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Current Opinion in Genetics & Development

Linking genotype, cell behavior, and phenotype: multidisciplinary perspectives with a basis in zebrafish patterns

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Zebrafish are characterized by dark and light stripes, but mutants display a rich variety of altered patterns. These patterns arise from the interactions of brightly colored pigment cells, making zebrafish a self-organization problem. The diversity of patterns present in zebrafish and other emerging fish models provides an excellent system for elucidating how genes, cell behavior, and visible animal characteristics are related. With the goal of highlighting how experimental and mathematical approaches can be used to link these scales, I overview current descriptions of zebrafish patterning, describe advances in the understanding of the mechanisms underlying cell communication, and discuss new work that moves beyond zebrafish to explore patterning in evolutionary relatives.

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Introduction

Because alterations in cellular dynamics lead to differences in organism appearance, pattern formation on animal skin is a useful system for studying cell behavior. Amenable to many experimental techniques and widely used for its biomedical applications, the zebrafish (*Danio rerio*) is a model organism for exploring pattern formation [1–4]. Zebrafish are characterized by dark stripes and light interstripes, but diverse patterns are found in mutant fish [1,2,5]. These patterns form due to the interactions of tens of thousands of brightly colored pigment cells.

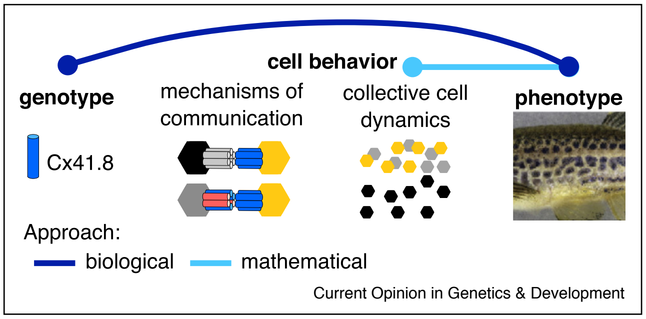
Writing for an interdisciplinary audience, in this review I discuss how experimental and mathematical-modeling approaches are being used to identify the genetic and cellular differences that underlie different phenotypes, linking scales that span from intracellular to evolutionary (Figure 1). With a focus on the literature from the last three years, I review self-organization in zebrafish, describe different modeling approaches to collective cell dynamics, highlight findings on the mechanisms that underlie cell communication, and discuss multidisciplinary perspectives on new pattern formation frontiers, including zebrafish's siblings in the *Danio* genus [9,10••,11], clownfish [12•], and lizards [13••].

Biology of self-organization

There are three main types of pigment cells that are involved in pattern formation in zebrafish: black melanophores (or melanocytes), yellow/orange xanthophores, and iridescent silver/blue iridophores. These cells belong to a wider set of chromatophores that are present in fish (and other cold-blooded animals) and also includes red erythrophores, white leucophores, and blue cyanophores [1,12•]. Interestingly, Lewis *et al.* [17••] recently showed that a subclass of leucophores develops from melanophores through transdifferentiation (meaning they transform from fully developed melanophores into a new cell type) and lines the edges of zebrafish's dorsal and caudal fins. While humans have one only one type of pigment cell (melanocytes, the originating cell for melanoma [3]), these six chromatophores form the basis of the patterns that are present across many fish and reptiles.

On zebrafish skin, adult patterning starts when the fish is approximately 3 weeks old [18,19]. As the fish nearly doubles in length over the next several weeks, chromatophores organize to produce stripes and interstripes sequentially from the horizontal myoseptum outward on the growing body (stripes also appear on two fins) [20]. Iridophores, which first appear along the horizontal myoseptum, help align the body pattern [19–21] (Figure 2b,c). As they disperse dorsally and ventrally, these iridescent cells take on different shapes and colors, providing signals to melanophores and xanthophores [19]. Experimentalists have employed diverse techniques, including mutational analysis, ablation, and transplantation, (e.g. [20,21,22•,23•,24,25]) to search for the cellular mechanisms that are involved in wild-type and mutant

Figure 1



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Overview and one example of how experimental and mathematical approaches can be used in tandem to link genotype [6,7], cell behavior, and phenotype. In this review, I describe biological and mathematical views on self-organization, discuss mechanisms of cell communication, and highlight pattern variability in mutant zebrafish and evolutionary relatives. Phenotype image adapted from [8] with permission from Elsevier, Copyright (2015) Elsevier Ltd.

patterning (Figure 2c–e). Together these approaches have uncovered an interaction network that includes local and long-range dynamics [24,26]. Cells interact through migration (repulsion or attraction), birth and differentiation, competition, and transitions in shape [19,21,25,27,28], and several recent models [16,29,30] have accounted for these behaviors phenomenologically.

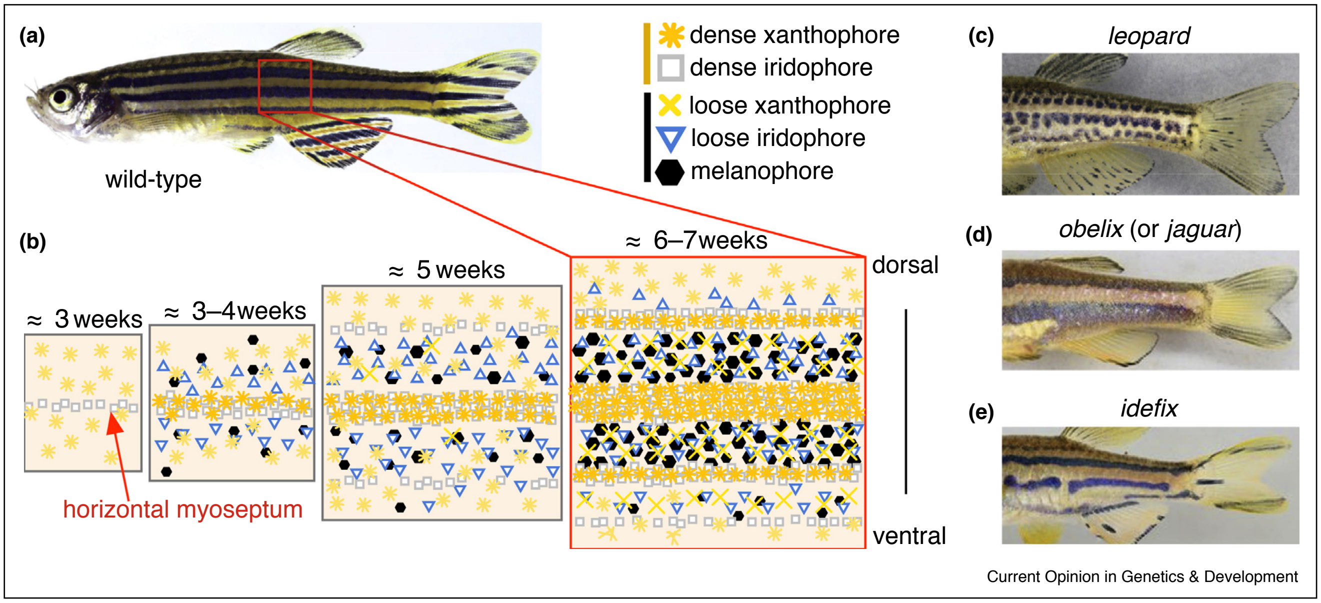
Mathematics of self-organization

Mathematical biologists have used three main approaches to describe wild-type and mutant pattern formation in zebrafish (Figure 3). On the microscopic side, agent-based models treat cells as individuals and track the (x, y)-coordinate of each cell's center (Figure 3b). Cells move continuously in space on growing domains according to differential equations, and stochastic rules govern differentiation, competition, and transitions in form [16,30]. Cellular automaton models [29,31], in comparison, treat space as a lattice and specify all cell behaviors using stochastic rules (Figure 3c).

On the macroscopic side, continuum models [24,29,32–34] describe cell (or morphogen) densities (Figure 3d). Although zebrafish have traditionally been studied using reaction–diffusion equations [24,29], recent models include non-local terms [33,34]. Motivated by *in vitro* observations [28], Woolley [32] also showed that patterns can arise through chase–run behavior with rotation in a system of partial differential equations (PDEs). As an alternative means of describing patterns that is, like continuum approaches, amenable to mathematical analysis, McCalla and von Brecht [35] studied the evolution of stripe–interstripe interfaces in a general model.

Models can be used to suggest wild-type cell interactions, and, by changing parameters, we can make experimentally

Figure 2



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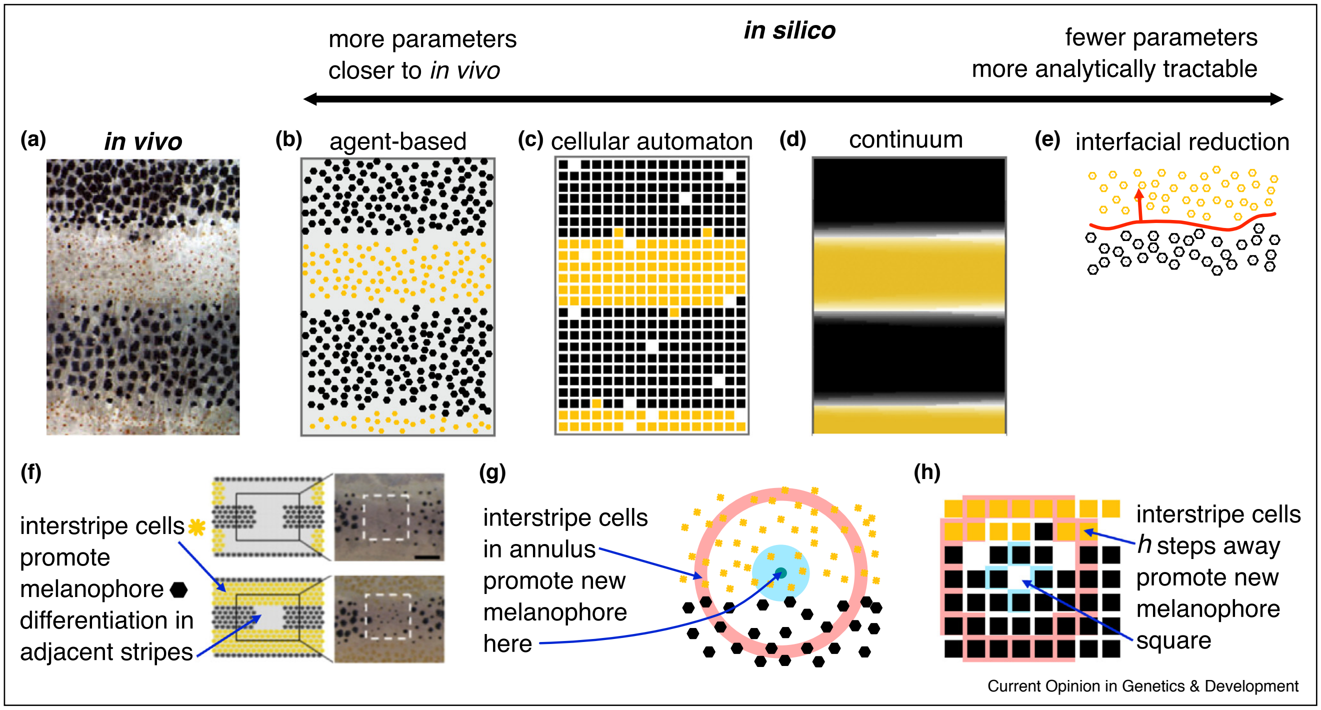
Stripe and mutant zebrafish patterns. **(a)** Wild-type zebrafish have stripe patterns that **(b)** form sequentially outward from the horizontal myoseptum due to the interactions of pigment cells [20]. **(c)** *Leopard* (encoding Cx41.8) [6,7], **(d)** *obelix* or *jaguar* (encoding Kir7.1) [6,14], and **(e)** *idefix* (encoding spermidine synthase) [15] patterns arise due to cell interactions that have been altered (often in unknown ways); see Figure 4. Images (a, c) adapted from [8] with permission from Elsevier, Copyright (2015) Elsevier Ltd.; image (b) adapted from [16], published by Springer Nature, and based on a schematic in [8]; and images (d, e) adapted from [15] and published by The Company of Biologists Ltd. Image (b) licensed under CC-BY 4.0 (https://creativecommons.org/licenses/by/4.0/) and images (d, e) licensed under CC-BY 3.0 (https://creativecommons.org/licenses/by/3.0/).

Figure 3



Mathematical (*in silico*) perspectives on pattern formation. Melanophores and xanthophores are shown, but the model [16] also includes iridophores. **(a)** *In vivo* pattern shows pigment cells. **(b)** Agent-based models describe the behavior of individual cells through stochastic rules and/or differential equations [16]. **(c)** In cellular automaton models, space is discretized, and rules govern the color of grid spaces [29]. **(d)** Continuum models describe cell (or morphogen) densities [32,33]. **(e)** Through an interfacial reduction, one tracks the evolution of stripe–interstripe boundaries (red curve) [35]. **(f)** Ablation experiments [24] have shown that interstripe cells promote melanophore differentiation at long range. **(g)** Agent-based [16] and **(h)** cellular automaton models [29] account for these long-range interactions phenomenologically. Image (a) reproduced from [20], licensed under CC-BY 3.0 (http://creativecommons.org/licenses/by/3.0) and published by The Company of Biologists Ltd.; image (g) adapted from [16] and licensed under CC-BY 4.0 (https://creativecommons.org/licenses/by/4.0/); image (f) adapted from [24].

testable predictions about the cell behaviors that may be altered in mutant patterns. Importantly, prior zebrafish models do so at a phenomenological — rather than mechanistic — level. For instance, the microscopic models [16,29] account for long-range interactions [21,24] by considering the types of cells that appear in an annulus around the position of interest (Figure 3f–h).

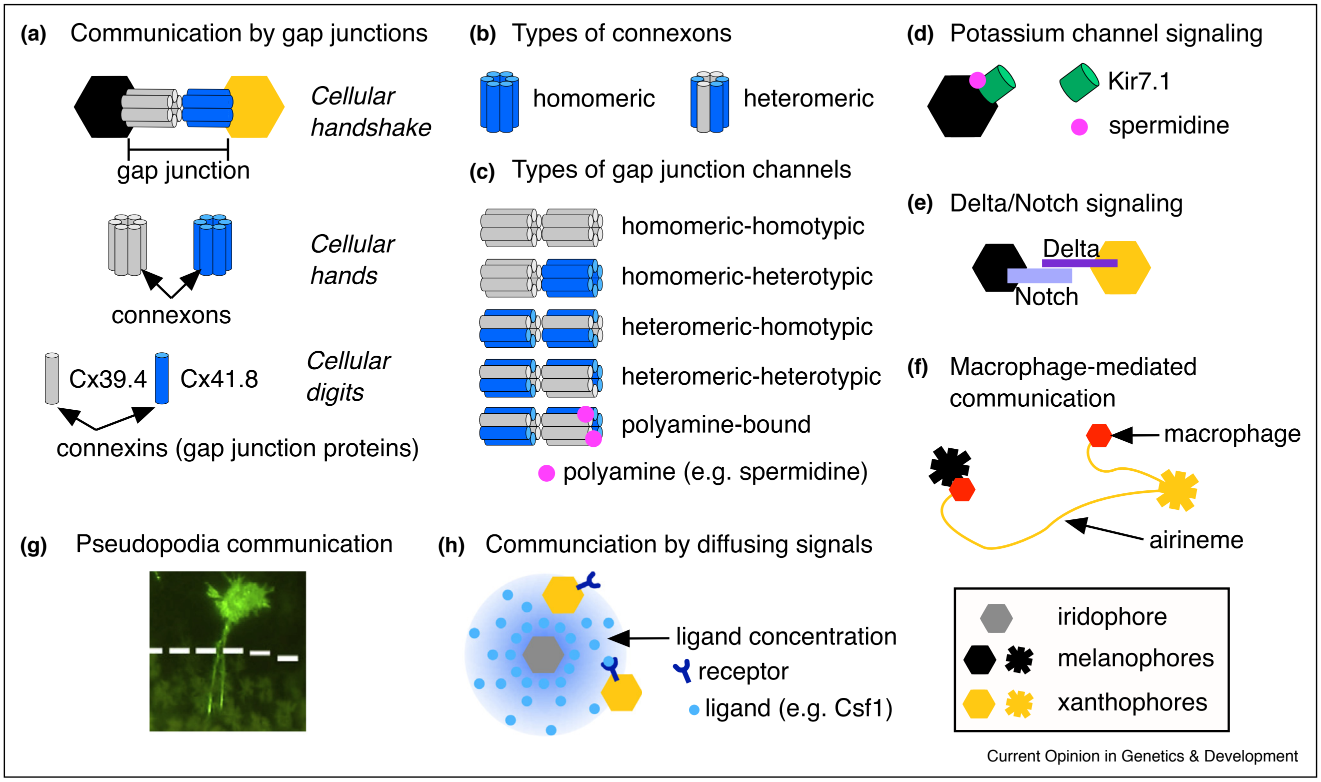
Mechanisms of cell communication

Down a scale from collective dynamics and phenomenological models, we can ask about the mechanisms that underlie how cells communicate, change their shape, and determine their fate. This is a complex and exciting problem, and I overview some of the ways that chromatophores interact in zebrafish.

Gap junctions are a form of communication between adjacent cells that can be thought of as cellular handshakes (Figure 4a–c). Each cell offers a connexon (e.g. hand) to form the channel, and individual connexons are composed of six connexins (e.g. fingers) [36••,37,38]. Two connexin proteins, Cx39.4 and Cx41.8, are required for melanophore–xanthophore communication, and mutations in the genes encoding these proteins produce the *luchs* and *leopard* phenotypes, respectively (Figure 2c) [36••,37,43]. Puzzling out what connexins make up connexons and what connexons form gap junctions is difficult, particularly because connexons can be either homomeric or heteromeric, meaning made up of the same or different connexins (Figure 4b). Moreover, pairs of connexons may come together with like or unlike connexons, forming a wide range of possible channels [36••,37]. In an exciting study, Usui *et al.* [36••] recently identified a minimal network of gap junctions that may regulate communication between melanophores and xanthophores.

Potassium channels provide another means of signaling (Figure 4d), and *obelix* (or *jaguar*; Figure 2d) zebrafish have a mutation in the gene encoding for an inwardly rectifying potassium channel (Kir7.1) [14]. Adding further complexity, polyamines may bind to gap junctions and potassium channels to regulate their function [15,36••]. The polyamine spermidine affects potassium channels as

Figure 4



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Mechanisms of cell communication. **(a)** Gap junctions provide a means of communication between adjacent cells [36••,37,38]. **(b)** Connexons are homomeric (consisting of identical connexin proteins) or heteromeric (consisting of a mix of connexins). **(c)** Pairs of connexons form channels, and polyamines (small diffusing factors) bind to gap junctions to regulate their function [15,36••]. **(d)** The *obelix* mutation (Figure 2f) encodes the Kir7.1 potassium channel [6,14]. Images (b, c, d) are based on schematics in [36••]. **(e)** Delta/Notch signaling may involve protrusions including **(f)** airinemes and **(g)** pseudopodia [2,26,39,40••]. Asterisk shapes denote cells in an early form, as airineme communication is state-dependent [39]. **(h)** Diffusing signals and secreted factors, expressed by pigment cells or the tissue environment, are involved in cell proliferation and fate [21,27,41,42•]; see [2] for a review. Image (g) adapted from [26], licensed under CC-BY 3.0 (http://creativecommons.org/licenses/by/3.0) and published by The Company of Biologists Ltd.

well as Cx39.4 gap junctions [36••], and the *idefix* mutant (Figure 2e) is associated with reduced spermidine levels [15].

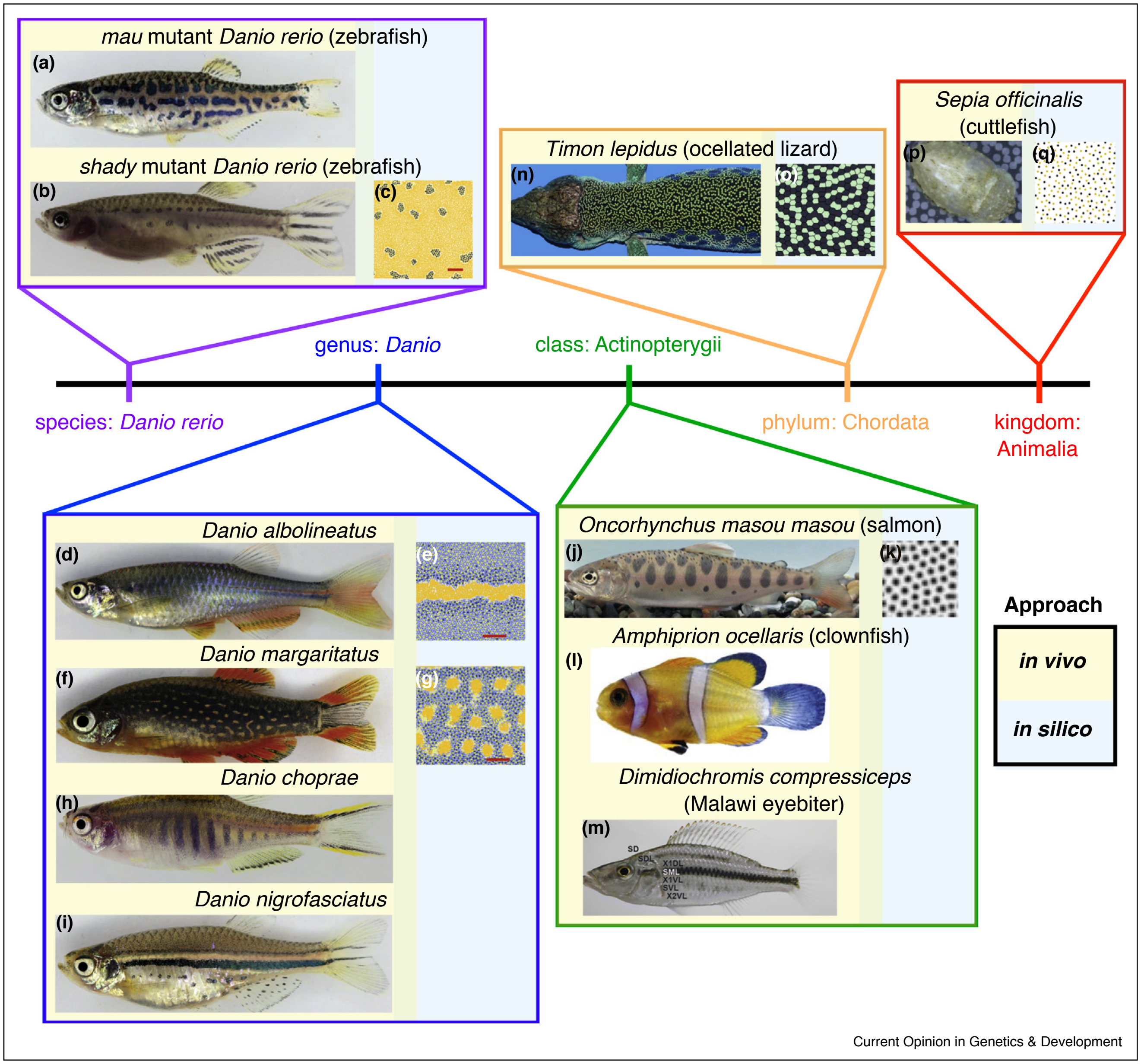
Cellular extensions make it possible for Delta/Notch signaling (a form of receptor–ligand communication that requires cellular contact; Figure 4e) to occur at long range [2,26,39,40••] (see the recent review [44]). In two novel studies, Eom *et al.* [39,40••] showed that early xanthophores extend fast projections (called airinemes) toward early melanophores with the help of macrophages (Figure 4f). Stable, long pseudopodia from melanophores have also been observed *in vivo* [26] (Figure 4g).

A variety of diffusing signals, external ligands, and receptors, including thyroid hormone [42•,45], Endothelin-3 [10••], colony-stimulating factor [27], and leukocyte tyrosine kinase [41,46,47] are involved in cell proliferation and differentiation (Figure 4h; see [1,2] for more comprehensive reviews). For example, Saunders *et al.* [42•] showed that thyroid hormone helps melanophores mature (to a non-dividing state) and causes xanthophores to acquire pigment. Interestingly, reduced levels of galanin lead to increased thyroid hormone, and a mutation that affects galanin signaling in the brain has recently been shown to have a global impact on the pigment cells across zebrafish [48•]. As I discuss next, mutations that involve some of these signals [10••,27,42•] have been associated with evolutionary relatives of zebrafish.

Diversity of patterns

There are several lines of new work that involve taking a broader view of pattern formation. If we look at zebrafish as a whole, there are differences in the patterns that appear in different regions of the fish [8,9,21]. For example, Eskova *et al.* [23•] recently showed that chromatophores from *mau* mutant zebrafish (encoding Aqp3a) can produce stripes when transplanted to a wild-type

Figure 5



Pattern diversity. I indicate *in vivo* patterns in yellow and their *in silico* counterparts in blue, noting that many organisms remain to be described mathematically. **(a–c)** Zebrafish mutants feature altered patterns, and distinct patterns may form in different regions of the fish [20]. **(d–i)** The *Danio* genus is becoming a newly accessible frontier in patterning [1,2,10••,11,16,17••]. **(j–m)** The Actinopterygii class contains many fish with horizontal stripe, vertical bar, or spotted patterns [5,12•,53,54•,56]. **(n–o)** Lizards have the same chromatophores as fish, and their patterns were modeled in [13••]. **(p–q)** The Animalia kingdom also contains cuttlefish, which change color depending on their environment [57••]. Image (a) reproduced from [23•] under CC-BY 3.0 (http://creativecommons.org/licenses/by/3.0) and published by The Company of Biologists Ltd.; images (b, d, f, h, i) reproduced from [8] with permission from Elsevier, Copyright (2015) Elsevier Ltd.; images (c, e, g) and (m) reproduced from [16] and [54•], respectively, published by Springer Nature, and licensed under CC-BY 4.0 (http://creativecommons.org/licenses/by/4.0/); images (n, o) and (p, q) adapted from [13••] and [57••] by permission from Springer Nature, Copyright (2017) and (2018) Springer Nature, respectively; images (j, k) reproduced from [53], Copyright (2010) Springer Nature, and licensed under CC BY-NC-SA 3.0 (http://creativecommons.org/licenses/by-nc-sa/3.0/); image (l) reproduced from [12•] with permission from John Wiley & Sons, Copyright (2019) John Wiley & Sons A/S.

environment, highlighting the role of the tissue environment in pattern formation [21] (Figure 5a). Moreover, the *shady* mutant [20,47] features a spotted body with striped fins (Figure 5b). Using an approach that could be applied to *shady* zebrafish in the future, Kozák *et al.* [49] analyzed a general reaction–diffusion model with spatially varying reaction parameters. New work [50–52] has also explored countershading, a pattern (separate from the fish's namesake stripes) that consists of a dark dorsal region and a light belly ventrally (see [1] for a review).

At the evolutionary scale, a collection of recent studies [10••,16,17••] (Figure 5d–i) have explored sibling species in the *Danio* genus (see reviews [1,2]). The agent-based model [16] describes zebrafish stripe formation and identifies specific cell behaviors that, when removed, produce *Danio margaritatus* patterns (Figure 5g). In a novel study, Spiewak *et al.* [10••] showed that differences between zebrafish and *Danio nigrofasciatus* (Figure 5i) involve changes in Endothelin-3, a ligand that is expressed by the skin and impacts the number of iridophores present. This work further highlights the role of the tissue environment on cell proliferation and dynamics [10••,23•].

If we widen our perspective further, we reach the taxonomic class that contains all of the fish that have rays in their fins, including zebrafish as well as clownfish [12•], salmon [53], cichlids [54•,55], and trout [56]. Along with the *Danio* genus, reef fish are emerging as exciting research frontiers [5]. In a new study, Salis *et al.* [12•] found that the white bars in clownfish contain iridophores (similar to the iridophores in interstripes in zebrafish). It has also been shown that two regulatory proteins (Sox5 and Sox10) have different roles in specifying cell fate in medaka and zebrafish [58•]. Lastly, Hendrick *et al.* suggested that differences in melanophore differentiation and migration help determine whether vertical or horizontal stripes form in cichlids [54•].

Beyond fish, two novel studies have combined experimental and modeling approaches to explore patterns in lizards and cephalopods (Figure 5n–q). First, Manukyan *et al.* [13••] linked a reaction–diffusion model to a cellular automaton description of scale color. The authors changed the diffusion coefficients in the reaction–diffusion system at scale boundaries, obtaining patterns with uniformly colored scales and reproducing *in vivo* results well. Second, Reiter *et al.* [57••] tracked features of individual chromatophores in time in cuttlefish. These authors also developed a minimal mathematical model for light and dark cells that change their color in the growing skin.

Conclusions

The zebrafish (*Danio rerio*) is a widely studied, experimentally tractable fish with important biomedical applications [1–3,46]. Within this species, there is a broad range of mutant patterns that form as the fish grows due to cell interactions that have been altered (often in unknown ways). Because they have been extensively studied in the lab, there is a wealth of biological literature available on zebrafish, and mathematicians [16,31,33] have used this to build models that make experimentally testable predictions about cell behavior. Although experimentalists have identified many of the genes involved in pattern formation in *Danio rerio*, much less is known about other fish.

Zebrafish patterning is an inherently multiscale, multidisciplinary problem that gives researchers the opportunity to uncover how genes, cell behavior, and organism appearance are related (Figure 1). Through a combination of experimental and mathematical-modeling approaches, some of the exciting challenges going forward will be continuing to uncover the detailed structure of communication channels [36••], the role of long extensions in cellular interactions [40••], and the impact of the tissue environment on pattern formation [23•]. At the phenotype scale, the rich diversity of patterns in fish of the *Danio* genus [10••,17••], as well as in more distant relatives of zebrafish like clownfish [12•], represent emerging pattern frontiers.

Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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Salis *et al.* determined that iridophores (not leucophores) are responsible for the white color in the vertical bars of clownfish.

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Lewis *et al.* showed that two separate classes of leucophores arise in zebrafish and several other *Danio* fish. One class seems to arise from xanthophores or their progenitors and the other (melanoleucophores) develops because adult melanophores transdifferentiate. Interestingly, melanoleucophores appear in prominent places on two fins, and experiments suggest other fish socially respond to the presence or absence of these cells.

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Laser ablation, used in prior mathematical models for development and testing, may affect the tissue environment beyond pigment cells. In this study, Aramaki and Kondo developed a less invasive technique for temporarily altering chromatophore arrangement through blue light. This approach can be emulated *in silico* in the future.

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Eskova *et al.* described *mau* mutant zebrafish, which feature spotty, broken stripes and short fins due to a gain-of-function mutation in the gene encoding for Aquaporin 3a. Transplantation experiments showed that mutant pigment cells are able to form stripes in a wild-type environment, highlighting the role of the tissue environment in pattern formation. These effects could be built into future mathematical models.

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