Reaction-Diffusion Model as a Framework for Understanding Biological Pattern Formation

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The Turing, or reaction-diffusion (RD), model is one of the best-known theoretical models used to explain self-regulated pattern formation in the developing animal embryo. Although its real-world relevance was long debated, a number of compelling examples have gradually alleviated much of the skepticism surrounding the model. The RD model can generate a wide variety of spatial patterns, and mathematical studies have revealed the kinds of interactions required for each, giving this model the potential for application as an experimental working hypothesis in a wide variety of morphological phenomena. In this review, we describe the essence of this theory for experimental biologists unfamiliar with the model, using examples from experimental studies in which the RD model is effectively incorporated.

Over the past three decades, studies at the molecular level have revealed that a wide range of physiological phenomena are regulated by complex networks of cellular or molecular interactions (1). The complexity of such networks gives rise to new problems, however, as the behavior of such systems often defies immediate or intuitive understanding. Mathematical approaches can help facilitate the understanding of complex systems, and to date, these approaches have taken two primary forms. The first involves analyzing every element of a network quantitatively and simulating all interactions by computation (1). This strategy is effective in relatively simple systems, such as the metabolic pathway in a single cell, and is extensively explored in the field of systems biology. However, for more complex systems in which spatiotemporal parameters take on importance, it becomes almost impossible to make a meaningful prediction. A second strategy, one that includes simple mathematical modeling in which the details of the system are omitted, can be more effective in extracting the nature of the complex system (2). The reaction-diffusion (RD) model (3) proposed by Alan Turing is a masterpiece of this sort of mathematical modeling, one that can explain how spatial patterns develop autonomously.

In the RD model, Turing used a simple system of "two diffusible substances interacting with each other" to represent patterning mechanisms in the embryo and found that such systems can generate spatial patterns autonomously. The most revolutionary feature of the RD model is its introduction of a "reaction" that produces the ligands (morphogens). If "diffusion" alone is at work, local sources of morphogens are needed to form the gradient. In such cases, the positional information made by the system is dependent on the prepattern (Fig. 1, A and B). By introducing the reaction, the system gains the ability to generate various patterns independent of the prepattern (Fig. 1C). Unfortunately, Turing died soon after publishing this seminal paper, but simula-

tion studies of the model have shown that this system can replicate most biological spatial patterns (4–6). Later, a number of mathematical models (4) were proposed, but most followed Turing's basic idea that "the mutual interaction of elements results in spontaneous pattern formation." The RD model is now recognized as a standard among mathematical theories that deal with biological pattern formation.

However, this model has yet to gain wide acceptance among experimental biologists. One reason is the gap between the mathematical simplicity of the model and the complexity of the real world. The hypothetical molecules in the original RD model have been so idealized for the purposes of mathematical analysis that it seems nearly impossible to adapt the model directly to the complexity of real biological systems. However, this is a misunderstanding to which experimental researchers tend to succumb. The logic of pattern formation can be understood with simple models, and by adapting this logic to complex biological phenomena, it becomes easier to extract the essence of the underlying mechanisms. Genomic data and new analytic technologies have shifted the target of developmental research from the identification of molecules to understanding the behavior of complex networks, making the RD model even more important as a tool for theoretical analysis.
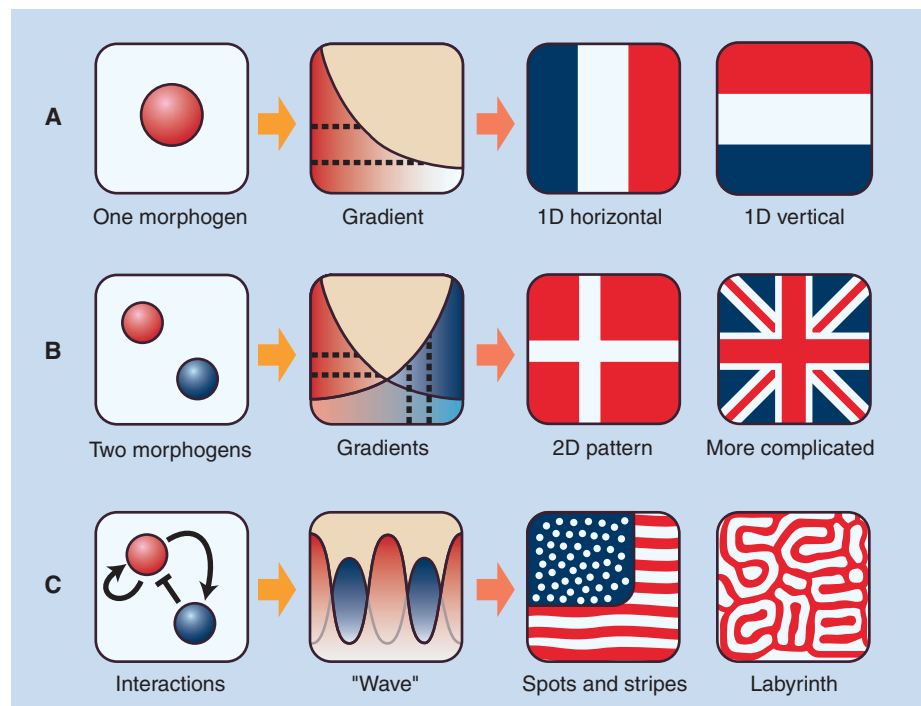
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Fig. 1. Schematic drawing showing the difference between the morphogen gradient model and Turing model. (**A**) A morphogen molecule produced at one end of an embryo forms a gradient by diffusion. Cells "know" their position from the concentration of the molecule. The gradient is totally dependent on the prepattern of the morphogen source (boundary condition). (**B**) Adding a second morphogen produces a relatively complex pattern; but with no interactions between the morphogens, the system is not self-regulating. (**C**) With addition of the interactions between the morphogens, the system becomes self-regulating and can form a variety of patterns independent of the prepattern. [Art work by S. Miyazawa]

In this review, we describe the RD model and its experimental applications, addressing some of the points biologists tend to question. We hope many biologists gain an appreciation for this beautiful theory and take advantage of it in their experimental work.

The Original Turing Model

At the beginning of his original paper (*3*), Turing stated that his aim was to show that the combination of known physical elements is sufficient to explain biological pattern formation. The elements selected by Turing were a theoretical pair of interacting molecules diffused in a continuous field. In his mathematical analysis, Turing revealed that such a system yields six potential steady states, depending on the dynamics of reaction term and wavelength of the pattern [Fig. 2A and Supporting Online Material (SOM) Text 1-1]. In case I, the system converges to a stable and uniform state. In case II, a uniform-phase oscillation of the morphogen concentration occurs. Such phase unification is seen in such systems as circadian rhythms (*7*) and the contraction of heart muscle cells (*8*). In case III, the system forms salt-and-pepper patterns, such as are made when differentiated cells inhibit the differentiation of neighboring cells. This is seen, for example, with differentiated neuroprogenitor cells in the epithelium of *Drosophila* embryos (*9*). Case IV represents an unusual state in which a pattern of the sort made in case III oscillates. No examples of this have been identified in a developmental system. In case V, a traveling wave is generated. Biological traveling waves caused by this mechanism include the spiral patterns formed by the social amoeba *Dictyostelium discoideum* on aggregation (*10*), and the wave of calcium ions that traverses the egg of the frog *Xenopus laevis* on sperm entry (*11*).

In case VI, stationary patterns are made. The finding of this type of wave is the major achievement of Turing's analyses, and these are usually referred to as Turing patterns. A Turing pattern is a kind of nonlinear wave that is maintained by the dynamic equilibrium of the system. Its wavelength is determined by interactions between molecules and their rates of diffusion. Such patterns arise autonomously, independent of any preexisting positional information. [The supporting materials contain an expanded explanation of the RD model for biologists who are unfamiliar with the mathematical description (SOM Text S1-1), as well as a mathematical explanation of how the periodic pattern arises in the system (SOM Text S1-2).] This property makes it possible to explain how patterns arise precisely in, for example, fertilized oocytes, which present little in the way of positional information. The ability of Turing patterns to regenerate autonomously, even after experimentally induced disturbances, is also important and of great utility in explaining the autonomy shown by pattern-forming developmental processes (*4*, *6*). In addition, through the tuning of parameters and boundary conditions, the sys-

tem underlying Turing pattern formation can generate a nearly limitless variety of spatial patterns. (Figure 2B shows representative two-dimensional patterns made by simulations with the RD model.) The intricate involutions of seashells (*5*), the exquisite patterning of feathers (*12*), and the breathtakingly diverse variety of vertebrate skin patterns (*13*) have all been modeled within the framework of the Turing model (Fig. 2C). The remarkable similarity between prediction and reality in these simulations points strongly to Turing mechanisms as the underlying principle in these and other modes of biological patterning. (User-friendly simulation software is available as supporting online material; a manual of the software is in SOM Text 1-3.)

Compatibility of RD and Gradient Models

Experimental biologists may recall the "gradient model," which has been quite effective in explaining pattern-formation events (*14*). In the classic gradient model, the fixed source of the morphogen at a specific position provides positional information (*14*) (Fig. 1). Although the assumption of a morphogen source appears to be different from the assumptions of the RD model, it can be introduced into the RD model quite naturally as a "boundary condition." In other words, the classic morphogen gradient model can be thought of as the specific case of the RD model in which the reaction term is removed. In many simulation studies, such boundary conditions are used to make the pattern more realistic (*6*). Recent experimental studies of morphogen gradients have shown that the precision and the robustness of the gradient are secured by the interactions of molecular elements (*15*). To model such situations, the authors used a mathematical framework that is essentially identical to that of the RD model. Both reaction and boundary conditions are essential to understanding complex real systems, and the RD model is useful for modeling such cases.

Applying the "Simple" RD Model to a Complex Reality

The hypothetical molecules in the original RD model (*3*) are idealized for the purposes of mathematical analysis. It is assumed that they control their own synthesis and that of their counterparts, and diffuse quickly across spaces that would be divided by cellular membranes. Obviously, it is quite difficult to apply such a model directly to complex living systems.

Concerted efforts to align theoretical models to real-world systems, however, have begun to bear fruit, pointing to a much broader range of situations in which the general principles underlying the Turing model might apply. Gierer and Meinhardt (*16*, *17*) showed that a system needs only to include a network that combines "a short-range positive feedback with a long-range negative feedback" to generate a Turing pattern. This is now accepted as the basic requirement for Turing pattern formation (*14*, *16*). This refinement leaves the types and numbers of reacting factors

unspecified, making it much simpler to envision systems that might fit the requirements.

The interacting elements need not be limited to molecules, or even to discrete entities; a circuit of cellular signals will do just as well (*18*). There is also no need for the stimulus to be provided via diffusion; other modes of transmission can achieve the same end result. Theoretical modeling has shown that a relayed series of direct cell-to-cell signals can form a wave having properties similar to one formed by diffusible factors (*19*). Other forms of signaling, including chemotactic cell migration (*20*), mechanochemical activity (*21*), and neuronal interactions [as in the Swindale model (*22*) of ocular dominance stripe formation], are also capable of forming Turing-like patterns. For all of these systems, a similar periodic pattern is formed when the condition of "short-range positive feedback with long-range negative feedback" is satisfied.

Why systems represented by apparently different equations behave similarly, and how much the capacity for pattern forming differs among them, are the important subjects from the mathematics perspective. But if the dynamics of the systems are nearly the same, experimental researchers can select any of the models as their working hypothesis. In the case of fish skin patterning, although experiments apparently suggest the involvement of a nondiffusing signal transduction mechanism, the simplest RD model can predict the movement of the pattern during fish growth (*23*) and the unusual patterns seen in hybrid fish (*24*).

Finding Turing Patterns in Real Systems

During embryogenesis, a great variety of periodic structures develop from various nonperiodic cell or tissue sources, suggesting that waves of the sort generated by Turing or related mechanisms may underlie a wide range of developmental processes. Using modern genetic and molecular techniques, it is possible to identify putative elements of interactive networks that fulfill the criteria of short-range positive feedback and long-range negative feedback, but finding the network alone is not enough. Skeptics rightly point out that just because there is water, it doesn't mean there are waves. No matter how vividly or faithfully a mathematical simulation might replicate an actual biological pattern, this alone does not constitute proof that the simulated state reflects the reality. This has been another major hurdle in identifying compelling examples of Turing patterns in living systems. The solution, however, is not so complicated; to show that a wave exists, we need to identify the dynamic properties of the pattern that is predicted by the computer simulation. Experimental demonstrations have focused on pattern formation in the skin, because the specific characteristics of Turing patterns are more evident in two dimensions than in one.

Turing Patterns in Vertebrate Skin

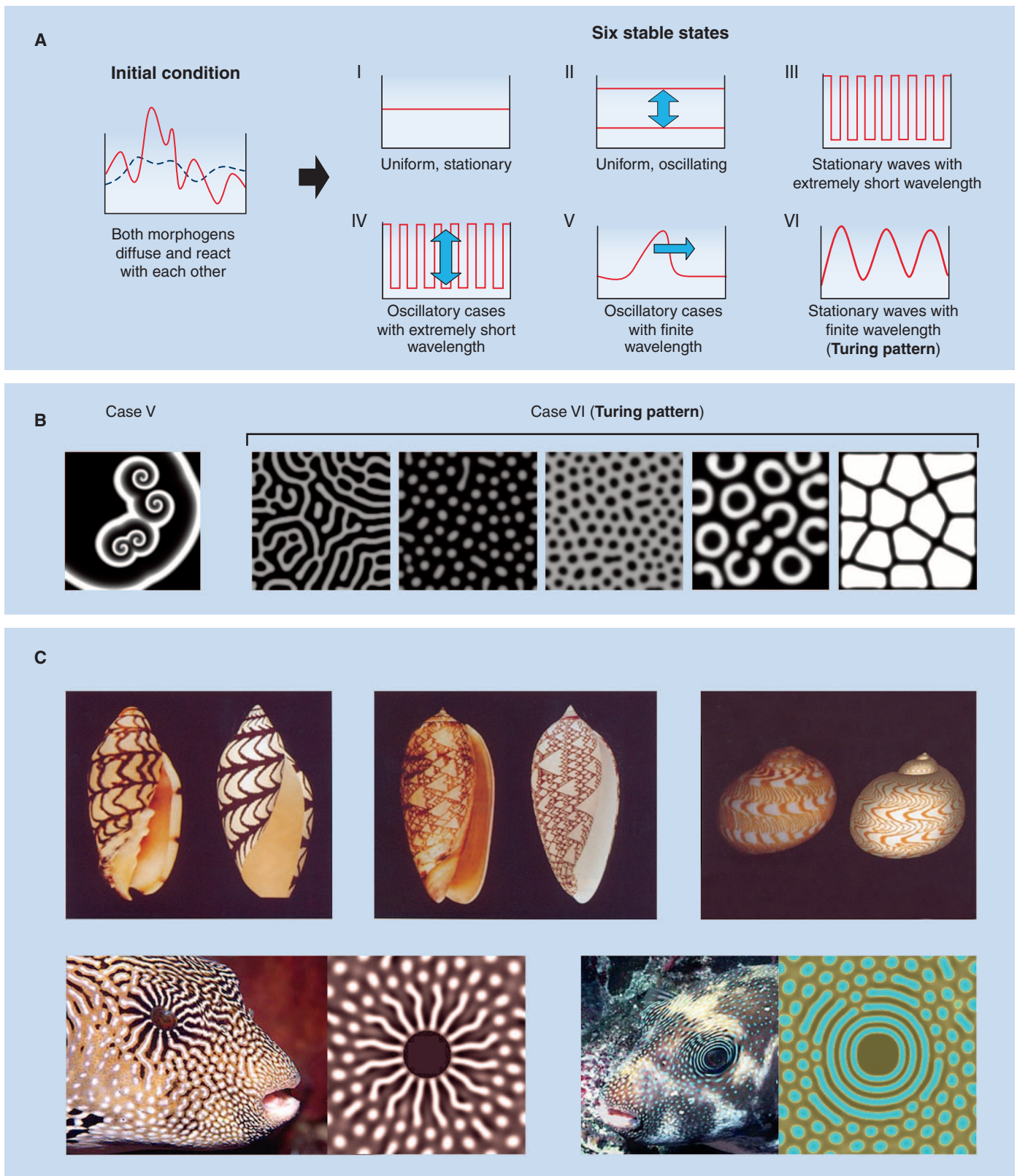Observation of the dynamic properties of Turing patterns in nature was made by Kondo and Asai

Fig. 2. Schematic drawing showing the mathematical analysis of the RD system and the patterns generated by the simulation. (**A**) Six stable states toward which the two-factor RD system can converge. (**B**) Two-dimensional patterns generated by the Turing model. These patterns were made by an identical equation with slightly different parameter values. These simulations were calculated by the software provided as supporting online material. (**C**) Reproduction of biological patterns created by modified RD mechanisms. With modification, the RD mechanism can generate more complex patterns such as those seen in the real organism. Simulation images are courtesy of H. Meinhardt [sea shell pattern (5)] and A. R. Sandersen [fish pattern (13)]. Photos of actual seashells are from Bishougai-HP (http://shell.kwansei.ac.jp/~shell/). Images of popper fish are courtesy of Massimo Boyer (www.edge-of-reef.com).

in a study of horizontal stripes in the tropical fish, *Pomacanthus imperator* (*25*). They have recently shown that this dynamic nature is shared by many fish species, including the well-established model organism, zebrafish. Although zebrafish stripes may appear to be stationary, experimental perturbation of the pattern triggers slow changes (*23*). Following laser ablation of pigment cells in a pair of black horizontal stripes, the lower line shifts upward before stabilizing in a Bell-like curve (Fig. 3A) (*23*). As a result, the spatial interval between the lines is maintained, even when their direction changes. This striking behavior is predicted by simulation (Fig. 3B).

Fortunately, the zebrafish is amenable to a variety of experimental approaches that may lead to the identification of the circuit of interactions that generates these patterns (*26*). Work to date has shown that the skin patterns of this fish are set up and maintained by interactions between pigmented cells (*26*). Nakamasu worked out the interaction network among the pigment cells. Although the shape of the network is different from that of the original Turing model, it fits the short-range positive, long-range negative feedback description (*18*). (The mutual inhibition between black and yellow cells behaves as a positive feedback loop, as the expansion of black cells weakens their counterpart.) Identification of the genetic factors involved in the zebrafish pigmentation is under way (*26*), and it is hoped that this will clarify the details of the signaling network beneath the waves in the skin of fish, and potentially all vertebrates. Many similar surface patterns are seen in invertebrates and plants. We suppose that in these cases an essentially similar mechanism (RD mechanism) is involved, although their molecular basis may be different.

Other well-studied examples include the regular disposition of feather buds in chick (*27*), and of hair follicles in mice (*28*). Jung *et al*. showed that the spatially periodic pattern of feather buds regenerates even when the skin is recombined from dissociated cells (*27*). In the case of mouse hair follicles, alteration of the amount of putative key factors changes the pattern in a manner predicted by computer simulation. Sick *et al*. used overexpression and inhibition of Wnt and Dkk in fetal mouse to study how such perturbations might affect the patterning of follicle formation

(*28*). By simulation, they first predicted that a ringed pattern of Wnt gene expression that does not occur in nature would result if ectopic production of a Wnt protein were to be controlled appropriately, and then confirmed this experimentally. They suggested that Wnt serves as a short-range activator, and Dkk as a long-range inhibitor, in this system (*28*). This pair of factors

functions in various patterning processes as well, making this a critically important result for the intimations it provides of a wider role for Turing patterns in development (Fig. 4). Interestingly, the growth of new hair relying on interactions between neighboring follicles proceeds even in adult mice, and in one case a mutant was identified in which a traveling wave of hair formation gradually moved across the skin over the life of animals carrying this mutation (*29*). Plikus *et al*. (*29*) have shown how the factors FGF (fibroblast growth factor) and BMP (bone morphogenetic protein) function to generate the traveling wave (case V in the Turing model).

Other Potential Turing-Driven Developmental Phenomena

Establishment of right-left asymmetry in vertebrates is triggered by the unidirectional rotation of cilia at the node, followed by the interaction of Nodal and Lefty that amplify and stabilize faint differences in gene expression (*30*, *31*). Nodal enhances both its own expression and that of Lefty, and Lefty inhibits the activity of Nodal. The fact that Lefty spreads further than Nodal suggests that the inhibitory interaction propagates more quickly than does its activating counterpart, which as we have seen, indicates that this system fulfills the fundamental requirements for Turing pattern formation (*30*) (Fig. 4).

In vertebrate limb development, precartilage condensation, which is later replaced by skeletal bone, occurs periodically along the anterior-posterior axis of the distal tip region. Because this patterning occurs without any periodic prepattern, the Turing model has long been suggested to describe the underlying mechanism (*32*). In this system, transforming growth factor–β (TGF-β) has been invoked as a candidate for the activator molecule (*33*). TGF-β can stimulate its own production and trigger precartilage condensation, and the sites of incipient condensation exert a laterally acting inhibitory effect on chondrogenesis. Although no candidate inhibitor has been identified, an interaction network comprising TGF-β function and precartilage condensation may satisfy the short-range activation and long-range inhibition criteria (*33*). Miura and Shiota have shown that nearly periodic spatial patterns of chondrogenesis occur in the culture of dissociated limb cells in vitro,
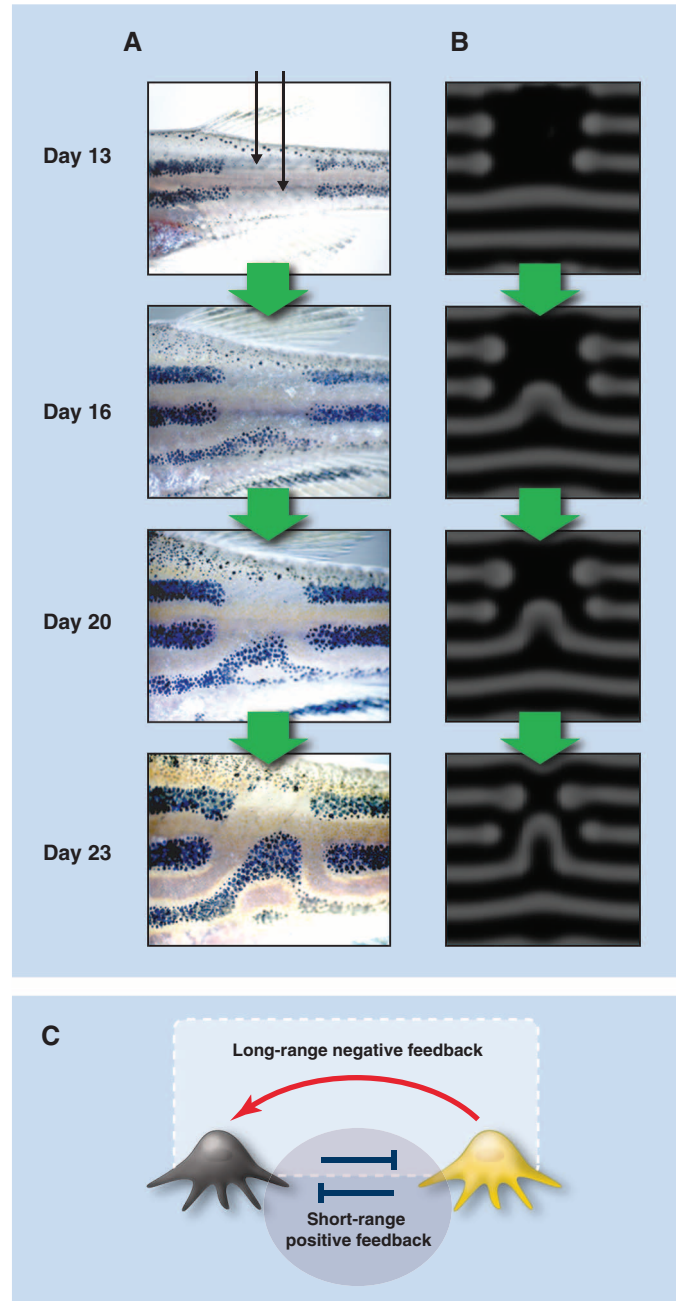

Fig. 3. Movement of zebrafish stripes and the interaction network among the pigment cells. The pigment pattern of zebrafish is composed of black pigment cells (melanophores) and yellow pigment cells (xanthophores). The pattern is made by the mutual interaction between these cells. (**A**) Melanophores in the two black stripes were ablated by laser, and the process of recovery was recorded. (**B**) Results of simulation by the Turing model. (**C**) Interaction network between the pigment cells deduced by experiments. The red arrow represents a long-range positive (enhancing) effect, whereas the blue line with the end bar represents a short-range negative (inhibitory) effect. A circuit of two negative interactions functions as a positive feedback.
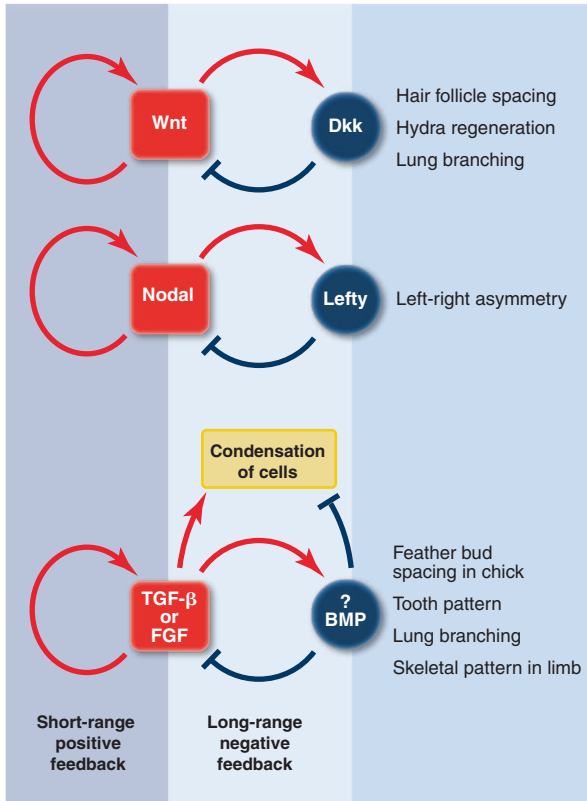
Fig. 4. Possible networks of protein ligands may give rise to Turing patterns in the embryo. Shown above are candidates for the RD mechanism proposed by molecular experiments. For a detailed explanation of each network, refer to the text and the articles listed in the references. In all these cases, the network is identical to that of the activator-inhibitor model proposed by Gierer and Meinhardt (*17*). Condensation of cells by migration into a local region causes sparse distribution of cells in a neighboring region. This can also function as long-range inhibition. (Note that the involvement of the RD mechanism in some of the phenomena above has not been fully accepted by experimental researchers.)

and that the addition of TGF-β changes the pattern in a manner consistent with the predictions of Turing's model (*34*).

Wnt and Dkk are also essential for lung branching in vertebrates (*35*) and play a key role in head regeneration in *Hydra* (*36*), in which involvement of the Turing mechanism has long been suggested by theoretical and experimental studies. Involvement of the same molecules does not directly prove that the same dynamic mechanism is at work. However, because they constitute the self-regulated system that is robust to artificial perturbations, it is reasonable to expect that the Turing mechanism may underlie these phenomena as it does those in the skin. Watanabe *et al.* have shown that signal transduction via the gap junction (connexin41.8) plays a key role in pigment pattern formation in zebrafish (*37*). Loss of function

of the connexin gene (*leopard*) reduces the spatial periodicity and changes the pattern from stripes to spots (*37*). A mutation in another gap junction gene, *connexin43*, shortens each fin ray, resulting in shortened fins (*38*). Although the detailed molecular mechanism underlying this phenomenon has yet to be elucidated, it is possible that the same mechanism functions in other processes of animal development. The above examples are by no means an exhaustive list of the candidates currently being examined as potential biological Turing patterns. More detailed discussions are provided in (*4*) and (*6*).

Identification of the specific dynamics of the RD system is critical to showing the applicability of the Turing mechanism to the formation of a given pattern. In the case of pigment pattern formation, it was possible to disturb the pattern and observe the process of regeneration. In most other systems, such observation is complicated because experimental perturbations may be lethal. This is one reason why it is difficult to demonstrate RD activity in some biological systems. (In SOM Text 1-4, we have summarized the important points for researchers to keep in mind when they use the RD model as the working hypothesis.) However, recent technological advances in imaging technologies are assisting such studies. Moreover, the artificial generation of Turing patterns in cell culture should be possible in the near future as the result of synthetic biology (*39*). Turing was born in 1912 and published his RD model in 1952. Although he was unable to witness the impact of his hypothesis on the work of contemporary biologists, we are hopeful that with an increased acceptance among experimental biologists of the principles he first elucidated, we will see Turing's mechanism take its place as a model for the understanding of spatial pattern formation in living systems.

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40. We thank D. Sipp for suggestions and help in preparation of the manuscript. We also thank H. Meinhardt, A. R. Sandersen, and P. Przemyslaw for figures reproduced from their original works.