8.1 Reaction-Diffusion Models of Pattern Formation in Developmental Biology

**Abstract.** In this paper we present mathematical approaches to understand a symmetry break and formation of spatially heterogeneous structures during development. We focus on the models given by reaction-diffusion equations and approach the question of possible mechanisms of development of spatially heterogeneous structures. We discuss two mechanisms of pattern formation: diffusion-driven instability (Turing instability) and a hysteresis-driven mechanism, and demonstrate their possibilities and constraints in explaining different aspects of structure formation in cell systems. Depending on the type of nonlinearities, we show the existence of Turing patterns, the maxima of which may be of the spike or plateau type, and the existence of transition layer stationary solutions. These concepts are discussed on example of morphogenesis of the fresh water polyp *Hydra*, which is a model organism in developmental biology.

**Keywords.** Hysteresis, Pattern Formation, Reaction-diffusion Equation, Receptor-based Model, Turing Instability

**2010 Mathematics Subject Classification.** 35K57, 35Q92, 92C15

8.1.1 Introduction

Spatial and spatio-temporal structures occur widely in physics, chemistry and biology. In many cases, they seem to be generated spontaneously. Understanding the principles of development and design in biology is among the crucial issues not only in developmental biology but also in the field of regenerative medicine. In order to develop methods for intelligent engineering of functional tissues, the main principles of development and design have to be understood.

Recent advances made in genetic and molecular biology have led to detailed descriptions of a number of events in embryological development. Although genes control pattern formation, genetics alone is insufficient to understand which physio-chemical interactions of embryonic material produce the complex spatio-temporal signaling

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cues which ultimately determine the cell’s fate. Since the establishment of symmetry breaking by cell polarity in developing tissues is determined by quantitative integration of multiple signals in a highly dynamical and self-organized process, it can be hardly understood using conventional molecular biology methods alone. The role of mathematical modeling is to verify which processes are sufficient to produce the patterning. Model mechanisms can suggest to the embryologist possible scenarios as to how, and sometimes when, a pattern is laid down and how the embryonic form might be created. Modeling also allows to make experimentally testable predictions and may provide alternative explanations for the observed phenomena.

In other areas of biology, such as neurophysiology or ecology, mathematical modeling has led to many discoveries and insights through a process of synthesis and integration of experimental data, see [37] and references therein. Also in developmental biology, mathematical models in developmental biology many different morphologies have been the subject of mathematical modeling. Some of the biological systems have attained the status of a paradigm in theoretical work [3, 7].

One such example, which shows how the study of model mechanisms can suggest real scenarios for the process of pattern formation, is limb development [37]. A mechanochemical model describes the diffusion, haptotaxis and advection of mesenchymal cells which evolve in a developing limb bud and which eventually become cartilage. The other developmental process for studying pattern formation and different aspects of embryogenesis is the segmentation of the insect embryo [3, 12]. The models based on chemotaxis and the response of cells to gradients in the chemotactant were applied to study the life cycle of the slime mould Dictyostelium discoideum and emergence of concentrated patterns of cell density [11, 13, 42]. More recently, models of morphogenesis have been applied to understand the growth of tumors. They involve a wide range of biological phenomena such as cell-adhesion and cell traction, angiogenesis, pattern formation in cancer and macrophage dynamics [5, 37].

All these models, although based on different biological hypotheses, have many common mathematical features and are mostly based on a few views of pattern generation. One is the chemical prepattern approach involving hypothetical chemicals (morphogens) which diffuse and react in such a way that spatial heterogeneous patterns can evolve from the uniform steady states. Coupling diffusion process of signaling molecules with nonlinear dynamics of intracellular processes and cellular growth and transformation leads to receptor-based models, which differ from the usual reaction-diffusion systems. Next, the mechanochemical approach takes into account mechanical forces and properties of cells and tissues. Another class of models rely on taxis, e.g., chemotaxis or haptotaxis, and the response of cells to gradients in the concentration of signaling molecules in the environment [1, 30].

Different models are able to produce similar patterns. The question is how to distinguish between them so as to determine which may be the relevant mechanisms. Of course the first necessary condition is that the model must produce observed patterns. But then it is important to design new experiments, which could allow for model
validation and therefore would also lead to the verification of model hypotheses and biological theories.

Whereas different developmental processes are involved in different organisms, it is striking how conserved the processes are across different taxonomic units such as the phyla. Also, the same processes are involved in various diseases, in particular in cancer. Molecules found to be oncogenic factors also play an important role in developmental processes. This unity and conservation of basic processes implies that their mathematical models can have an impact across the spectrum of normal and pathological development.

In this paper we present different approaches to model development and regeneration of the fresh-water polyp *Hydra*. We focus on the framework of continuous models given by partial differential equations. In particular, we employ reaction-diffusion equations and discuss their performance in the context of key experiments. One of the objectives is to understand how the structure of nonlinear feedbacks determines qualitative behavior of the system, in particular existence of stable spatially heterogenous patterns. We discuss different mechanisms of pattern formation, i.e., diffusion-driven instability and hysteresis-driven pattern formation. The first class of models uses special features of diffusion, which results in the destabilization of the spatially homogeneous steady state and emergence of spatial heterogeneity. The second mechanism of pattern formation in such systems is based on the existence of multiple steady states and hysteresis in the intracellular dynamics. Diffusion of the signaling molecules tries to average different states and is the cause of spatio-temporal patterns.

### 8.1.2 Mechanisms of Developmental Pattern Formation

One of the crucial issues in developmental biology is to understand how coordinated systems of positional information are established during an organism’s development and how cells in the organism respond to the associated signaling cues, processes which ultimately result in the subdivided and patterned tissues of multicellular organisms [35, 36, 46]. Experiments suggest that during development cells respond to local positional cues that are dynamically regulated. The hypothesis is that cells differentiate according to positional information [46]. The question is how this information is supplied to the cells.

There exists a number of models for pattern formation and regulation based on the idea that positional information is supplied to cells by a diffusing biochemical morphogen [7, 37, 46]. It links the expression of target genes with local concentrations of morphogen molecules (ligands). Different concentrations of morphogens are able to activate transcription of distinct target genes and thus cell differentiation. However, both regulatory and signaling molecules (ligands) act by binding and activating receptor molecules which are located in the cell membrane (or, with lipophilic ligands, in the cytoplasm) [20, 36]. This observation leads to a hypothesis that the positional value of the cell may be determined by the density of bound receptors which do not diffuse [35].
8.1.3 Motivating Application: Pattern Control in *Hydra*

One of the most frequently discussed organisms in theoretical papers on biological patterns formation is the fresh-water polyp *Hydra*. What is peculiar about *Hydra*?

*Hydra*, a fresh-water polyp, is one of the oldest and simplest multi-cellular organisms equipped with typically animal cells such as sensory cells, nerve cells and muscle cells. The animal has an almost unlimited life span and regeneration capacity. Similar to plants, in *Hydra* tissue there are stem cells that are constantly dividing and regenerating the adult structures of the polyps. This unlimited growth indicates that *Hydra* does not undergo senescence and, in this sense, it is biologically immortal [4]. Morphogenetic mechanisms active in adult polyps are responsible for the regenerative ability and the establishment of a new body axis. Research on *Hydra* might reveal how to selectively reactivate the genes and proteins to regenerate human tissues. The fact that no tumor formation or other malignancies have been reported for *Hydra* so far, indicates that growth control and tissue homeostasis in normal *Hydra* polyps are very efficient.

The developmental processes governing formation of the *Hydra* body plan and its regeneration are well understood at the tissue level [35, 36]. Therefore, experiments performed on *Hydra* provide a good ground for testing the abilities and limitations of mathematical models. We may distinguish three main experiments:

- **De novo pattern formation.**
  It was shown that normal *Hydra* can regenerate from random cellular aggregates [10, 36] (see Figure 8.1). Reorganization does not result from a spatial rearrangement, but it is an effect of concerted changes in the functional state of the cells. The cells do not sort with respect to the positional origin along the body axis [39]. These experiments suggest that there exist mechanisms which define new centers of head organizing activity within an initially chaotic mass of cells.

- **Cutting experiments.**
  *Hydra* has a high capacity to regenerate any lost body part, which occurs mainly by the re-patterning of existing tissues and is an example of morphallaxis [46]. The lack of growth requirements for regeneration is shown in heavily irradiated polyps. No cell divisions occur, but the animals can still regenerate normally. Consequently, the mechanism of pattern formation in *Hydra* seems to be independent of growth. Overlapping cut levels show that the same cells can form either the gastric region, or the head, or the foot, according to their position along the body axis (see Figure 8.2). The experiment shows that after a transverse cut both parts of the animal can regenerate [35]. Moreover, the polarity is maintained even in small pieces of the body. A tissue piece containing 150–300 epithelial cells, i.e., about 1 percent of normal polyp, regenerates a complete *Hydra* [41]. However, below this size no regeneration takes place. There are also observations showing that the time required for the regeneration decreases with increasing tissue size [41].
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Figure 8.1. Time evolution of aggregates from randomly arranged *Hydra* cells. Several polyps were disintegrated into a suspension of isolated cells, which subsequently were allowed to reestablish contact. Within two weeks the aggregate reintegrated itself into intact animals [Courtesy of W. Müller].

Figure 8.2. Cutting experiment. *Hydra* regenerates after a transverse cut of cells of the gastric region (from both upper and lower half of the body column). In one experiment (left-hand side) the lower body column is removed, in the second experiment (right-hand side) the upper part is removed. The cut levels are not identical but somewhat different to show that one and the same group of cells (marked in grey) can form a foot (left-hand side), or a head (right-hand side) or a gastric segment (original state in the middle). The function of the cells depends on the position along the body column [Courtesy of W. Müller].

• **Grafting experiments.**
Grafting experiments show how disparities between the positional value of the transplant and the surrounding host tissue result in the head or foot formation leading to development of new organisms with multiple heads or feet [35,36] (see Figure 8.3).
To conclude, experiments of this kind suggest that the cells respond to local positional cues that are dynamically regulated. It leads to the hypothesis of Wolpert on positional information [46] (compare Figure 8.4). The question is how this information is supplied to the cells and which mechanisms control the formation of spatially heterogeneous structures in the positional information, and consequently patterns of cell differentiation.

In the remainder of this paper we will address this question based on the results of mathematical models employing different hypotheses. Since the exact molecular mechanism of pattern formation in *Hydra* is unknown, the proposed models are hypothetical. They attempt to answer the following questions:
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- What minimal processes are sufficient to produce *de novo* patterns?

- Which models are able to capture the results of the above experiments?

The problem was first approached by Wolpert [46], who suggested a gradient model to account for head formation, in which at the head end a morphogen $S$ is emitted. The morphogen spreads by diffusion and is distributed down the body. This diffusing chemical induces formation of the head. The proposed model corresponds to the assumption that morphogens are secreted only by a group of cells in some restricted region of a tissue and then transported in an adjacent tissue. While there is experimental evidence of such signaling in other systems, such as, for example, *Drosophila* wing imaginal disc or Spemann organizer, it is not the case in *Hydra de novo* development from the dissociated cells [36].

8.1.4 Diffusive Morphogens and Turing Patterns

The question of *de novo* pattern formation in a homogenous tissue was addressed by Turing in his pioneering paper [45]. He proposed a hypothesis that can be stated as follows: *When two chemical species with different diffusion rates react with each other, the spatially homogeneous state may become unstable, thereby leading to a nontrivial spatial structure.*

The idea looks counterintuitive, since diffusion is expected to lead to the uniform distribution of the particles. Mathematical analysis of reaction-diffusion equations provides an explanation for the phenomenon postulated by Turing. The proposed mechanism of pattern formation is related to a local behavior of solutions of a reaction-diffusion system in the neighborhood of the constant solution that is destabilized via diffusion. Patterns arise through a bifurcation, which we call diffusion-driven instability (DDI). They can be spatially monotone corresponding to the gradients in positional information or spatially periodic.

**Definition 8.1** (Turing instability). A system of reaction-diffusion equations exhibits DDI (Turing instability) if and only if there exists a constant stationary solution, which is stable to spatially homogenous perturbations, but unstable to spatially heterogenous perturbations.

The original idea was presented by Turing on the example of two linear reaction-diffusion equations of the form

$$\frac{\partial u}{\partial t} = D \Delta u + Au \quad \text{in } \Omega,$$

$$\partial_n u(t, 0) = 0 \quad \text{on } \partial \Omega,$$

$$u(0, x) = u_0(x),$$

(8.1)

where $u \in \mathbb{R}^2$ is a vector of two variables, $D$ is a diagonal matrix with nonnegative coefficients $d_u, d_v$ on the diagonal, the symbol $\partial_n$ denotes the normal derivative...
(no-flux condition), and $\Omega$ is a bounded region. Here the only constant steady state is $(0, 0)$.

Following Turing [45], we can formulate the following result on DDI:

**Theorem 8.2** (Allan Turing). Assume that $\text{tr} A < 0$, $\det A > 0$ and $d_v > 0$. There exists $d_u > 0$ (small enough) such that the constant steady state $(0, 0)$ is unstable for the reaction-diffusion equation (8.1).

It can be proven using a spectral decomposition of the Laplace operator with homogeneous Neumann boundary conditions and calculating the eigenvalues of obtained finite dimensional operator.

Due to the local character of Turing instability, the notion has been extended in a natural way to the nonlinear equations using linearization around a constant positive steady state. However, in case of nonlinear systems we may deal with the existence of multiple constant steady states. In such cases we observe the existence of heterogenous structures far from the equilibrium and the global behavior of the solutions cannot be predicted by the properties of the linearized system, e.g., [27, 43]. In fact we can observe a variety of possible dynamics depending on the type of nonlinearities. On the other hand, Turing instability can also be exhibited in degenerated systems such as reaction-diffusion-ODE models or integro-differential equations, for example, shadow systems obtained through reduction of the reaction-diffusion model [26, 27].

Following all these observations and original character of Turing’s system we define the Turing patterns in the following way:

**Definition 8.3.** By Turing patterns we refer to the solutions of reaction-diffusion equations that are

- stable,
- stationary,
- continuous,
- spatially heterogenous and
- arise due to the Turing instability (DDI) of a constant steady state.

It can happen in a reaction-diffusion system with DDI that all nonconstant stationary solutions are unstable and then the solution converges to another constant solution or to a dynamical structure such as a spike pattern [26, 43]. In case of at least three equations, the system can also exhibit a Turing-type Hopf bifurcation, which leads to spatio-temporal oscillations [19].
8.1.4.1 Activator-inhibitor Model

The most famous realization of Turing’s idea in a mathematical model of biological pattern formation is the activator-inhibitor model proposed by Gierer and Meinhardt [7]. The model aims to explain head formation in *Hydra* due to a coupling of a local activation to a long-range inhibition process. An activator promotes head formation and increases itself autocatalytically. An inhibitor acts as a suppressant against the self-enhancing activator to prevent the system from unlimited growth. In this approach the positional value is interpreted as the density of the activator. Gradients of morphogens are formed by the DDI mechanism. Each of the various body parts is assumed to be under control of a separate activator-inhibitor system (for details, see [32]). The basic activator-inhibitor model takes the form

\[
\frac{\partial a}{\partial t} = D_a \frac{\partial^2 a}{\partial x^2} + \rho_a \frac{a^2}{h} + \sigma_a - \mu_a a,
\]

\[
\frac{\partial h}{\partial t} = D_h \frac{\partial^2 h}{\partial x^2} + \rho_h a^2 + \sigma_h - \mu_h h,
\]

(8.2)

where \(a\) and \(h\) denote the concentrations of the activator and the inhibitor, respectively. The parameters \(\sigma_a\) and \(\sigma_h\) describe de novo production, \(\mu_a\) and \(\mu_h\) are the rates of degradation and \(\rho_a\) and \(\rho_h\) the parameters of the activator-inhibitor interactions. The model and several of its modifications were applied in the study of various topics from developmental biology (see, e.g., [33, 34]). Due to its interesting mathematical features and emerging singularities, the model has also attracted a lot of attention from the side of mathematical analysis, e.g., [31, 43].

The activator-inhibitor theory operates with purely hypothetical morphogens. As we can see in Theorem 8.2, the key mechanism of Turing-type patterns is that an inhibitor diffuses faster than an activator. However, dynamics and complex tissue topologies are likely to prevent the establishment of long-range inhibitor gradients. Furthermore, diffusion rates of typical morphogens are often found to be quite small [9], i.e., do not allow significantly varying diffusion rates as required by the Turing mechanism. In the case of *Hydra*, while recently Wnt can be identified as an activator [10], a long-range inhibitor is missing [21, 34]. These observations support the search for a different inhibitory mechanism such as mechanical inhibition [6] or different than DDI mechanism of pattern formation [24]. In the context of *Hydra* experiments it is also important to note that the shape of Turing patterns depends on the size of the domain and diffusion rather than on initial conditions. Therefore, one of the difficulties of the Turing-type models is their inability to reproduce the experiments resulting in multiple head formation in *Hydra* [23].
8.1.5 Receptor-based Models

Another type of mathematical models for pattern formation follows the hypothesis that the positional value of the cell is determined by the density of cell-surface receptors, which regulate the expression of genes responsible for cell differentiation [35], see Figure 8.5.

![Figure 8.5. Bound receptors density determining “positional value”: the head is formed if the density of bound receptors is high (above some threshold). Consequently, in normal development we expect a gradient-like distribution of bound receptors [Courtesy of W. Müller].](image)

The receptor-based models are based on the idea that epithelial cells secrete ligands (a regulatory biochemical), which diffuse locally within the interstitial space and bind to free receptors on the cell surface [23, 29]. It results in a bound receptor that can be removed from the cell surface due to degradation or internalization, or dissociate back to free receptors and ligands. Both ligands and free receptors are produced within the whole tissue and undergo natural decay.

The first receptor-based model for *Hydra* was proposed by Sherrat, Maini, Jäger and Müller in [40] in the following form (SMJM model),

\[
\begin{align*}
\frac{\partial}{\partial t} a &= D_a \frac{\partial^2}{\partial x^2} a + s_a(x) - \mu_a a - k_e a e - k_a a f + k_d b, \\
\frac{\partial}{\partial t} f &= k_d b - k_a a f + k_i [\alpha(x) + \beta b - f], \\
\frac{\partial}{\partial t} b &= k_a a f - (k_d + k_i) b, \\
\frac{\partial}{\partial t} e &= D_e \frac{\partial^2}{\partial x^2} e + s_e(x) - \mu_e e,
\end{align*}
\]

defined on a bounded one-dimensional domain with zero-flux boundary conditions for \(a\) and \(e\). The variables \(f\), \(b\), \(a\) and \(e\) denote the density of free receptors, bound receptors, biochemical (ligands) and enzyme, respectively. In order to achieve the required
local competition phenomenon, it is assumed in the SMJM model that the terms describing de novo production of free receptors, ligands and enzyme depend on the position of the tissue along the body axes $x \in [0, L]$ and also on $y(x)$, which denotes position from which the tissue at location $x$ originates. The functions describing the production of new receptors and production of enzyme are linearly decreasing in $x$. The production of ligands is assumed to be constant on $4/5$ of the domain and then decrease linearly to zero. It is assumed that

\[
\alpha(x) = \alpha_1[1 - y(x)/L] + \alpha_2 y(x)/L,
\]
\[
s_a(x) = s_1[1 - y(x)/L] + s_2 y(x)/L,
\]

where $s_a(x)$ is constant for $y(x) \in [0, \frac{4L}{5}]$ and decreases linearly to zero for $y(x) \in [\frac{4L}{5}, 1]$. The combination of these two parallel gradients enables the model to capture some results of grafting and cutting experiments. Thus, the model functions not because of nonlinear interactions between receptors and ligands, but because of the assumption that cells produce new molecules depending on the position they had in the donor organism. In conclusion, the SMJM model is not a model for de novo pattern formation.

Later, receptor-based models without imposing initial gradients were proposed by Marciniak-Czochra [23]. In general, equations of such models can be represented by the following initial boundary-value problem,

\[
\begin{align*}
\frac{\partial u}{\partial t} &= D \Delta v + f(u, v) \quad \text{in } \Omega, \\
\frac{\partial v}{\partial t} &= g(u, v) \quad \text{in } \Omega, \\
\frac{\partial u}{\partial n} &= 0 \quad \text{on } \partial \Omega, \\
u(x, 0) &= u_0(x), \quad \quad \quad \quad \quad \quad \quad v(x, 0) = v_0(x),
\end{align*}
\]

where $u$ is a vector of variables describing the dynamics of diffusing extracellular molecules and enzymes, which provide cell-to-cell communication, while $v$ is a vector of variables localized on cells, describing cell surface receptors and intracellular signaling molecules, transcription factors, mRNA, etc. $D$ is a diagonal matrix with positive coefficients on the diagonal, the symbol $\partial_n$ denotes the normal derivative (no-flux condition), and $\Omega$ is a bounded region.

A rigorous derivation, using methods of asymptotic analysis (homogenization) of the macroscopic reaction-diffusion models describing the interplay between the non-homogeneous cellular dynamics and the signaling molecules diffusing in the intercellular space has been undertaken in [25, 29]. It is shown that receptor-ligand binding processes can be modeled by reaction-diffusion equations coupled with ordinary differential equations in the case when all membrane processes are homogeneous within the membrane.

As shown in [23] and also more recently highlighted in [16], receptor-based models may exhibit Turing-type instability. The simplest receptor-based model takes into account only one type of diffusive signaling molecules. The basic model of this type,
for one-dimensional epithelial sheet, takes the form

\[
\begin{align*}
\frac{\partial}{\partial t} r_f &= -\mu_f r_f + p_r (r_f, r_b) - b r_f l + d r_b, \\
\frac{\partial}{\partial t} r_b &= -\mu_b r_b + b r_f l - d r_b, \\
\frac{\partial}{\partial t} l &= \frac{1}{\gamma} \frac{\partial^2}{\partial x^2} l - \mu_l l - b r_f l + p_l (l, r_b) + d r_b,
\end{align*}
\]

(8.5)

with zero flux boundary conditions for \( l, \partial_x l(t, 0) = \partial_x l(t, 1) = 0 \).

The model takes into account dynamics of free and bound receptors on cell membranes, denoted by \( r_f(t, x) \) and \( r_b(t, x) \), respectively, and diffusing signaling molecules denoted by \( l(t, x) \). The original model operated with purely hypothetical molecules. However, in case of Hydra pattern formation we may associate the variables to Frizzled receptors and Wnt ligands [17]. The hypothesis that Wnt is a diffusing ligand is supported by experimental evidence [10, 21].

The effects of intracellular dynamics are modeled via nonlinear functions describing production of new signaling molecules and free receptors, \( p_l \) and \( p_r \), respectively. Besides, the kinetics describe binding at the rate \( b \), dissociation at the rate \( d \) and natural decay at the rates \( \mu_f, \mu_b \) and \( \mu_l \). \( \gamma = \frac{L^2}{d_l} \) is a scaling coefficient depending on the domain length \( L \) and the diffusion coefficient \( d_l \).

As it was stated in [28, Proposition 3.1], a generic system of two ordinary differential equations coupled with a reaction-diffusion equation exhibits DDI if there exists a positive, spatially constant steady state, for which the following conditions are satisfied

\[
\begin{align*}
-\text{tr} (A) &> 0, \\
-\text{tr} (A) \sum_{i < j} \det (A_{ij}) + \det (A) &> 0, \\
-\det (A) &> 0, \\
-\det (A_{12}) &> 0,
\end{align*}
\]

(8.6)–(8.9)

where \( A = (a_{ij})_{i, j=1,2,3} \) is the Jacobian matrix of the system without diffusion linearized around this constant positive equilibrium and \( A_{ij} \) is a submatrix of \( A \) consisting of the \( i \) th and \( j \) th column and \( i \) th and \( j \) th row.

Conditions (8.6)–(8.8) are necessary for the stability of the steady state in the absence of diffusion. Inequality (8.9) is a sufficient and necessary condition for destabilization of this steady state. These conditions guarantee that the model (8.5) exhibits diffusion-driven instability if the function \( p_r \) evaluated at the steady state satisfies \( p_r (\tilde{r}_f, \tilde{r}_b) < \tilde{r}_f \frac{\partial}{\partial r_f} p_r (\tilde{r}_f, \tilde{r}_b) \), which, in particular, holds for \( p_r (r_f) = m_1 r_f^{\alpha+1} \) for \( \alpha > 0 \). This inequality can be interpreted as an autocatalysis at the steady state in the first equation of the system. This condition leads to the instability of those constant solutions, for which an autocatalysis occurs.
Figure 8.6. Spatial profile of $r_b$ for different initial perturbations and a fixed $\gamma$. On the left-hand side we present the initial condition and on the right-hand side the final pattern originating from such an initial condition. The perturbation in initial data is of order $10^{-2}$ (it can be arbitrarily small) while the final peak is of height $8 \times 10^4$. (a) Different initial conditions and corresponding solutions are depicted using matching line styles. The location of the peak strongly depends on the initial condition. (b) The results for the initial conditions with two maxima or two minima—the result is always one peak (depicted using matching line styles).

Following the classical Turing idea, one expects stable patterns to appear around the constant steady state in the system with DDI property. Interestingly, in numerical simulations of model (8.5), diffusion-driven instability of the constant steady state leads to the emergence of growth patterns concentrated around discrete points along the spatial coordinate, which take the mathematical form of spike-type spatially inhomogeneous solutions [23]. The structures are not robust and depend strongly on initial conditions. In some cases, blow up occurs. Definitely, the observed solutions are not Turing patterns, see Figure 8.6.

Recent analytical studies of the reaction-diffusion-ODE models with only one nonzero diffusion coefficient revealed that monotone or periodic stationary solutions can be constructed for most interesting models [8, 26, 27]. However, the same mechanism that destabilizes constant solutions of these models also destabilizes non-constant solutions [26]. Consequently, there exist no stable continuous stationary solutions for the initial boundary-value problems for ordinary-PDE systems as the one in (8.5). While in some other applications such dynamical spike patterns are of biological relevance [27, 28, 38], they cannot be applied to describe pattern formation in Hydra.

Considering two diffusing signaling factors leads to a four-variable receptor-based model exhibiting Turing patterns [23]. In this model it is additionally assumed that
there exists a second diffusing substance, functioning as an enzyme as in the SMJM model [40], which is secreted by cells, diffuses along the body column and degrades the ligands. The equations have the following form

\[
\frac{\partial}{\partial t} r_f = -\mu_f(r_f) + p_r(r_f, r_b) - b(r_f, l) + d(r_b),
\]

\[
\frac{\partial}{\partial t} r_b = -\mu_b(r_b) + b(r_f, l) - d(r_b),
\]

\[
\frac{\partial}{\partial t} l = d_l \frac{\partial^2}{\partial x^2} l - \mu_l(l) - b(r_f, l) + p_l(r_f, r_b) + d(r_b) - b_e(l, e),
\]

\[
\frac{\partial}{\partial t} e = d_e \frac{\partial^2}{\partial x^2} e - \mu_e(e) + p_e(l, r_b),
\]

\begin{equation}
\text{(8.10)}
\end{equation}

with zero flux boundary conditions for \(l\) and \(e\). Here \(e\) denotes the density of enzyme, \(b_e\) the rate of binding of ligands and enzyme, \(p_e\) the rate of production of enzyme, \(\mu_e\) the rate of decay of the enzyme, \(d_e\) is the diffusion coefficient for enzyme, and the other terms are as in the three-variable model.

The role of the enzyme is to remove the biological regulator, i.e., ligand, before it binds to the receptors on the cell surface. It is important to stress that this model cannot be simplified to an activator-inhibitor system of the type (8.2). The four-variable receptor-based model consists of two subsystems. The reaction-diffusion subsystem describing ligand and enzyme dynamics is not of the activator-inhibitor type and cannot produce diffusion-driven patterns itself. It is the ODEs subsystem that causes destabilization of a constant solution and emergence of a Turing pattern.

In such a model, patterns can evolve due to the DDI, even if no self-enhancement of free receptors nor ligands is assumed. \(p_r\) is assumed to be a function of \(r_b\), since it is known from a number of other biological contexts that there can exist a positive feedback loop between the density of bound receptors on the cell surface and the subsequent expression of new receptors [15, 44]. Also no assumption on the range of enzyme diffusion is needed.

These observations show that including receptor dynamics in the model of interacting diffusing signaling molecules allows to relax the assumptions on the range of diffusion and type of nonlinear interactions necessary for a formation of stable spatially heterogeneous patterns. In particular, it seems that although the Wnt antagonist Dickkopf found in Hydra tissue [2] does not satisfy the assumptions of the inhibitor from the Gierer–Meinhardt model [34], its interactions with Wnt signaling may lead to a stable gradient-like pattern formation as in the receptor-based model.

In the four-variable receptor-based model, similarly to the activator-inhibitor model, the spatially homogeneous steady state bifurcates into the spatially inhomogeneous solution which has a maximum at the one end and a minimum at the other end for some range of the domain size. This model is robust and the final pattern does not depend on small perturbations of initial conditions. Pattern formation phenomenon is similar to
that in the activator-inhibitor model of Gierer and Meinhardt (8.2) with the difference that maxima of the pattern have the shape of plateaux and not spikes as was the case in (8.2). It is related to uniform boundedness of solutions in model (8.10). Models with Turing patterns can describe self-organization of *Hydra* cells (see Figure 8.8, left-hand panel) and are able to simulate the cutting experiments (see Figure 8.7). Concluding, introduction of a second diffusing biochemical species improved performance of the model. The four-variable receptor-based model can explain at least as much as the

**Figure 8.7.** Numerical simulation of the cutting experiment in the four-variable receptor-based model. Left-hand panel: Initial data (dashed line) corresponding to a surgical removal of the lower part (half) of the body column. We observe that a reorganization of the “gradient” on a smaller domain corresponds to the formation of a new “foot”. Right-hand panel: Initial data (dashed line) corresponding to a surgical removal of the upper part (half) of the body column. We observe that a reorganization of the “gradient” on a smaller domain corresponds to the formation of a new “head”.

**Figure 8.8.** Left-hand panel: *De novo* gradient-like pattern formation in four-variable receptor-based model. Right-hand panel: Simulation of a transplantation experiment for the initial data corresponding to the head grafting. The final distribution shows the transplant disappearance.
activator-inhibitor model regarding \textit{de novo} pattern formation and basic experiments. Numerical studies of both models based on Turing mechanism showed that the grafting experiments could not be explained within such an approach without changing the size of domain (or diffusion coefficient), which does not reflect experimental conditions (see Figure 8.8, right-hand panel).

\section*{8.1.6 Multistability}

Transplantation experiments suggest that there are a number of locally asymptotically stable patterns depending on the past history. The patterns may have multiple peaks, which depend on the local cues induced by the grafted tissue. Such experiments suggest a mechanism of pattern formation based on multistability in intracellular signaling. Coupling diffusion with a kinetics system with multiple stable steady states and hysteresis may lead to the coexistence of different patterns for the same parameters but depending on the initial conditions. Such a hypothesis was incorporated in the receptor-based model proposed in [24] by replacing the function $p_l$, describing the rate of production of diffusing signaling molecules, by a new variable modeled using an additional ordinary differential equations. The model includes a hysteresis-based relation in the quasi-stationary state in the ODEs subsystem, i.e., $g(u, v) = 0$ in the system of equations (8.4) (see Figure 8.9).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure8.9.png}
\caption{A typical configuration of the kinetic functions in a receptor-based model (8.4) with hysteresis in the quasi-stationary ODEs subsystem.}
\end{figure}

The model suggests how the nonlinearities of intracellular signaling may result in spatial patterning. It allows for formation of gradient-like patterns corresponding to the normal development as well as emergence of patterns with multiple maxima describing transplantation experiments (see Figure 8.10). Numerical simulations show the existence of stationary patterns resulting from the existence of multiple steady states and switches in the production rates of diffusing molecules, see Figure 8.10, left-hand panel. The patterns observed in such models are not Turing patterns. In fact, the system does not need to exhibit DDI. Indeed, in most cases its constant steady states do not
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Figure 8.10. Simulations of the receptor-based model with hysteresis. Formation of a gradient-like pattern corresponding to a normal development and head formation in *Hydra* (left-hand panel) and formation of two heads pattern for the initial conditions corresponding the transplantation experiment (right-hand panel).

change stability. In such models, spatially heterogenous stationary solutions appear far from equilibrium due to the existence of multiple quasi-steady states.

Properties of a hysteresis-based mechanism of pattern formation have been recently studied in a minimal version of the model consisting of one reaction-diffusion equation coupled to one ODE [17]. In such a model, infinitely many stationary solutions can be constructed. Such solutions are discontinuous in the nondiffusing variables. The shape of spatial structures can be very irregular, since it depends strongly on the initial conditions. Therefore, the model can simulate the effects of transplantation experiments and formation of multiple heads, see Figure 8.10, right-hand panel.

On the other hand, it was shown that the system with multistability but reversible quasi-steady states in the ODE subsystem, i.e., $g(u, v)$ globally invertible, cannot exhibit stable spatially heterogeneous patterns. Hysteresis is necessary to obtain stable patterns.

8.1.7 Discussion

Transplantation and tissue manipulation experiments provided data for models of patterning in *Hydra*, starting with the positional information ideas of Wolpert [46], the activator-inhibitor model of Gierer and Meinhardt [7, 32–34] and, finally, receptor-based models of Marciniak-Czochra [17, 23, 24]. Each model has shed light on different but overlapping aspects of self-organization and regeneration. Now, it is possible to state which conceptual elements have to be present in a complete model, although modeling of *Hydra* regeneration still involves quite a few unsolved problems. In the framework of reaction-diffusion systems there are essentially two ways in which a system of identical cells can start to differentiate:
There is a critical number of cells (size of domain), above which the spatially homogeneous attractor loses stability, which leads to “spontaneous” spatial patterning. It is the case for the models with the Turing instability. Such models can explain de novo pattern formation since for some set of parameters and the domain size value, the final pattern is the same and does not depend on the initial perturbation.

There is an external inducing signal which drives the system into a new, spatially inhomogeneous state. Such a signal originates from another group of already differentiated cells and it must be strong enough to trigger differentiation. It corresponds to a sufficiently strong initial perturbation of the homogeneous steady state. This type of initialization of the pattern-forming mechanism is involved in the model with hysteresis.

The experiments showing de novo formation of Hydra from the dissociated cells could suggest a Turing-type mechanism. On the other hand, transplantation experiments suggest coexistence of different spatially inhomogeneous stationary patterns which grow up for different initial conditions. Experiments show that large perturbations (but within the range of the values of the solution itself) of the gradient-like solution should lead to another solution. The observations may be explained by models with multiple steady states exhibiting hysteresis. In such models solutions depend on the initial condition similarly to the Hydra resulting from the grafting experiment depends on the graft position and not on the size of the animal.

The question is whether these two kinds of experiments can be explained using the same mechanism and whether it could be the combination of the already considered mechanisms. To clarify these issues new models including a more detailed description of cell-to-cell and intracellular signaling should be developed. Mathematical understanding of the relation between the structure of nonlinearities and the dynamics of model solutions shall be helpful both in building new models and also in designing experiments that might help to verify different hypotheses.

Many uncertainties exist regarding the biological foundations of the models. Further biological discoveries are needed to gain insight into the molecular nature of cell communication and of the positional value. To understand the morphogenesis in Hydra it is necessary to bridge the gap between experimental observations at the cellular level and those at the genetic and biochemical levels.

The advent of new techniques in molecular biology has recently made it possible to advance the understanding of the development of multicellular organisms. Large scale expression screening helps to identify new factors involved in embryonic development. Recently, expression analysis during regeneration and budding indicated a pivotal role of the Wnt (wingless gene) pathway in the Hydra head organizer [10]. Also the evidence of Dkk (Dickkopf) signaling in Hydra regeneration was provided [2]. Experimentally observed patterns of Wnt and Dkk gene expression give rise to many new questions. New aspects of Wnt-Dkk signaling, such as bi-stability in Wnt dynamics and switches in the Dkk functionality depending on the cellular context, were also
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recently found in other model organisms [14, 18, 22]. Mathematical modeling should integrate these concepts and observations into a new model of pattern formation controlled by the intracellular dynamics of Wnt-Dkk signaling.

In conclusion, growth and pattern formation provide a great source of interesting and novel mathematical problems, while mathematics can be used as a tool to explore different mechanisms and processes underlying these phenomena. The use of realistic models may help to understand many complex processes.

Bibliography


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