligand initiates the transmission of a signal across the plasma membrane by inducing a change in the shape or conformation of the intracellular part of the receptor, often leading to activation of an enzymatic activity contained within the receptor or expose a binding site for other signaling proteins within the cell. For example, the extracellular ligand-receptor complex AR results in the activities of its intracellular part AR_{in} leading to activation of protein G (See Figure 2.2). This process may be modeled as

$$\frac{d[AR_{in}]}{dt} = k_1[AR] - k_{-1}[AR_{in}], \tag{2.8}$$

$$\frac{d[G]}{dt} = k_2[AR_{in}] - k_{-2}[G] \tag{2.9}$$

where k_i, $i = -2, -1, 1, 2$ are rate constants. Often the morphogen signal pathway may interact with components in other pathways. For example, protein E from the other pathway binds with G leading to loss of active G:

$$\frac{d[G]}{dt} = k_2[AR_{in}] - k_3[G][E] + k_4[GE] - k_{-2}[G] \tag{2.10}$$

where k_3 is the on rate and k_4 is the off rate. The equations for $[E]$ and $[GE]$ are omitted.

One of the key steps during signal transduction is transcription: a protein, called transcription factor, binds to specific DNA sequences to control copy of genetic information from DNA to mRNA. This function may be achieved by one transcription factor alone or binding with other transcription factors in a complex, through promoting (as an activator) or blocking (as a repressor) function of RNA to specific genes. Assume that G is a transcriptional factor and W is the mRNA encoding information from certain gene through the transcriptional factor G, a simple linear model for an activator takes the form:

$$\frac{d[W]}{dt} = r_{trans}[G] - r_2[W] \tag{2.11}$$

where r_{trans} is the transcription rate and r_2 is the degradation rate.

2.5 Feedback regulations

Many intracellular signaling molecule activities may involve feedback regulation, that is, concentration of a protein or mRNA depends on its downstream responses. Assume that in equation 2.11 the transcriptional rate r_{trans} is proportional to the promoter activity that may depend on W, the product of G, such feedback may be modeled through a Hill function

$$r_{trans} = Hill(W) \tag{2.12}$$

where

$$Hill(x) = a_{min} + \frac{a_{max} - a_{min}}{1 + (x/\gamma)^m} \tag{2.13}$$

where a_{min} is the minimal value of the Hill function, a_{max} is its maximal value, γ is the half maximal effective concentration of x allowing such regulation, and m is the Hill exponent that

controls the slope of the response. For small m, the Hill function is a graded function of x; for large m, the Hill function has a ultra-sensitive response; When $m > 0$, the Hill function is a decreasing function representing negative feedback; When $m < 0$, the Hill function is an increasing function representing positive feedback.

Feedback regulations may also occur to regulate properties of ligand, receptor and non-receptor, for example, through synthesis or degradation of receptor, ligand, and non-receptor. In the case that feedback is on the synthesis of receptor through mRNA, W, one may write

$$V_R = Hill(W) \tag{2.14}$$

where the parameters in the Hill function are usually different from regulation to regulation.

2.6 Parameters

Although the diffusion coefficient of morphogen may be estimated experimentally, the exact values are difficult to obtain due to complexity of extracellular environment and other aspect of the *in-vivo* developmental system (Lander et al. 2002). The individual reaction rate, such as k_{on} and k_{off}, is usually difficult to measure, however, the ratio of k_{on}/k_{off} may be estimated through *in-vitro* experiments. The effective synthesis rate and degradation rate may be estimated based on experimental observation on the net influx and out-flux of the mass observed in experiments. Usually a range for each parameter may be estimated instead of individual specific values. The parameters in the feedback regulations are difficult to obtain, in particular, γ, the half maximal effective concentration, directly depends on solution of the system while its value also affects the solution. Overall, exploration of a developmental system using a large set of parameters within biological plausible ranges is an effective approach of using models to characterize their properties qualitatively and quantitatively for testing biological hypotheses.

2.7 An example

One of the developmental systems which has been studied using modeling is the dorsoventral axis patterning during early *Drosophila* embryo development (Mizutani et al. 2005). Several zygotic genes are involved in the regulatory network of the developmental system. Among them, decapentaplegic (Dpp) promotes dorsal cell fates such as amnioserosa and inhibits development of the ventral central nervous system; and another gene Sog promotes central nervous system development. In this system, Dpp is produced only in the dorsal region while Sog is produced only in the ventral region. For the wild-type, the Dpp activity has a sharp peak around the mid-line of the dorsal with the presence of its "inhibitor" Sog. Intriguingly, mutation of Sog results in a loss of ventral structure as expected, but, in addition, the amnioserosa is reduced as well. It appears that the Dpp antagonist, Sog, is required for maximal Dpp signaling (Ashe and Levine 1999).

An integrated modeling and experiment study was performed for robustness and temporal dynamics of the morphogens under various genetic mutations (Mizutani et al. 2005). The model (Mizutani et al. 2005) used a one-dimensional geometry of the perivitelline space of the *Drosophila* embryo. Analytical study for the one-dimensional model was also carried for steady states (Lou et al. 2005). To examine an experimental observation on over-expression of the receptors along the anterior-posterior axis of the embryo (Mizutani et al. 2005), a two-dimensional model was developed (Lander et al. 2009b) for the Dpp activities outside the area of elevated receptors in a *Drosophila* embryo. For the sake of analytical study, the two-dimensional model investigated in (Lander et al. 2009b) is a simplified version of the models presented below.

Let $[L], [S], [LS], [R], [LR]$ denote the concentration of Dpp, Sog, Dpp-Sog complexes, free receptors, and Dpp-receptor complexes, respectively. Following the modeling principle in sub-sections (2.1 -2.6), the Dpp-Sog system is governed by the following reaction-diffusion equations:

$$\begin{aligned}
\frac{\partial [L]}{\partial T} &= D_L \left(\frac{\partial^2 [L]}{\partial X^2} + \frac{\partial^2 [L]}{\partial Y^2} \right) - k_{on}[L][R] + k_{off}[LR], \\
&\quad -j_{on}[L][S] + (j_{off} + \tau j_{deg})[LS] + V_L(X,Y), \\
\frac{\partial [R]}{\partial T} &= -k_{on}[L][R] + k_{off}[LR] - k_{1deg}[R] + V_R(X,Y), \\
\frac{\partial [LR]}{\partial T} &= k_{on}[L][R] - (k_{off} + k_{2deg})[LR], \\
\frac{\partial [LS]}{\partial T} &= D_{LS} \left(\frac{\partial^2 [LS]}{\partial X^2} + \frac{\partial^2 [LS]}{\partial Y^2} \right) + j_{on}[L][S] - (j_{off} + j_{deg})[LS], \\
\frac{\partial [S]}{\partial T} &= D_S \left(\frac{\partial^2 [S]}{\partial X^2} + \frac{\partial^2 [S]}{\partial Y^2} \right) - j_{on}[L][S] + j_{off}[LS] + V_S(X,Y)
\end{aligned} \tag{2.15}$$

in the domain $0 < X < X_{max}$ and $0 < Y < Y_{max}$. X axis is the anterior-posterior axis of the embryo, and Y axis is the dorsal-ventral axis. The boundary conditions for $[L]$, $[LS]$, and $[S]$ are no-flux at $X = 0$ and $X = X_{max}$, and periodic at $Y = 0$ and $Y = Y_{max}$. $V_R(X,Y)$, $V_L(X,Y)$ and $V_S(X,Y)$ are the synthesis rates for receptors, Dpp, and Sog, respectively; D_L, D_{LS}, D_S are diffusion coefficients; τ is the cleavage rate for Sog; and other coefficients are on, off and degradation rate constants for the corresponding bio-chemical reactions (Mizutani et al. 2005).

Another similar model for BMP gradients is the dorsal-ventral patterning of the zebrafish embryo, in which a three-dimensional approximation of the zebrafish embryo shape was developed (Zhang et al. 2007). Numerical simulations have to be utilized for studying those models.

3 Numerical methods

The model described above takes the general form:

$$\frac{\partial \mathbf{u}}{\partial t} = D\Delta \mathbf{u} + \mathbf{F}(\mathbf{u}), \tag{3.1}$$

where $\mathbf{u} \in \mathbf{R}^m$ represents the morphogen species, $D \in \mathbf{R}^{m\times m}$ is the diffusion constant matrix, Δ is the Laplacian, and $\mathbf{F}(\mathbf{u})$ describes the bio-chemical reactions.

This system is usually very stiff due to the drastically different time scales associated with the reactions among the different extracellular and intracellular molecules in a developmental system. For such stiff systems, typical temporal explicit schemes require very small time-step sizes and typical implicit temporal schemes require solving large nonlinear systems, regardless of choices of spatial discretization. As a result, simulations for long time dynamics of morphogen system are computationally prohibitive using standard numerical approach.

A class of semi-implicit temporal schemes based on integration factor approach is particularly suitable for solving this type of stiff reaction-diffusion equations (Nie et al. 2006; Nie et al. 2008). In this implicit integration factor (IIF) method, the diffusion terms are treated exactly while the reactions are treated implicitly leading to excellent stability conditions without many extra computational costs. As a result, large time-step sizes can be used in IIF method even for very stiff systems. To use this method, one first discretizes the spatial variables in the Laplacian operator to reduce the PDE system to a system of ODEs:

$$u_t = \mathcal{C}u + \mathcal{F}(u) \tag{3.2}$$

where $\mathcal{C}u$ is a finite difference approximation of $D\Delta\mathbf{u}$. Let N denote the number of spatial grid points for the approximation of the Laplacian $\Delta\mathbf{u}$, then $u(t) \in R^{N\cdot m}$ and $\mathcal{C}$ is a $(N \cdot m) \times (N \cdot m)$ matrix representing a spatial discretization of the diffusion.

IIF method, in principle, can be constructed for any order of accuracy (see (Nie et al. 2006; Nie et al. 2008) for a list of methods in different order). The second order IIF method takes a simple form:

$$u_{n+1} = e^{\mathcal{C}\Delta t}\left(u_n + \frac{\Delta t}{2}\mathcal{F}(u_n)\right) + \frac{\Delta t}{2}\mathcal{F}(u_{n+1}). \tag{3.3}$$

This method is unconditionally stable, that is, no stability constraint is imposed on the time-step size for the stability reason. This method can also be utilized with spatially adaptive mesh refinement (Liu and Nie 2010). The second order one as shown in (3.3) has been applied to several developmental systems and the simulations have shown excellent efficiency and accuracy (Nie et al. 2006; Nie et al. 2008; Liu and Nie 2010).

4 Discussions

One of major questions in developmental system is how morphogen gradient and developmental patterning achieve robustness and precision (Lander et al. 2009a). Mathematical modeling and computational analysis can be used for investigating a large, diverse, and growing number of robustness strategies among which some of them are difficult for experimental tests. One example is

a study (Lander et al. 2009a) of self-enhanced morphogen clearance strategy whose usefulness is found to come less from its ability to increase robustness to morphogen source fluctuations than from its ability to overcome noise leading to robust establishment of threshold positions.

Another interesting question is dynamics of morphogen gradients (Kanodia et al. 2009). A mathematical model of the gradient in dorsoventral patterning constrained its parameters by experimental data suggests that the patterning gradient is dynamic and, to a first approximation, can be described as a concentration profile with increasing amplitude and constant shape (Kanodia et al. 2009).

Modeling may also be used to identify specific role of particular feedback regulation in cellular responses of a morphogen system. Through exploring the capability of models with nine different mechanisms for signal transduction with feedback along with eight combinations of geometry and gene expression pre-patterns to reproduce proper BMP signaling output in wild-type and mutant embryos (Umulis et al. 2010), the modeling study shows one particular positive feedback coupled with experimentally observed embryo geometry provides best agreement with experiments, leading to insights into mechanisms that guide developmental patterning (Umulis et al. 2010).

As more modeling and computation are successfully employed for better understanding of developmental patterning in recent years, several key elements and complexity in morphogen system have yet been well addressed in modeling morphogen patterning. Growth (Baker and Maini 2007), which is usually intimately linked with morphogens that pattern the tissue, requires more sophisticated modeling and computational techniques. Noises, which present in both spatial and temporal form existing in extracellular environment and during intracellular interactions, demand new machineries in stochastic differential equations. And cell-cell communications, such as Notch-Delta signaling, necessitate efficient multi-scale and hybrid modeling techniques that couple discrete cells, continuum of morphogens, and intracellular signal transductions. All of theses provide great opportunity for development of new modeling techniques, mathematical tools and concepts, and computational methods.

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