not exclude a particular role of UVA in melanomagenesis. However, the finding that G:C to T:A transversions were much less common (<4000) indicates that oxidative DNA damage is a much less common mutation-inducing type of DNA damage in melanomagenesis than pyrimidine dimers.

With so much oxidative DNA damage generated by ultraviolet light and in particular by UVA, one may ask why there are not more mutations typical for oxidative DNA damage found in melanoma. While many oxidative DNA lesions are not or only poorly mutagenic, 8-oxoG, the most studied type of oxidative DNA base damage has a clearly established potential to generate mutations. The answer to the question is probably that the frequency of mutation formation at sites of 8-oxoG is much lower than that at sites of pyrimidine dimers.

Studying mechanism of mutation formation in primary melanocytes is a very important endeavor and should be investigated, as conducted by Wang et al., at all steps of the photocarcinogenesis cascade, including DNA damage formation, cellular responses to DNA damage, and mutation formation. Differences in any of those steps between melanocytes and other skin cells remain highly plausible. In that regard, the influence of different types of melanin on UVA mutagenesis with pheomelanin as photosensitizer and eumelanin as photoprotector may be worth studying. Current evidence, however, favors a much higher contribution of pyrimidine dimers to mutation formation during melanomagenesis than of oxidative DNA damage. In that regard, at the moment, melanoma does not appear to be different from other types of UV-induced skin cancer.

References

Courdavault, S., Baudouin, C., Charveron, M., Favier, A., Cadet, J., and Douki, T. (2004). Larger yield of cyclobutane dimers than 8-oxo-7,8-dihydroguanine in the DNA of UVA-irradiated human skin cells. Mutat. Res. *556*, 135–142.

Hocker, T., and Tsao, H. (2007). Ultraviolet radiation and melanoma: a systematic review and analysis of reported sequence variants. Hum. Mutat. *28*, 578–588.

Pleasance, E.D., Cheetham, R.K., Stephens, P.J. et al. (2010). A comprehensive catalogue of somatic mutations from a human cancer genome. Nature *463*, 191–196.

Rünger, T.M. (2008). C to T transition mutations are not solely UVB-signature mutations, because they are also generated by UVA. J. Invest. Dermatol. *128*, 2138–2140.

Rünger, T.M., and Kappes, U.P. (2008). Mechanisms of mutation formation with long-wave ultraviolet light (UVA). Photodermatol. Photoimmunol. Photomed. *24*, 2–10.

Wang, Y., DiGiovanna, J.J., Stern, J.B., Hornyak, T.J., Raffeld, M., Khan, S.G., Oh, K.S., Hollander, M.C., Dennis, P.A., and Kraemer, K.H. (2009). Evidence of ultraviolet type mutations in xeroderma pigmentosum melanomas. Proc. Natl Acad. Sci. USA *106*, 6279–6284.

Turing patterns: how the fish got its spots

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From the spiraling florets of sunflowers to the vivid stripes of the zebra, periodic patterns abound in nature. But how can such patterns, so complex and diverse, arise during the development of an organism? Fascinated by this question, Alan Turing, an English computer scientist and philosopher, turned his attention to mathematical biology during the final years of his life. Turing pioneered the idea that stochastic activity at the molecular level could result in large-scale organization. In the now famous Turing model, published in 1952, a locally self-enhanced molecule diffuses and activates its inhibitor at long range (Figure 1A). As a consequence of the differential diffusion rates of these two molecules, regular patterns can emerge even from an initially homogeneous state. Turing proposed that this single pattern formation mechanism, called 'reaction-diffusion,' generates many of the patterns observed in nature, including the colorful designs that adorn many animals (Figure 1B).

As envisioned by Turing, many animal color patterns have been replicated with commendable accuracy by computer simulations driven solely by reaction–diffusion dynamics. By simply adjusting the initial parameters of the system (e.g. diffusion rates, molecular concentrations), a wealth of possible patterns can be generated, including regular stripes, spots, rosettes, and reticulated designs (Figure 1C; Kondo and Miura, 2010). Because these simulated patterns resemble color patterns seen in nature, Turing's mechanism has been widely accepted as an elegant and simple solution to explain the complexity (and diversity) of natural patterns. But as striking as this correlation is, one still must ask whether these artificial patterns capture biological processes.

In their work recently published in *Nature Communications*, Miyazawa and colleagues elegantly address this question by testing reaction–diffusion theory with real organisms. Miyazawa et al. (2010) capitalize on the observation that two differently patterned fish—the white-spotted char (*Salvelinus leucomaenis*) that displays light spots on a dark background and the masu salmon (*Oncorhynchus masou masou*) that has dark spots on a light background—occasionally hybridize in Japanese rivers. Surprisingly, their hybrid offspring are not spotted at all, but exhibit a distinct pattern—a 'labyrinthine' design of contorted stripes (Figure 2). Moreover, this case is not unique, as labyrinthine patterns are observed in other salmonid hybrids if their parents are inversely spotted. But more remarkably, the authors clearly demonstrate that this unusual labyrinthine pattern is precisely what reaction–diffusion theory predicts. First, using mathematical simulations, the authors determined the precise parameter values that recapitulate the patterns

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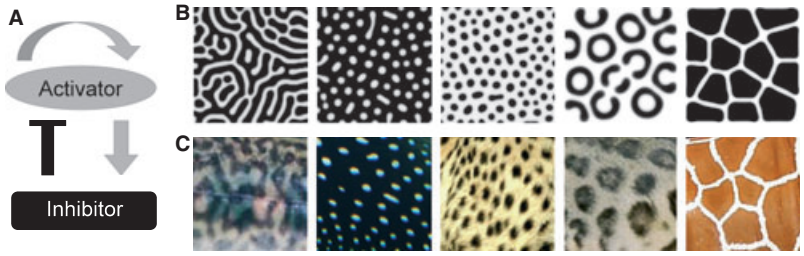

Figure 1. Reaction–diffusion simulations reproduce color patterns found in nature. (A) Reaction–diffusion mechanism relies on the interaction between an activator (gray) and its inhibitor (black), whose expression and/or activity is triggered by the activator. (B) Periodic patterns can be produced in reaction–diffusion computer simulations (modified from Kondo and Miura, 2010). (C) Computer-generated patterns resemble color patterns observed in nature. From left to right: hybrid fish (*Salvelinus leucomaenis* × *Oncorhynchus masou masou*), [modified from Miyazawa et al. (2010)], marine beta (*Calloplesiops altivelis*), cheetah (*Acinonyx jubatus*), bengal cat (*Felis catus*), and giraffe (*Giraffa camelopardalis reticulate*) (photographs taken from wikipedia.org).

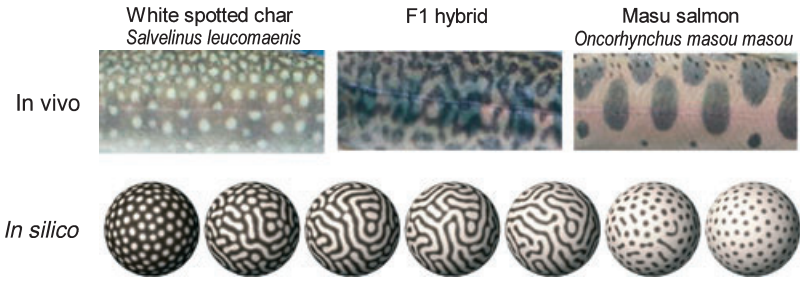

Figure 2. Reaction–diffusion simulations predict hybrid fish patterns. Top row: Color patterns of salmonid species (white-spotted char and masu salmon) and their hybrid offspring. Bottom row: As reaction–diffusion simulation parameters are gradually adjusted, the resulting artificial patterns change from light spots (reproducing the white-spotted char pattern) to labyrinthine patterns (resembling the hybrid) to dark spots (similar to the masu salmon). Modified from Miyazawa et al. (2010).

observed in the pure species. Then, using the intermediate parameters of the parental values (as intermediate values could mimic a hybridization event), they predict the color pattern phenotype of hybrids. In this '*in silico* hybridization' experiment, labyrinthine patterns similar to those observed in natural hybrids were produced (Figure 2). Thus, labyrinthine patterns result from the blending of two complementary spotting patterns, both theoretically and empirically. The authors go on to propose that such a 'pattern blending' mechanism may also contribute to speciation: hybrids with labyrinthine patterns may be reproductively isolated from the parental species if, for example, color pattern is involved in mate choice. While its relevance to salmonid diversification remains purely speculative, this work adds to the accumulating empirical evidence for Turing processes driving color patterning in fish (Kondo and Miura, 2010).

The next challenge, now in the hands of geneticists and developmental biologists, is to determine the identity of the in vivo activating and inhibitory molecules. Turing postulated that two molecular players were sufficient to generate a reaction-diffusion-based pattern. Indeed, a two-molecule interaction has been implicated in recently studied systems: the patterning of feather branching in chick and the spacing of hair follicles in the mouse skin (reviewed in Kondo and Miura, 2010). As to the identity of the molecules controlling color patterns, some clues can be gained from other patterning systems. Developmental biologists have identified numerous molecules involved in organogenesis whose mode of action is compatible with the requirements of a reaction–diffusion system. So-called 'morphogens' are often locally self-activating but diffuse at long range to trigger differential tissue responses according to their concentration and location. One the most famous and best characterized morphogens, Wingless, has recently been shown to be locally expressed and to diffuse along developing Drosophila wings to control pigment patterning (Werner et al., 2010). Moreover, in mice, spacing of dermal appendages in the skin (such as hair follicles) appears to be controlled by differential expression levels of WNT and its inhibitor DKK in the only described example of a reaction–diffusion mechanism in vivo. The WNT signaling pathway therefore constitutes an excellent candidate for reaction-diffusion-based color patterning in vertebrates.

Knowing the molecules, in turn, will provide more realistic parameters to the mathematicians. Here, Miyazawa and colleagues predicted the hybrid phenotype by simply choosing parameter values intermediate between the two pure species, as might result from genetic co-dominance, incomplete dominance, or (as suggested by the authors) polygenic inheritance. However, this need not be the case – geneticists since Mendel have known that hybrids are rarely the average of their two parents, because dominance and epistasis are common. Therefore, a next step for mathematicians would be to generate an array of *in silico* hybridizations that attempt to assimilate knowledge of genetics and molecular biology into their parameters. For instance, what pattern would result from the hybridization of parents with different morphogen diffusion rates (e.g. one fast and one slow)? Their progeny would have ½ fast-diffusing and ½ slow-diffusing morphogens, which is quite different from a homogenous intermediate rate of diffusion when spatial position is important. In return, more biologically realistic simulations would provide hints to molecular geneticists as to the underlying mechanism of action of these interacting proteins – mathematics and genetics can be mutually enlightening.

Beyond knowing the precise molecular players and their interactions, a complete understanding of color pattern formation will require understanding the developmental processes controlling the actions of these molecules. How do these molecules produce patterns – for example, how do they affect pigment cell behavior; what are their sources; what limits their diffusion; and how do their localized interactions produce a whole-organism pattern? Because most traditional laboratory models do not display regular color patterns, the developmental mechanisms largely have

remained a black box. However, with recent advances in genome sequencing and the increasing availability of molecular tools in 'non-model' organisms, it is now conceivable to describe – and quite possibly functionally test – *how* these interacting molecules produce patterns.

Ultimately, however, we strive to understand not only how patterns are formed but also why and how they evolve. Why do different salmon species have and maintain such distinct color patterns? In a recent attempt to understand the evolution of color patterns, Allen et al. (2010) surveyed felid patterns and used reaction–diffusion simulations to determine which pattern elements best correlate with species' habitat and behavior. Their comparative analyses suggest that different feline patterns serve as background-matching camouflage, although not all species nicely conform to such predictions. More comparative and experimental work to disentangle the ultimate mechanisms driving patterning variation in cats, fish, and other species is sorely needed (but admittedly represent the hardest question to answer).

And, finally, what are the precise molecular changes responsible for the diversity of patterns observed among species in nature? If the Turing model is both correct and general, then the evolution of color patterns may result from a small number of simple genetic changes – even a single mutation that affects the expression or diffusion of one of the interacting molecules could produce a wholly unique pattern. Do these changes occur in the activator or inhibitor molecules themselves or somewhere upstream in their respective pathways? One way forward is to use a genetic-mapping approach to determine how many and which loci are responsible for changes in the reaction–diffusion mechanisms that give rise to striking differences in skin pigmentation. A second round of laboratory-based crosses between salmonid hybrids or an association study in a naturally admixed river population would be a first step in localizing the genomic regions associated with the color pattern differences reported by Miyazawa and colleagues. Such a study would complement the ongoing work on the genetic architecture of color pattern formation in tabby cats (Eizirik et al., 2010) and would elucidate to what extent changes in patterning molecules and mechanisms are shared among vertebrate groups.

While the clever work of Miyazawa and colleagues solidifies the biological relevance of Turing's mathematical predictions, unanswered questions still remain: what are the precise molecular changes and evolutionary processes responsible for the amazing diversity of color patterns – from fish to felines – we observe in nature?

References

Allen, W.L., Cuthill, I.C., Scott-Samuel, N.E., and Baddeley, R. (2010). Why the leopard got its spots: relating pattern development to ecology in felids. Proc. Roy Soc. Lond. B. Published online before print October 20, 2010, doi: 10.1098/rspb.2010.1734

Eizirik, E., David, V.A., Buckley-Beason, V., Roelke, M.E., Schaffer, A.A., Hannah, S.S., Narfstrom, K., O'Brien, S.J., and Menotti-Raymond, M. (2010). Defining and mapping mammalian coat pattern genes: multiple genomic regions implicated in domestic cat stripes and spots. Genetics *184*, 267–275.

Kondo, S., and Miura, T. (2010). Reaction-diffusion model as a framework for understanding biological pattern formation. Science *329*, 1616–1620.

Turing, A.M. (1952). The chemical basis of morphogenesis. Phil. Trans. Roy. Soc. Lond. Series B. *237*, 37–72.

Werner, T., Koshikawa, S., Williams, T.M., and Carroll, S.B. (2010). Generation of a novel wing colour pattern by the Wingless morphogen. Nature *464*, 1143–1148.

A new delta-COP on the block – linking pigmentation defects with neurodegeneration

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The study of mouse coat color mutants, which were appreciated as early as the 11th century BC and which were actively bred by mouse fanciers during the 1800s, has contributed tremendously to the understanding of many fundamental biologic processes, including intracellular membrane trafficking and organelle transport. Importantly, structural and functional abnormalities in membrane trafficking pathways have also been linked to neurodegenerative disease. For example, membrane trafficking is directly affected by α-synuclein, a protein that is intimately linked to the pathogenesis of Parkinson's disease (Auluck et al., 2010). Moreover, mutations affecting the function of cytoskeletal motors that drive membrane transport can cause diverse forms of neurodegeneration, including the motor neurodegenerative disease amyotrophic lateral sclerosis (ALS; Perlson et al., 2010). ALS is also caused by mutations in *Fig4*, which encodes a lipid phosphatase that is critical for retrograde traffic from endosomes to Golgi (Chow et al., 2009).

In a recent study, Xu et al. used a forward genetic approach to shed more light on the mechanisms by which defects in membrane trafficking can lead to neurodegenerative disease. Given the prior examples of defects in membrane trafficking pathways that cause both neurological and pigmentation defects (see e.g. Chow et al., 2009), the authors postulated that the additional presence of a pigmentation defect in mouse mutants that display a neurodegeneration phenotype would be a strong predictor that the underlying problem was in membrane trafficking.

Coverage on: Xu, X., Kedlaya, R., Higuchi, H., Ikeda, S., Justice, M.J., Setaluri, V., and Ikeda, A. (2010). Mutation in archain 1, a subunit of COPI coatomer complex, causes diluted coat color and Purkinje cell degeneration. PLoS Genet. *6*, e1000956.

doi: 10.1111/j.1755-148X.2010.00818.x