Review

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Is pigment patterning in fish skin determined by the Turing mechanism?

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More than half a century ago, Alan Turing postulated that pigment patterns may arise from a mechanism that could be mathematically modeled based on the diffusion of two substances that interact with each other. Over the past 15 years, the molecular and genetic tools to verify this prediction have become available. Here, we review experimental studies aimed at identifying the mechanism underlying pigment pattern formation in zebrafish. Extensive molecular genetic studies in this model organism have revealed the interactions between the pigment cells that are responsible for the patterns. The mechanism discovered is substantially different from that predicted by the mathematical model, but it retains the property of 'local activation and long-range inhibition', a necessary condition for Turing pattern formation. Although some of the molecular details of pattern formation remain to be elucidated, current evidence confirms that the underlying mechanism is mathematically equivalent to the Turing mechanism.

How do skin patterns form?

The beauty and variety of animal pigmentation patterns have an attraction not only for the public, but also for developmental biologists interested in understanding the formation of such patterns [1,2]. Two characteristics have piqued the curiosity of developmental biologists about the underlying mechanism of pattern formation. The first is the variation in patterns among closely related species. For example, one can see by going to an aquarium that pigment patterns of fish vary extensively among species, even those belonging to the same genus. Yet, the genomes of all of these species are similar, particularly within a genus, so it is unlikely that a different mechanism underlies each different pattern. This suggests that a single underlying mechanism can produce all of the various types of skin pattern.

The second characteristic is that, in many cases, there are no internal body structures corresponding to the pigment pattern. This means that there are few landmarks in the animal body for the pigment cells to follow, and that development of patterns of pigmentation in the skin appears to occur without any influence from internal anatomy. In other words, pigment pattern formation requires a self-organizing process that develops spatial pattern autonomously, without any prepattern. Known mechanisms of embryology fail to explain these two characteristics of animal skin patterning, but an old mathematical model may provide an explanation.

In 1952, the English mathematician Alan Turing presented a unique theoretical model that suggests a mechanism for spatial pattern formation in organisms [3]. Now known as the Turing model or the reaction-diffusion model, it has become the most accepted theoretical model for the formation of animal pigmentation patterns [3]. The original Turing model is a simple system comprising two diffusible substances that interact with each other while diffusing at different rates. Turing demonstrated, by mathematical analysis, that this simple mechanism is autonomously able to generate a type of stable wave [3]. Later mathematical studies, using computer simulations, proved that this hypothetical mechanism could autonomously generate a variety of 2D patterns that are similar to the pigmentation patterns in animal skin [4–6]. Meinhardt and Gierer found that, for patterns to emerge, the network of interactions of the Turing substances need to involve both local activation through autocatalysis and long-range inhibition, which are now accepted as necessary conditions for the autonomous formation of pigment patterns [7]. A detailed explanation of the mechanism involved in the Turing model, which is outside the scope of this work, is available elsewhere [8–10].

A significant number of biologists were intrigued by the consistent ability of this mathematical model to produce the periodic patterns often found in organisms. However, when the segmentation-related stripes of *Drosophila* that were originally assumed to be the product of the Turing mechanism were found to be produced by the positional information mechanism instead, most biologists became more skeptical of the Turing model.

Then, in 1995, Kondo and Asai showed that the stripes of the emperor angelfish split continuously during its growth, and that this dynamic change in the pattern of the stripes was precisely predicted by the Turing model [11]. Similar rearrangements in pigmentation patterns were also observed in other species, including zebrafish (Figure 1) [12–14]. Discovery of these dynamic properties of pigment patterning reinvigorated interest in the mathematical model, and gave it credence as one of the most feasible approaches to understanding the processes involved in this patterning.

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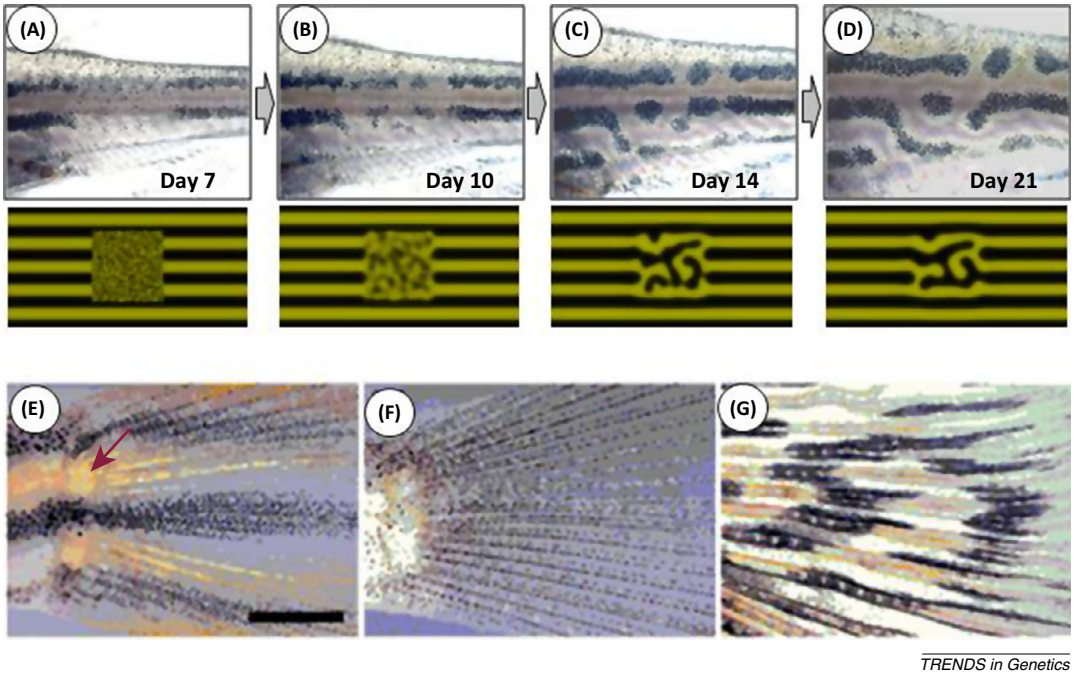

Figure 1. Regeneration of labyrinthine pattern in adult zebrafish suggests involvement of a self-organizing mechanism. **(A–D)** Regeneration process of the pigmentation pattern induced by laser ablation. Laser ablation killed all three types of pigment cell in a square area of an adult zebrafish. At day 7 (A), melanophores and xanthophores developed randomly. (B–D) The stripes then developed, but the directionality was lost. Note that the spacing between the regenerated stripes is almost the same as the original spacing. **(E)** Normal fin stripes developed in temperature-sensitive *csf1ra* mutant (fms^{174A}) fish when cultured at the permissive temperature. **(F)** When the activity of *csf1ra* was lost, no pattern developed in caudal fins. **(G)** When the activity of *csf1ra* was recovered in adult fish, *de novo* generation of stripes occurred. Directionality of the stripes was also lost in fins. Reproduced from [14] (A–D) and [13] (E–G).

Nevertheless, although the similarities between the mathematically produced patterns and the patterns of pigmentation found in nature are impressive, they are not definitive evidence. The original Turing model is too abstract to be adapted in a straightforward manner to real-life systems, and some of the properties of the diffusing substances required by the model cannot be expected to be found in living organisms. For example, in the model, the rates of reaction and diffusion need to be stable [8]. Analysis of computer simulations revealed that a change of less than 10% in parameter values can cause a drastic change in the resulting pattern [5]. However, in a real biological system, the values of such parameters are more likely to fluctuate, which would make the resulting patterns irregular.

Therefore, experimental studies have been carried out to identify the driving force behind the patterning of the skin [11,15]. A full understanding of the detailed mechanism that gives rise to the variety of animal patterns is expected to yield a new principle in the field of developmental biology, and to provide valuable experimental feedback to the field of mathematical biology. It is noteworthy that reaction-diffusion is not the only possible mechanism for autonomous pattern formation. For example, the effect of mechanical stress on biological pattern formation has recently been suggested to have a role in the arrangement of cell arrays [16] and in the cracked appearance in the skin of reptiles [17]. However, the Turing mechanism is the most generally accepted and widely applicable model [18,19], and further elucidation of its precise nature would provide insights that might guide the investigation of other possible mechanisms. In this review, we summarize numerous experimental results and show how the abstract theoretical idea of a Turing-like mechanism has gradually been confirmed over the past 15 years.

Zebrafish as a model organism to study pigment patterning

Among the many animal species that have skin patterns, the zebrafish is the only model organism for which a variety of techniques for genetic manipulation [20,21] and a library of mutant lines [22–24] are both available. As a result of these obvious advantages, studies involving zebrafish have been the leading source for information about patterning in skin pigmentation [25]. These studies have also produced several mutants, and have uncovered the involvement of numerous genes in the regulation of pattern formation [26–51].

Zebrafish have distinct stripes, both on their bodies and on their fins (Figure 2, wild type). Stripes in the fin comprise melanophores, which produce a black pigment, and xanthophores, which produce a yellow pigment, localizing to adjacent, nonoverlapping regions [25,52]. Zebrafish also have cells called iridophores; in fins, the distribution of these cells does not correspond with the pattern of the stripes [46]. However, in the body trunk, two specific types of iridophore, both of which are silver (or blue) in color, colocalize with melanophores and xanthophores [53]. Flat, L-type iridophores are localized beneath melanophores in the stripe region, and columnar, S-type iridophores are localized as clusters beneath xanthophores in the interstripe region [53]. The configurations of the various pigment cells in the body and in the fins of zebrafish have been analyzed in detail by electron microscopy [53,54].

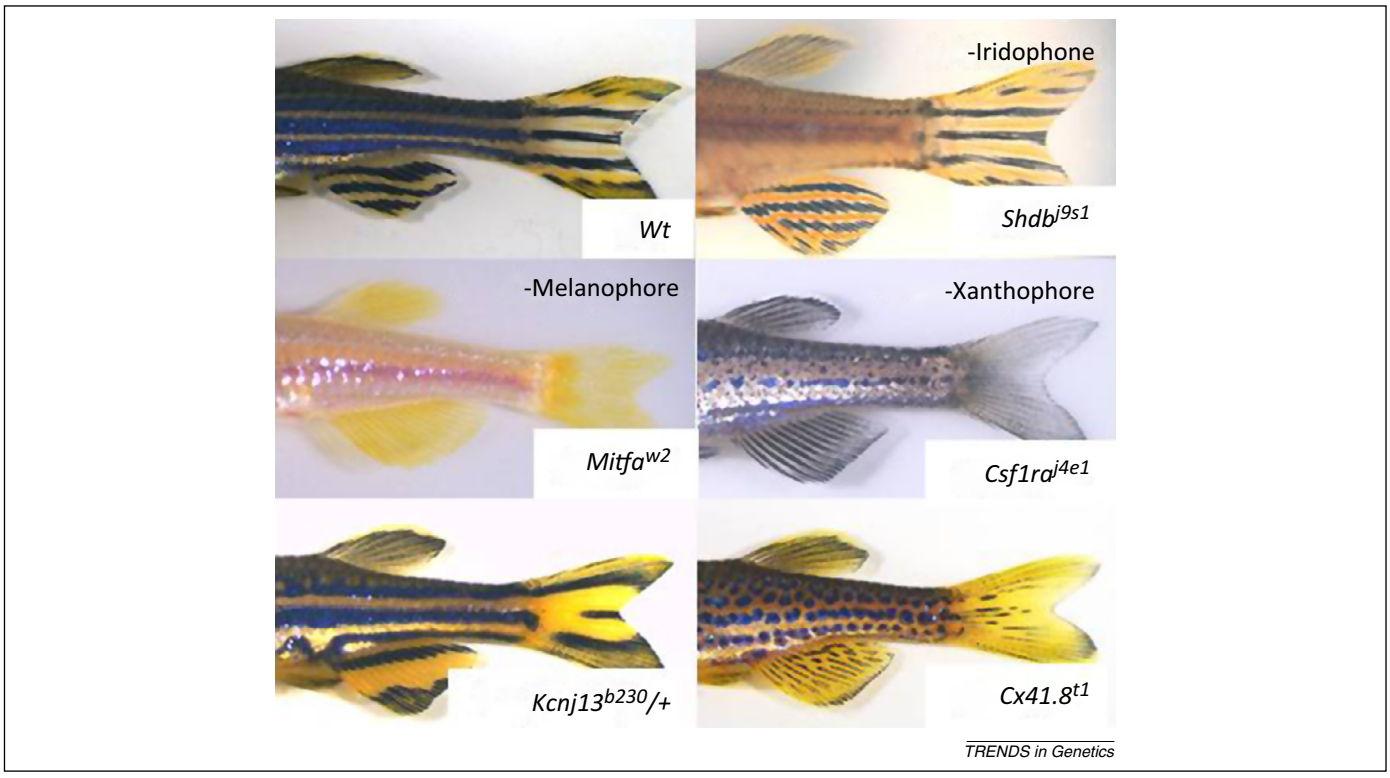

Figure 2. Pigmentation patterns in zebrafish mutants. Pigmentation patterns in body and fins. Wild type (WT) zebrafish exhibit normal stripe patterns in both the body and fins. In mutants lacking melanophores (*mitfa*^{w2}) or xanthophores (*csf1ra*^{j4e1}), patterns are lost in both the body and fins. In mutants lacking iridophores (*shd*^{j9s1}), normal stripes form in fins. Mutants, such as*kcnj13*^{b230} and *cx41.8*^{t1}, form distinct but altered patterns. Patterns in the body and fins are similar, suggesting essentially identical mechanisms underlie each. Picture of *shd* is modified from [46]. Original mutant name for each gene is as follows, *shd=shady, mitfa=nacre, csf1ra=panther, kcnj13=jaguar/obelix, cx41.8=leopard*.

Involvement of pigment cells in pattern formation

When one of the pigment cell types that constitute the pattern of stripes in zebrafish is lost due to a mutation, the entire pattern is lost, suggesting that the pattern is induced by the mutual interaction between the pigment cells. In zebrafish mutants lacking melanophores (Figure 2, *nac*) [42] or xanthophores (Figure 2, *pfe*) [43], no distinct patterns form in the body or in the fins. In zebrafish lacking iridophores, the pattern of stripes in the body is lost, but the stripes in the fins are unaffected [45,46,55,56] (Figure 2, *shd*). Therefore, in the fins, melanophores and xanthophores are sufficient to form stripes, but in the body trunk, iridophores are also required.

This is the point at which the opinions of researchers diverge. Some researchers assume that the patterning mechanism is different in the body trunk from that in the fins. However, the stripes in the fins are continuous with those in the body, and their widths are almost identical. Moreover, in some mutants [e.g., *cx41.8*(*leopard*) and *kcnj13(jaguar/obelix)*], the pattern changes in the same way in the body and in the fins (Figure 2). These observations suggest that the same mechanism underlies the patterning in both body parts. A reasonable explanation would be that the core mechanism of this patterning involves melanophores and xanthophores, and that it needs the assistance of iridophores to function in the body trunk, but not in the fins.

Interactions between pigment cells

To illuminate the patterning mechanism, the exact nature of the actual interactions between the pigment cells first needs to be elucidated. Given that the interactions between melanophores and xanthophores can drive pattern formation, at least in the fins, we focus first on these cells. The contribution of iridophores to body patterning is discussed thereafter.

To understand the role of the interactions between melanophores and xanthophores in stripe formation, Maderspacher and Nuesslein-Volhard produced mosaic fish by transplanting these cells from wild type zebrafish, *leopard* mutants, which tend to have spots instead of stripes, or *jaguar/obelix* mutants, in which melanophores and xanthophores fail to segregate properly [57]. By studying the pigmentation patterns in these fish and in fish derived from related experiments, they found that activity of the *jaguar/obelix/kcnj13* gene is required in melanophores for these cells to aggregate and to segregate from xanthophores [57]. They also found that the *leopard/cx41.8* gene is required in both cell types for the proper interactions to occur between melanophores and xanthophores [57]. They concluded that both genes are required for correct pattern formation to occur via local interaction between adjacent pigment cells [57].

In another study, Parichy and Turner used a zebrafish line carrying a temperature-sensitive allele of *csf1ra* to ablate xanthophores conditionally in adult fish; they showed that, once xanthophores are lost, melanophores gradually die both in the body trunk and in the fins [13]. These experiments demonstrated that melanophores require continuous signaling from xanthophores to survive in adult stripes [13]. Interestingly, when xanthophores were allowed to recover, melanophores also returned,

and the pattern of stripes was regenerated [13]. Similarly, in a complementary experiment, Parichy and Turner transplanted wild type (*csf1ra*$^{+}$) cells into *csf1ra*$^{-}$ host fish, and confirmed that melanophore stripes developed around the donor-derived cells in host fish [13]. These findings provide unambiguous evidence that the *de novo* generation of stripes (in a labyrinth pattern) by melanophores and xanthophores is possible even in adult fish.

Nakamasu *et al.* used a laser to ablate pigment cells in various targeted regions to observe the effects of the loss of neighboring cells on the survival and development of melanophores and xanthophores [15]. This experimental design made it possible to determine the effect of the distance between interacting cells on the nature of each interaction in the overall interaction network [15]. The researchers found that neighboring melanophores and xanthophores compete for survival and development, such that xanthophores actively eliminate adjacent melanophores; at the same time, xanthophores exhibit a long-range enhancing effect on melanophore survival and development [15]. Therefore, the interaction between xanthophores and melanophores is reversed depending on the space between the cells.

Given that the version of laser ablation used in these experiments specifically kills those cells that have colored pigments, iridophores are effectively resistant to the treatment. However, it was recently noticed that some (approximately 20%) of the iridophores located beneath the killed xanthophores also die. Therefore, some loss of iridophores also occurs in this laser experiment. However, the complementary experiment by Parichy and Turner involving transplantation of wild type cells into *csf1ra*$^{-}$ host fish [13], and at least one *in vitro* experiment [58], indicate that the effects of xanthophores on melanophores is sufficient to explain the observed phenomena.

Identification of feedback loops consistent with the Turing model

The inferred interaction network between melanophores and xanthophores is shown in Figure 3. There are two feedback loops in this network. One involves mutual inhibitory interactions that function locally. Given that this feedback loop contains two negative interactions, it produces a similar result to that of a positive feedback loop. The second feedback loop is one in which melanophores exhibit a local, negative effect on xanthophores, while the latter induce a long-range, positive effect on the former; this feedback loop has the overall effect of a long-range negative feedback loop. The combination of these two feedback loops produces an outcome that is consistent with the necessary conditions for pattern formation in the Turing model.

One of the amazing properties of the Turing mechanism is that, within the system, a variety of patterns can be formed by changing or tuning only a single parameter (Figure 4). Watanabe and Kondo tested whether this property of the theoretical system has a role in pigment patterning in zebrafish [59]. The *leopard* (*cx41.8*) gene, which encodes the gap junction protein Connexin 41.8 [41], mediates interactions between melanophores and xanthophores [57]. Watanabe and Kondo expressed

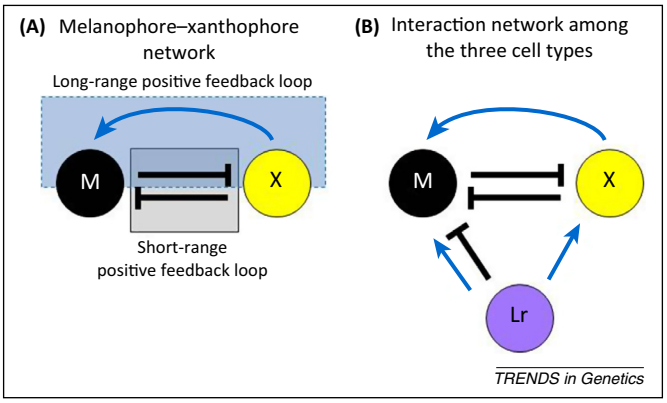

Figure 3. Putative interaction network of pigment pattern formation deduced from various experiments. **(A)** Interaction network in the fins. Two feedback loops are identified. The distance of the interactions is determined by the length of the cell projections. This network satisfies the necessary conditions of Turing pattern formation. **(B)** Interaction network in the body. In addition to the network of melanophores and xanthophores, iridophores are required to generate the pattern. How the iridophores influence the dynamics of the whole network is still not clear. The arrows in the model are based on the assumption that these cells behave in the same way in both the fins and the body trunk. Given that laser ablation often induces severe necrosis of the fins, results from such experiments involving the fins are not available. Parichy and Turner demonstrated the existence of a long-range interaction in fins [13], but the short-range effect is only based on an *in vitro* experiment [60], and needs to be confirmed as being present in zebrafish fins *in vivo*. Abbreviations: Ir, iridophore; M, melanophore; X, xanthophore.

various alleles of this gene, each of which exhibited a different level of activity, in zebrafish, to 'tune' the melanophore–xanthophore interaction, and successfully generated a variety of patterns (including the spotted pattern expected of the *leopard* allele, rings, the labyrinth pattern, and others) in the transgenic zebrafish [59]. These results further suggested that a Turing-like mechanism is responsible for pigment pattern formation. This 'tuning' of *cx41.8* activity also produced various stripe patterns, with altered numbers, widths, or positions of the stripes, demonstrating that these parameters are determined by the interaction between melanophores and xanthophores.

Mechanisms of cell–cell interactions

Recent attempts to identify the molecular and cellular factors involved in the cell–cell interactions that are responsible for pattern formation have revealed some unexpected cellular events and roles in the interaction network. For example, Inaba *et al.* isolated pigment cells from fins and plated them in a culture dish to study the *in vitro* behavior of mixed melanophores and xanthophores [60]. They found that xanthophores extend dendrites toward melanophores, and that contact with a xanthophore dendrite induces the melanophores to migrate away from the xanthophores (Figure 5) [60]. Using a fluorescent dye to detect depolarization of the melanophore membrane, they showed that this is a specific response to the contact between these two cell types [60]. Yamanaka and Kondo extended this *in vitro* assay, and observed that xanthophores actively follow the melanophore [61]. This 'chase and run' behavior does not occur to the same extent with *leopard* and *jaguar* mutant pigment cells [61], suggesting that this *in vitro* behavior is relevant for pigment pattern formation.

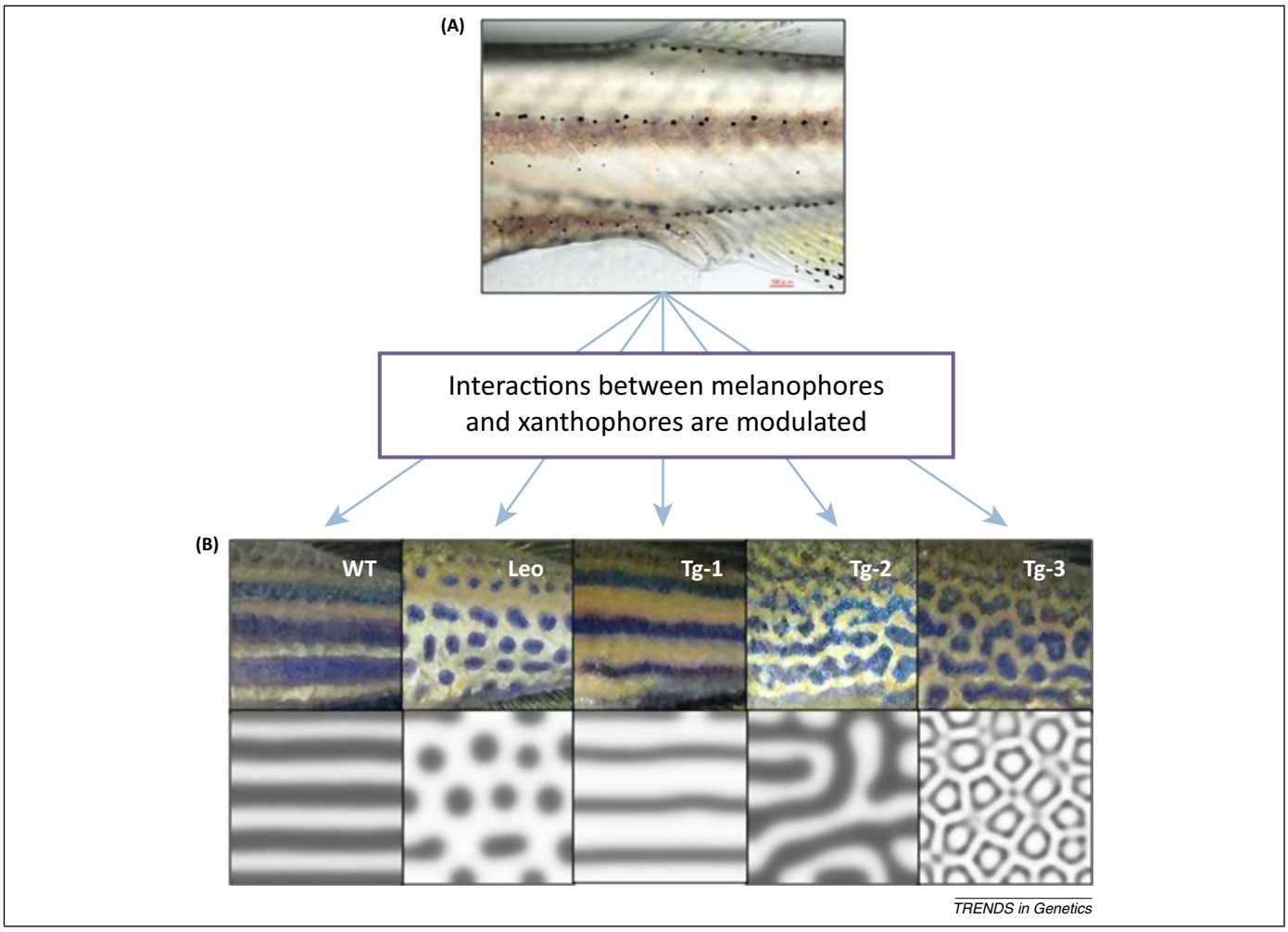

Figure 4. Tuning of melanophore–xanthophore interactions generates a series of patterns without changing iridophore activity. **(A)** Mid-larval zebrafish from all *leopard* variant mutants display an almost identical mid-larval pattern. **(B)** However, depending on the extent of melanophore–xanthophore interaction (via *cx41.8*), they formed different adult patterns. In most cases, the mid-larval pattern disappears. Abbreviations: Tg-1, Tg(*mitfa*$^{-}$*cx41.8*)*cx41.8*$^{t1/t1}$; Tg-2, Tg(*mitfa*$^{-}$*cx41.8M7*)*cx41.8*$^{+/+}$; Tg-3, Tg(*mitfa*$^{-}$*MKLcx41.8*)*cx41.8*$^{tq270/+}$. Modified from [59].

The implication of this *in vitro* behavior is that contact-induced migration is the driving force for the segregation of melanophores and xanthophores (Figure 5). In the environment of real skin, where cells cannot move as freely as in the culture dish, the direct contact of the cells might cause other behaviors. In mid-larval (approximately 30-day-old) fish, melanophores actively migrate out of the xanthophore territory, just as they do in the culture dish, whereas the 'chasing' by xanthophores appears to be less active [62]. The death of cells located in the territory of other cell types also significantly contributes to the completion of segregation [62]. Given that cell death is induced by those cells that are in close proximity, it might also be induced via direct contact by xanthophore dendrites (Figure 6) [15,62]. Regardless, it is evident that the local, mutual interaction between melanophores and xanthophores occurs via xanthophore dendrites directly touching melanophore membranes [58], not by the transmission of a diffusible molecule.

Just as the above findings shed light on the local interactions between pigment cells, other studies have attempted to uncover aspects of long-range interactions that determine stripe width. Hamada *et al.* [58] investigated the identity of the putative long-range signaling molecules (Figure 7). By conducting gene-chip analyses, they found that *deltaC* and *notch1a* are expressed exclusively in xanthophores and melanophores, respectively [58]. When the relevant Notch signaling pathway is inactivated with the specific inhibitor *N*-[*N*-(3,5-Difluorophenacetyl)-L-alanyl]-*S*-phenylglycine t-butyl ester (DAPT), melanophores die in the same manner as when xanthophores are ablated with lasers [15] or eliminated by genetic manipulation [13,55]. Ectopic expression of DeltaC or the Notch1a intracellular domain in melanophores generated wider black stripes [58], suggesting that Notch signals, most likely originating in distant xanthophores, promote melanophore survival, and implying that the long-range signaling between xanthophores and melanophores involves Notch1a and DeltaC. Hamada *et al.* visualized the *in vivo* contour of melanophores and found that melanophores extend long projections toward xanthophores (Figure 7) [58]; this direct contact allows a membrane-bound ligand to transmit an apparent long-range signal.

Although these reports confirmed the validity of the Turning model in biological systems in general, they overturned the specific expectation of the original Turing model that signaling at a distance must occur via diffusion

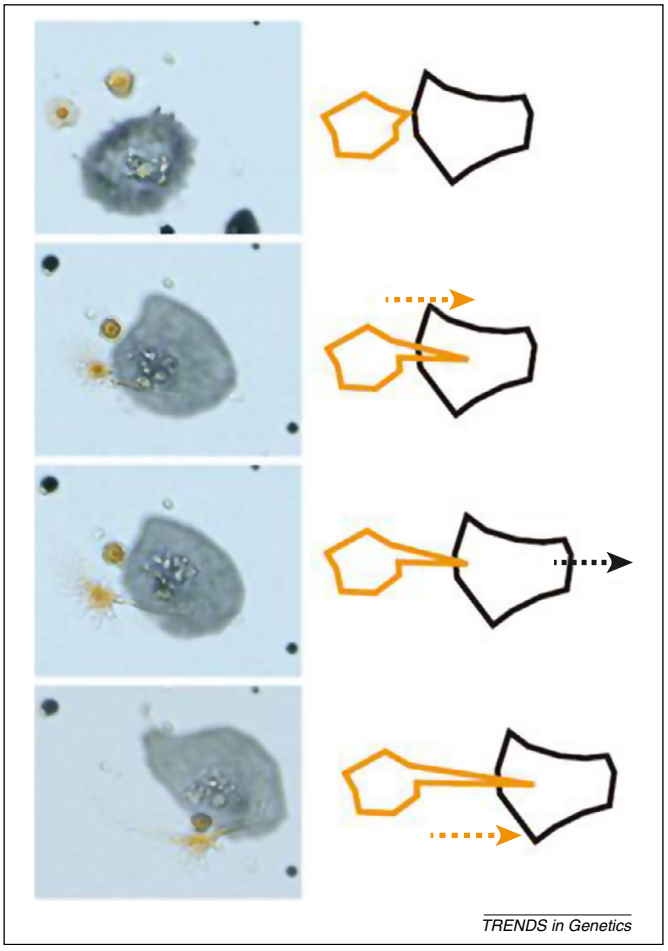

Figure 5. Run-and-chase behavior observed in *in vitro* culture of melanophores and xanthophores. Melanophores and xanthophores are isolated from fins and then incubated in a culture dish. When a melanophore touches a xanthophore, the xanthophore is activated to elongate dendrites toward the melanophore, which induces a concerted behavior in both cells. The melanophore is depolarized upon contact with xanthophore dendrites, which induces it to migrate to escape from the xanthophore. The xanthophore also migrates to maintain contact with the melanophores, resulting in a specific 'run-and-chase' behavior.

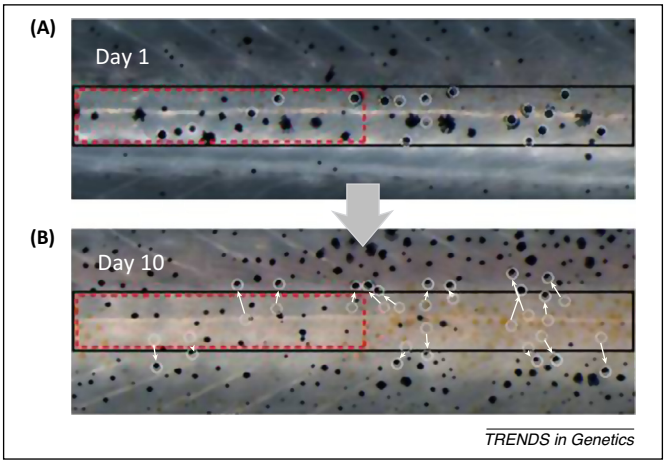

Figure 6. Exclusion of melanophores from future yellow stripe observed *in vivo*. In mid-larval fish, segregation of pigment cells is incomplete. Melanophores located at the wrong position are excluded by migration or cell death. **(A)** Several melanophores distributed in the future yellow stripe in mid-larval fish. In the left region surrounded by the dotted lines, xanthophores were removed via laser. **(B)** In the right region, most of the melanophores died or migrated toward the black stripe regions. In the left region where the xanthophores were killed, migration or cell death rarely occurred relative to the control region. This experiment showed that xanthophores are responsible for the exclusion of melanophores. Modified from [62].

[3]. However, diffusion is not critical to generating a pattern of pigmentation, even by the Turing mechanism [7]. The combination of short dendrites and long projections that was found to exist in zebrafish (Figure 7) can mimic the effect of interactions between slow- and fast-diffusing molecules. As long as a biological system retains the conditions of local activation and long-range inhibition (Figure 3), it has the properties of the original Turing model [3]. Moreover, the finding that long cell projections have a role in patterning does not exclude a possible involvement of diffusible substances in the patterning mechanism. However, because a cell projection is more stable than a diffusion gradient, the latter appears to be a more reasonable mechanism for determining the width of stripes than the former.

The role of iridophores in body stripes

In the fins, where the pigment pattern comprises melanophores and xanthophores, the interaction network described above is sufficient to explain pattern formation. However, in the body trunk, pigment patterns cannot form without iridophores [45,46,55,56]. Recent investigations focused on iridophores are gradually revealing the role of this cell type in pigment pattern formation. Iridophores are the first cell type to form clusters in the hypodermis of the body trunk [55,63]. Detailed tracking of the onset of pattern formation revealed that xanthophores arrive after and colocalize with iridophores, and that melanophores actively avoid iridophores, such that xanthophores and melanophores end up distributed in neighboring regions [55,64]. Therefore, iridophores are capable of influencing the final pigment pattern. Moreover, Patterson and Parichy determined that the signaling molecule Csf1 is expressed by iridophores, and that it is required for xanthophores to develop and cluster [55]. They also found that an array of iridophores defines the boundaries of melanophore stripes [55]. These findings indicate that an extended version of the pigment cell network might exist in which three cell types might interact (Figure 3), and it is certainly possible to generate patterns in the Turing model through the interaction of three elements [8]. Similarly, the results of single cell tracking of pigment cells by Singh *et al.* suggest that the iridophore itself is able to stimulate the pigment pattern to self-organize [63]. This is an interesting suggestion because it is theoretically possible to generate a Turing pattern through the interaction between the two types of iridophore, but further experimental evidence is required to confirm this possibility. We expect future studies will clarify these issues.

Directionality of stripes

Local interactions, such as those responsible for pattern formation in the Turing model, cannot establish the global directionality of the pattern. Specifying the direction of the stripes first requires settling on an orientation, based on some specific biological indicator [15]. In the absence of directionality, a labyrinthine pattern develops [15]. In contrast to the body trunk, which grows uniformly during

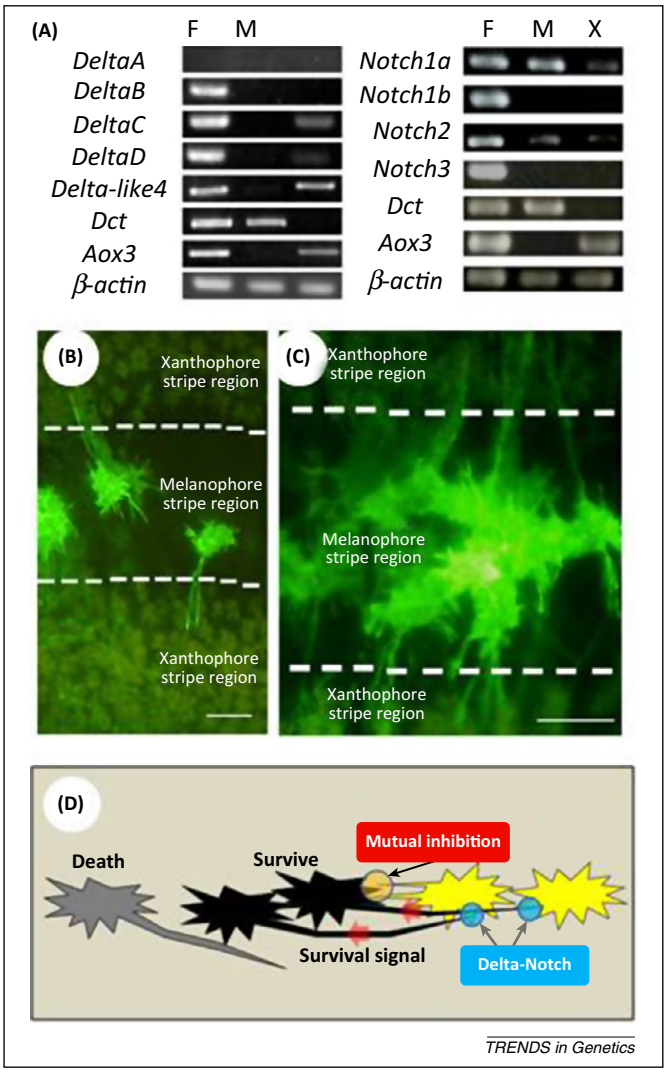

Figure 7. Molecular basis of the long-range signal that determines the stripe width. **(A)** RT-PCR detection of mRNA related to Delta-Notch signaling. mRNA is isolated from fin tissue (F), melanophores (M), and xanthophores (X). *deltaC* and *delta-like 4* are expressed exclusively in xanthophores, and *notch1a* is expressed in melanophores. *dct* and *aox3* are the marker genes for the presence of melanophores and xanthophores. **(B,C)** Membrane-associated GFP is expressed in melanophores located in the middle of black stripes. Melanophores extend long projections toward the yellow stripe region to touch xanthophores directly. **(D)** Schematic of the cell–cell interaction mediated by short dendrites and long projections. Modified from [58].

development, the fins grow mainly at the end (i.e., they undergo appositional growth). In such a case, the growing edge can provide the necessary directionality. However, in the body trunk, some initial condition must provide the required orientation.

In mid-larval fish, iridophore precursors migrate through the horizontal myoseptum to form a single, horizontal band of iridophores. As explained above, melanophores and xanthophores that arrive in the same region later are influenced by the localization of already-present iridophores, suggesting this mid-larval pattern of iridophores acts as the initial mediator for stripe patterning. Multiple experiments support this possibility [55,63,64]. In mutant fish in which the horizontal myoseptum is lost, the mid-larval pattern does not form, and adult fish develop a labyrinth-like pattern [35,56]. When all three types of pigment cell are killed using a laser, regeneration of the pattern occurs, but the directionality is lost [14,15]. This result also indicates that without the mid-larval pattern, the orientation or directionality of the stripes cannot be determined (Figure 1).

A Turing mechanism is consistent with the current model

As discussed above, experimental studies over 15 years have gradually revealed the underlying mechanism of pigment pattern formation in zebrafish. Some findings were expected based on the original Turing model, but others were not.

Artificial ablation of pigment cells induced the dynamic regeneration of the pigment pattern that is specifically predicted based on the mathematical model [14], and the interaction network that is deduced from various cellular analyses satisfies the conditions that are necessary to form the pattern predicted by the Turing model [15]. Moreover, modulation of the activity of a single component of the interaction network in zebrafish by genetic manipulation generated the same variety of patterns as a simulation of the Turing model [59].

By contrast, some findings were unexpected. The original Turing model comprises two chemical substances that react and diffuse. However, in fish pigment patterns, the functional elements are pigment cells that differentiate, migrate, proliferate, and die. Moreover, in the biological organism, direct cell–cell contact, via local or extended cellular projections, replaces the process of diffusion in the original model. Therefore, strictly speaking, formation of pigment patterns in this biological system does not occur via the classic Turing mechanism. However, the properties of the experimentally defined system are mathematically analogous to that of the original Turing model. Therefore, we conclude that the process that generates the pigmentation pattern in zebrafish is equivalent to the Turing mechanism.

Concluding remarks and future directions

Although the actual biological mechanism for pigment pattern formation has been outlined, many of the molecular details remain unknown. The nature of the signal transduction at the tip of the cell projection should be verified through more experiments of a definitive nature, and it is possible that some important interactions have not yet been identified. The role of iridophores is also an important subject for further study, and our understanding of the putative network that is currently known to function in fins may need to be updated to include iridophores. Additionally, how the three-element system functions to form pigment patterns is an interesting question in mathematics. We hope that future experimental and theoretical investigations will clarify the complete mechanism of pigment pattern formation.

Defining these mechanisms in detail is not a trivial issue in biology. Theoretically, every possible type of pattern can be generated by tuning components of the core Turing mechanism, but it is also possible, even likely, that additional factors influence the variety of pigment patterns observed in nature. To understand the origin of pattern variation between different species, one must obtain detailed molecular information. Moreover, recent studies

have suggested that the Turing mechanism underlies many different patterning processes in development [65–67]. It is also likely that the identification of all of the factors involved in zebrafish pattern formation will be useful in discovering the molecular mechanisms underlying pigment patterning in other biological systems.

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