Is pigment cell pattern formation in zebrafish a game of cops and robbers?

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Biological self-organization generates enormous diversity from relatively few conserved pathways controlling differential gene expression, raising the prospect that understanding extensive biological complexity relies, in turn, upon understanding relatively simple regulatory frameworks. Consequently, the regulation of biological self-organization has attracted enormous attention, not only from biologists but also from physical and mathematical scientists enthused by exploring the emergence of complex structure from, ultimately, the zygote. Pigment cell patterning, in particular, presents a remarkable opportunity within these broader studies to link genotype to phenotype and this has been exploited in numerous recent investigations documenting the impact of genetic mutations on patterning in a number of fish species.

As ever, the novel results from such studies presents further challenges. Zebrafish patterning, and more generally fish pigmentation, appears to behave very similarly to a theoretical model introduced by Alan Turing (Turing, 1952), which hypothesized how structure may emerge in the embryo. Turing's eponymous mechanism may be readily interpreted in terms of a pair of interacting, diffusible ligands one of which, the self-activator, induces its own production but has limited transport compared to the other ligand, the self-inhibitor, which also inhibits its own production. In addition, this mechanism requires specific interaction kinetics, for instance that the self-activator antagonizes the self-inhibitor, which in turn promotes the self-activator.

However, genetic knockouts, which radically alter zebrafish pigmentation patterns, do not code for diffusible factors, but instead for membrane-bound proteins associated with potassium channels and gap junctions (e.g. Watanabe et al., 2006). Thus, the first challenge is understanding the link between the protein nanoscale, associated with ion channels and gap junctions, with the phenotypic scale of pigment markings. The second challenge is understanding why such a fundamentally different mechanism behaves similarly to a pair of reacting and diffusing ligands, which may offer insight into why Turing's model agrees with numerous biological observations, even though its confirmation at the molecular biology level is lacking thus far. In turn, this raises the intriguing and increasingly recognized prospect that Turing's mechanism at the macroscale may be realized by very different biological physics at the nanoscale.

Indeed, the authors (Yamanaka and Kondo, 2014) have been suggesting for a few years that zebrafish pigment cells may be acting as the components within a Turing mechanism (Yamaguchi et al., 2007), motivating detailed studies of how zebrafish xanthophores and melanophores interact, as their segregation into rows characterizes the standard stripes exhibited by wildtype zebrafish. The authors compared and contrasted the individual movements of these cells in isolation and together with the pigment cells from zebrafish *jaguar* and *leopard* mutants. In particular, in wildtype, xanthophores move more slowly than melanophores but the former extrude pseudopodia which, on contact with a melanophore, induce an escape mechanism in the melanophore and a chasing behaviour in the xanthophore. However, the xanthophore is ultimately outrun due to its slower speed. Furthermore the direction of the melanophore escape is rotated relative to the chasing xanthophore, leading to a circling behaviour (Figure 1).

The mutant pigment cells exhibit different behaviours, leading to a demonstrated correspondence between observed cell behaviour on the one hand and the mesoscale patterning on the other. The importance of this correspondence should not be understated as it provides valuable clues for the first challenge, namely an empirical link between the protein-level effects and cellular behaviour, in turn offering the prospect of completing the link to phenotypic pigment patterning. The authors also tentatively pursue this direction, suggesting that the xanthophore–melanophore chase can induce patterning. The suggested basic principle appears to be that the faster melanophore (perhaps reinterpreted as outlaws, dressed in black, as in all good and not-so-good films), will continually outrun the slower xanthophores (the law enforcers, with sun-bleached apparel) until such point that melanophores no longer need to run, thus segregating the cells (akin to the criminals remaining on the run until arrival in outlaw territory).

However, a fundamental property of any patterning mechanism is that the final pattern is stable and this property is conspicuously absent in this mechanism. The melanophores will, via the random motion observed empirically, make a foray into regions densely populated by xanthophores; however, in

Figure 1. A simulation trajectory of a single melanophore (black) and xanthophore (yellow) using the rules presented in Yamanaka and Kondo (2014); note the circling while the cells are close before they separate due to the faster melanophore migration.

Coverage on: Yamanaka, H. and Kondo, S. (2014). In vitro analysis suggests that difference in cell movement during direct interaction can generate various pigment patterns in vivo. *PNAS* doi/1017/pnas.13154116111

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doing so, the xanthophores follow the melanophores once more, destabilizing the pattern, akin, in the analogy, to outlaws continually returning to the scene of the crime, only to be chased further away, dragging the law enforcers with them into outlaw territory (Figure 2).

This is seen in our simulations, together with the reported circling behaviour of a single pair of an interacting melanophore and xanthophore (see figures). The simulations illustrated in the figures are based on a 'run and tumble' model in which isolated cells choose their direction from a uniform random distribution and travel a fixed distance, whilst the directions of interacting melanophores and xanthophores are biased according to the observational data supplied by Yamanaka and Kondo. Consequently, these observations of even simply wildtype cell behaviour in isolation do not yet explain pattern-forming tendencies observed in zebrafish and do not yet fulfil the first challenge, and thus the second challenge is still out of reach too.

More generally, Yamanaka and Kondo's suggested mechanism preserves cell number (except in the case of *leopard* mutants); however, it is difficult to see how Turing's ideas can be caricatured by, ultimately, interaction-dependent transport, preventing the inhibition–promotion kinetic interactions that traditionally characterize Turing's mechanism. As such, the proposed mechanism is much more closely related to cell sorting, although possessing very different characteristics compared to traditional cell-sorting drivers, such as differential adhesion and chemotaxis. Nonetheless, even for sorting cells, Figure 2 demonstrates that additional constraints on cell behaviour are required. Even when parameters are altered to match the speed and direction estimates for melanophore–xanthophore interactions in the *jaguar* and *leopard* mutant experiments, the large cell number simulations hardly differ from that shown in Figure 2.

Despite such observations and limitations of this specific work by Yamanaka and Kondo, their work is unquestionably an important study and should be viewed within the context of their on-going investigations, where they have further demonstrated long-range interactions by melanophore projection (Hamada et al., 2014). Essentially, this work provides valuable data that can contribute to the grand challenge of linking genetic expression to large scale structure in developmental biology, as well as assessing whether Turing's mechanism may be implicit in this patterning process even if diffusible ligands are not present.

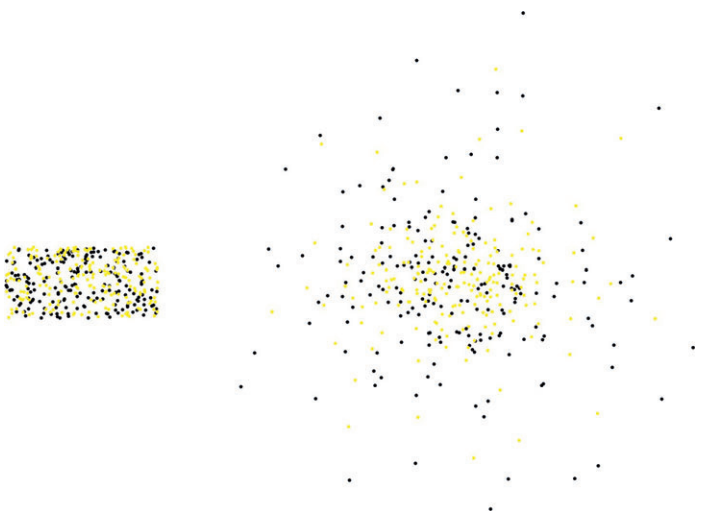

Figure 2. A simulation of 200 melanophores (black) and xanthophores (yellow) using the rules presented in Yamanaka and Kondo (2014). Left. The initial distribution, which is well mixed. Right. The pattern after 100 hours; note the diffuse edge of melanophores with numerous xanthophores in the periphery.

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More generally, this form of experimental study is very important in enabling the development of dynamical models for biological self-organization, which can be properly parametrized and validated against experiment.

Theoretical challenges also emerge from the work of Yamanaka and Kondo, as it is a non-trivial task to assess whether the observed cell-based rules may be incorporated into existing modelling frameworks, traditionally used to explore differential adhesion cell sorting and Turing's mechanism. Alternatively, novel modelling paradigms may be required. In turn, progress in such endeavours should enable extensive mathematical tools to be brought to bear in the analysis of cell sorting and patterning through general individual cell-based rules, and their connections to genetic differences, rather than interpreting results based on the inspection of limited numbers of numerical simulations.

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