TECHNICAL COMMENT

FISH PIGMENTATION

Comment on "Local reorganization of xanthophores fine-tunes and colors the striped pattern of zebrafish"

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Mahalwar *et al*. (Reports, 12 September 2014, p. 1362) observed the onset of pigment pattern formation in zebrafish. They concluded that their data do not support our Turing mechanism–based model and presented an essentially different mechanism. Here, we clarify their misunderstanding that may have caused their conclusion and explain past experimental data that do not support their proposed mechanism.

Recent observations regarding the cell behaviors of zebrafish pigment from the Singh and Nüsslein-Volhard research group concluded that the current model based on the Turing mechanism cannot explain stripe development (*1*, *2*). Rather, they presented an essentially different model, in which the stripes depend on a prepattern specified by iridophores. This conclusion reflects a misunderstanding of the current Turing-based model and its predictions (*3*). In fact, the reported observations do not contradict the current Turing model. Here, we clarify these issues and discuss the validity of the two models with respect to experimental observations.

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First, they claim that the current Turing-based model cannot apply to zebrafish because the model assumes random initial conditions (*1*). This claim might result from misunderstanding of the model. We selected different initial conditions depending on the phenomenon to be simulated. When we simulated zebrafish stripe formation (*4*), we selected a single horizontal stripe, analogous to the early pattern of iridophores (*1*, *2*, *5*, *6*), as an initial nonrandom condition. In case of regeneration that gives rise to labyrinthine patterns, we selected random initial conditions (*7*). It is a merit of the Turing model that it can explain both development and regeneration.

Second, they rarely observed cell migration during pattern formation and used this to argue against the Turing-based model (*1*). Because we and others observed the migration of pigment cells and their precursors, we wonder if this conflict might be from a difference in some experimental conditions (*8*–*11*). More fundamentally, however, migration is not a crucial requirement of our Turing-based model. The core element of the model is an interaction network having properties of short-range activation and long-range inhibition (*12*). In principle, any cellular behavior might be accommodated. Following this idea, we have never presented any detailed simulation in which a specific cellular behavior is included. The arrows in our model represent rough relationships (inhibition or enhancement) between the pigment cells (*13*). In our previous papers (*4*), we suggested that the differentiation, death, and repulsive migration are the possible behaviors of actual cell-cell interactions (*4*, *14*). Because pigment pattern arises in many different

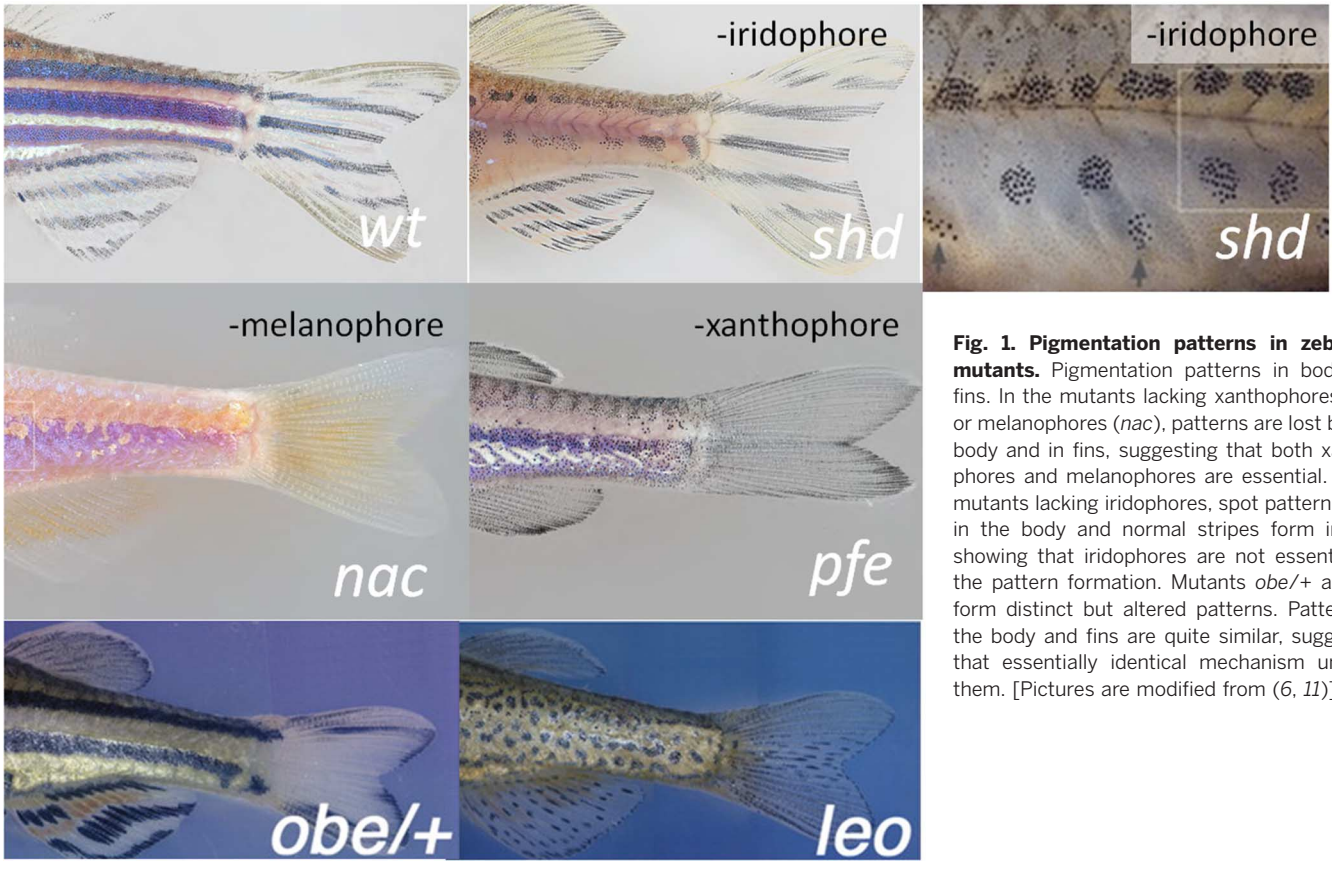

Fig. 1. Pigmentation patterns in zebrafish mutants. Pigmentation patterns in body and fins. In the mutants lacking xanthophores (*pfe*) or melanophores (*nac*), patterns are lost both in body and in fins, suggesting that both xanthophores and melanophores are essential. In the mutants lacking iridophores, spot pattern forms in the body and normal stripes form in fins, showing that iridophores are not essential for the pattern formation. Mutants *obe*/+ and *leo* form distinct but altered patterns. Patterns in the body and fins are quite similar, suggesting that essentially identical mechanism underlie them. [Pictures are modified from (*6*, *11*)]

situations—in body, in fins, and in the process of regeneration—it seems natural that there is some difference in the actual cellular event. As long as the specific property is kept, the system can act as Turing mechanism (*12*).

The most important argument of theirs is that our current model does not contain iridophores (*1*, *2*). Zebrafish have stripes on both the body and the fins. The fin stripes are continuous with those on the body, and the width of the stripes is almost identical (Fig. 1., wt). In some mutants (leo and obe), the pattern in the body and the fins change in the same way (*11*). Therefore, it is highly probable that the same mechanism underlies body and fin stripe development. In the mutants without melanophores or xanthophores, patterns are lost in both the body and the fins. However, in the iridophore-free mutants (Fig. 1, shd), normal stripes form in the fins and spot pattern forms in the body (*5*, *6*), demonstrating that iridophores are not absolutely essential. This is not explicitly stated by them but is the reason that our current model consists of only melanophores and xanthophores. Although iridophores need to be added to explain the body patterning, it is unlikely that the major role of melanophores and xanthophores is drastically changed. (It is theoretically easy to construct a Turing mechanism with three elements.) As described below, past experiments revealed the major contribution of melanophores and xanthophores in the body trunk patterns.

They claim that the early iridophore distribution functions as a "prepattern" to establish all four to five adult stripes and that later cell-cell interactions merely sharpen stripe boundaries (*1*, *2*). However, transition of the patterns from juvenile to adult in some mutants indicates that cell-cell interactions can change the initial pattern (Fig. 2, leo, Tg-2, and Tg3). For example, *leopard* mutants develop normal iridophore prepatterns but gradually change to widely distributed adult spots.

The *leopard* gene mediates interactions between melanophores and xanthophores (*11*) and encodes the gap junction protein connexin41.8 (*15*). We constructed a series of *leopard* genes with varying activity to "tune" the melanophore-xanthophore interaction and successfully generated a variety of patterns (for example, spots, rings, and labyrinth) in transgenic zebrafish without changing the juvenile iridophores pattern (*15*). It is notable that all these patterns are common outcomes of the Turing mechanism but cannot be made by the prepattern mechanism. This "tuning" of the *leopard* gene also produced various stripe patterns with altered numbers, widths, and positions, demonstrating that these parameters are determined by the interaction between melanophores and xanthophores. One particular property of the juvenile pattern that cannot be changed by later rearrangement is the directionality of the stripes. When all the pigment cells are ablated artificially, zebrafish regenerate a labyrinthine pattern, or stripes without directionality, suggesting that the directionality of adult stripes is derived from the juvenile pattern (*7*, *9*).

Numerous other reports using cell transplantation, laser ablation, and other genetic approaches emphasize the contribution of melanophore-xanthophore interactions to adult patterns (*9*, *11*, *14*). The interaction network deduced from these studies shows that the interaction network satisfies the property of short-range activation and long-range inhibition, which is an essential requirement for Turing pattern formation (*13*).

Furthermore, we and others have shown the dynamic rearrangement of pigment patterns following laser or genetic ablation. These experiments demonstrate pattern autonomy that is specific to the Turing mechanism, which is the definitive reason we use the Turing model as the working hypothesis. Because of this dynamic property, Turing-based models can generate many kinds of patterns and can be applied to both development and regeneration of the pattern (*4*). This merit is important because if the Turing mechanism generally underlies these processes, we do not need to find new mechanisms for other pigmentation patterns. Although our current knowledge of the detailed cellular and molecular mechanism is far from complete (e.g., the unknown role of iridophores in the body), experimental analyses over the past 15 years support the Turing mechanism.

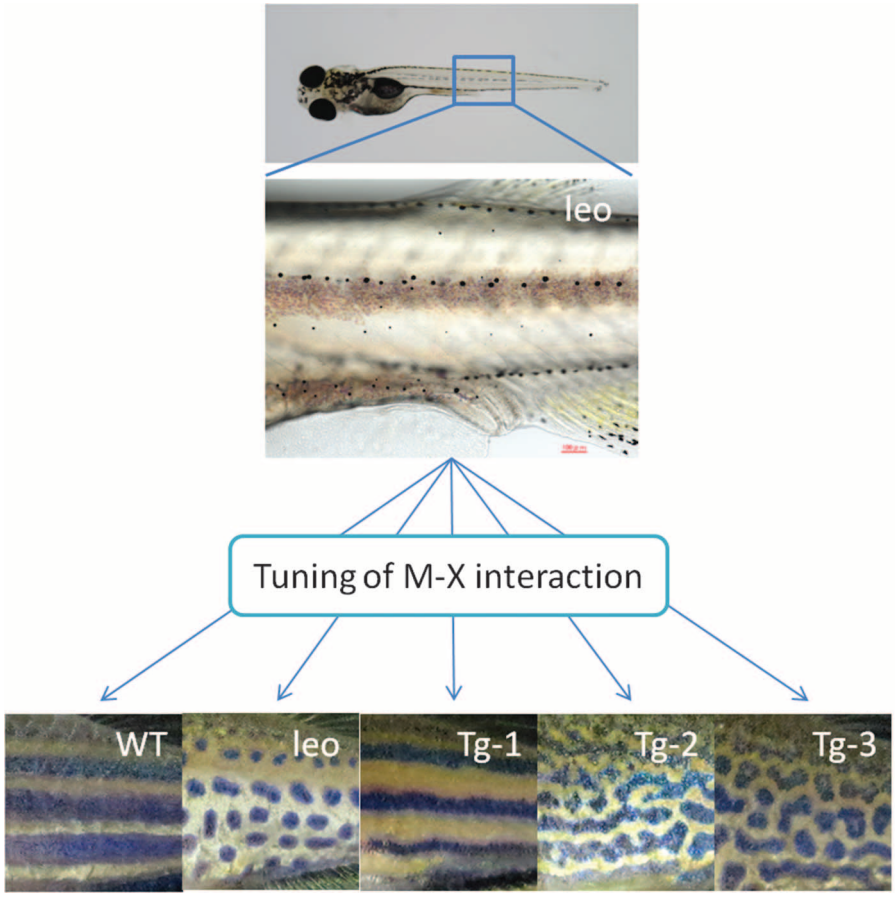

Fig. 2. Tuning of melano-xantho interactions generates a series of patterns without changing the activity of iridophores. Juvenile zebrafish of all leopard variants show almost identical juvenile pattern. However, depending on the activity of melano-xantho interactions, they form different adult patterns. In most cases, the juvenile pattern disappears. [Pictures are modified from (*15*)]

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