How the mouse got its stripes

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A central issue in developmental biology is the understanding of how various processes interact to produce spatio-temporal patterns in the embryo. To even observe these dynamical patterns directly is a challenge and, although technological advances in, for example, imaging techniques are beginning to address this problem, it is still far from trivial. A new strain of mouse that is deficient in hair formation (1) now makes it possible to visualize directly traveling waves of pigmentation propagating over the skin of the mouse. This new study adds an extra dimension to the challenge of elucidating the mechanisms that underlie embryonic pattern formation and provides further evidence for self-organization.

From an apparently homogeneous mass of dividing cells in the very early stages of development emerges the vast and sometimes spectacular array of patterns and structures observed in animals. The mechanisms underlying the coordination required for cells to produce pattern on a spatial scale much larger than a single cell is still largely a mystery, despite a huge amount of experimental and theoretical research. There is inherent in the oocytes positional information that must guide pattern, but cells that are completely dissociated and randomly mixed can recombine to form periodic spatial structures (2). This leads to the intriguing possibility that at least some aspects of spatio-temporal patterning in the embryo arise from the process of self-organization. The mathematician Alan Turing first proposed such a mechanism in his seminal 1952 article (3), in which he showed that a system of chemicals could evolve spontaneously into a spatial pattern. He further hypothesized that if these chemicals, which he termed morphogens, cued cell differentiation, then the patterns we see in nature would be the interpretation of chemical prepatterns. One year earlier, the Russian chemist Belousov showed experimentally that a system of reacting and diffusing chemicals could evolve into a spatio-temporal pattern of traveling waves and spirals. This reaction is known as the Belousov–Zhabotinskii (BZ) reaction (see ref. 4 for a brief review of the history of this reaction), and such spatio-temporal patterns have now been observed in a range of chemical systems.

A number of authors have shown that Turing-type models can exhibit an enormous diversity of patterns, many of them consistent with those observed in nature (5, 6), and Turing's original concept was developed more fully into the general patterning principle of short-range activation, long-range inhibition (7) (sometimes known as local-activation-lateral-inhibition, or LALI). In 1995, Kondo and Asai (8) showed that, in certain fish species, pigmentation stripes moved as the fish grew until the interstripe distance was about twice the original wavelength, at which point another stripe was initiated to preserve the original wavelength. This is consistent not only with the Turing-type models, but also with other LALI models. Now, in a fascinating new study, Suzuki *et al.* (1) show that traveling patterns, similar to those observed by Belousov, can actually be exhibited on the skin of certain mice. Although traveling patterns of hair formation in mice have been known for some time, this article carries out a detailed study of this phenomenon on a new mutant strain of mouse. In this mouse, hair follicle development terminates just after pigment begins to accumulate in the follicle. The immature follicles are discharged, new follicles begin to form, and the cycle of pigment accumulation resumes. This results at first in the pigmentation pattern on the skin oscillating synchronously. About 30 days later, they observe a broad traveling band of pigment moving along the skin of the mouse; the band then splits into two traveling bands moving in opposite directions. As the mouse matures, the bands narrow. Eventually, in the adult, each wave appears to arise from the armpit regions and spreads over the skin.

If morphogens cued cell differentiation, then natural patterns would be the interpretation of chemical prepatterns.

The progression from bulk (synchronous) oscillations to traveling bands is common in chemical systems and is well understood by using mathematical models. It is interesting to note that, although the study of pattern formation in chemistry is characterized by experiment and theory moving hand in hand, with theory informing experiment and vice versa, in developmental biology few experimentalists have embraced mathematical modeling. A notable exception is Kondo and his laboratory. In their present study, they show that the spacing of subsequent waves varies as in the BZ reaction and that waves collide also in a similar way to the BZ reaction. The phenomenon of traveling waves with variable wavelength also occurs during the aggregation phase in the slime mold *Dictyostelium discoideum*. There, a plausible explanation for this variation is that biochemical properties affecting wave speed change over the course of aggregation. However, mathematical modeling reveals that this is not necessary, because the change in period of the signaling cAMP waves is a natural consequence of the aggregation process interacting with the excitable medium from which the waves are generated (9).

The type of spatial patterning observed in Suzuki *et al.* (1) is more complicated than the sequential patterning that is typical in many areas of developmental biology. For example, pigmentation patterns on certain alligators begin at the head and form behind a traveling front of pattern initiation (10); feather germ patterns form in a hexagonal array behind a propagating maturation front (11). The fronts are unique in each of these cases, so the problem of collision does not arise. However, for photoreceptors in the developing *Drosophila* eye, one can make ectopic fronts that travel in different directions to the endogenous front and arrange for collisions between fronts. In this case, pattern formation self-organizes behind the endogenous and ectopic fronts (12). A very well studied example of patterning behind a front occurs in somitogenesis, where somites form in a well ordered spatio-temporal sequence from head to tail. Recently, it has been shown that waves of gene expression actually propagate in the opposite direction, narrowing as they move headway and defining, it seems, the caudal part of the next somite (13).

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Suzuki *et al.* (1) conjecture that there is a pacemaker around the armpit region. However, another possible explanation is that there could be a reentrant trajectory around the torso and arms, much as is thought to happen in a ring of cardiac tissue, or around a myocardial infarct leading to sustained activity in the ventricles (see, for example, ref. 14).

The ability of reaction–diffusion mechanisms of the type proposed by Turing to generate stable spatial patterns has been demonstrated in a number of chemical systems. For example, the chloride-iodide-malonic acid reaction yields stable patterns of stripes and spots exactly as predicted by the model (15, 16). However, whether such mechanisms act to specify pattern in biology remains hugely controversial. For example, although Turing-type models can provide very elegant explanations for the formation of periodic striped patterns of, for example, the pair-rule genes during *Drosophila* segmentation, experiments suggest that each stripe forms independently and that this pattern is the result of a complex cascade of interacting gradient-like elements. Examples such as this illustrate that, although self-organization may provide an elegant means of producing patterns *de novo*, pattern formation in biology may sometimes depend more on sequential elaboration of initially simple asymmetries (17).

Two stabilizing elements are combined to produce an instability.

The hypothesis that biological pattern formation is the result of self-organization, though highly controversial and largely ignored by experimental biologists, does raise a number of very important points. For example, in Turing's model, the chemicals are proposed to react in such a way that, in the absence of diffusion, they reach a stable, spatially uniform steady state. It is diffusion, normally assumed to be a homogenizing process, that actually drives the instability. That is, two stabilizing elements are combined to produce an instability. This is an example of an emergent property and shows that not only is it important to determine the individual components in a biological system, but it is at least equally as important to understand how they interact. The BZ reaction is another example. It is a relaxation oscillator, but researching the molecular basis of this reaction in the hope of finding the oscillating element would be fruitless. It is the integration of the elements that leads to the oscillator. Mathematical models can also provide a mechanistic understanding of observed developmental constraints (18). Perhaps the most famous of these constraints is that described by Murray (5), who showed that it is more likely to have a spotted animal with a striped tail than a striped animal with a spotted tail.

Of course, biology is much more complicated than chemistry, and so, whereas in the latter we now have several well identified and studied examples of self-organization, in the former we do not yet have the molecular detail at hand to support or refute the self-organization hypothesis. The article by Suzuki *et al.* in this issue of PNAS (1) does, however, provide another example of a biological pattern that can, at least, be mimicked by a mathematical model for self-organization. The growing number of examples of patterns that can be mimicked by simple models based on self-organization presents a challenge to experimentalists to determine the molecular circuitry that underlies these patterning events. Coat patterns form an ideal system in which to pursue this question because they are easily visualized compared with, say, patterns at gastrulation, and variants tend to be viable.

Progress is now being made through the use of genetics. For example, a wide range of mutant strains of zebrafish have been identified that exhibit altered pigmentation patterns. In the case of mutations of one gene in particular, the *leopard* gene, it is possible to match the observed allelic sequence by smoothly varying a parameter in a Turing-type model (19). This lends further support to the notion that a self-organizing process is at work in this system. In the present study, Suzuki *et al.* (1) identify the expression patterns of a number of genes that may play an important role in the spatio-temporal dynamics they observe. They also allude to the possible functions they may have in establishing the appropriate interactions for a self-organizing process.

As pointed out by Suzuki *et al.* (1), in a number of animals, each individual hair (or quill in the case of porcupines) has a roughly periodic pigmentation pattern. In such cases, this pattern could be the spatial read-out of a temporally oscillating process of pigmentation production. The biochemical pathways underlying melanin synthesis have been studied in detail (20), and a mathematical model for melanogenesis has been proposed (21) that takes into account several of the key elements involved. The resultant model, a system of ordinary differential equations, is of a type that can exhibit temporal oscillations. Incorporating spatial variation and coupling into such models may provide a framework in which one could study how the local dynamics that give rise to propagating patterns on an individual hair interact to give the global spatio-temporal picture. We may then be able to answer the age-old question of how the leopard got its spots (22).

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1. Suzuki, N., Hirata, M. & Kondo, S. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 9680–9685.
2. Miura, T., Komori, M. & Shiota, K., (2000) *Anat. Embryol.* **201,** 419–428.
3. Turing, A. (1952) *Philos. Trans. R. Soc. London B* **237,** 37–72.
4. Winfree, A. (1984) *J. Chem. Educ.* **61,** 661–663.
5. Murray, J. D. (2002) *Mathematical Biology* (Springer, Berlin), Vol. 2.
6. Meinhardt, H. (1995) *The Algorithmic Beauty of Sea Shells* (Springer, Berlin).
7. Gierer, A. & Meinhardt, H. (1972) *Kybernetik* **12,** 30–39.
8. Kondo, S. & Asai, R. (1995) *Nature* **376,** 765–768.
9. Höfer, T., Sherratt, J. A. & Maini, P. K. (1995) *Physica D* **85,** 425–444.
10. Murray, J. D., Deeming, D. C. & Ferguson, M. W. J. (1990) *Proc. R. Soc. London Ser. B* **239,** 279–293.
11. Davidson, D. (1983) *J. Embryol. Exp. Morphol.* **74,** 245–273.
12. Strutt, D. I. & Mlodzik, M. (1995) *Development (Cambridge, U.K.)* **121,** 4247–4256.
13. Palmeirim, I., Henrique, D., Ish-Horowicz, D. & Pourquié, O. (1997) *Cell* **91,** 639–648.
14. Keener, J. & Sneyd, J. (1998) *Mathematical Physiology* (Springer, Berlin).
15. Castets, V., Dulos, E., Boissonade, J. & De Kepper, P. (1990) *Phys. Rev. Lett.* **64,** 2953–2956.
16. Lee, K.-J., McCormick, W. D., Pearson, J. E. & Swinney, H. L. (1994) *Nature* **369,** 215–218.
17. Monk, N. A. M. (2000) *Endeavour* **24,** 170–173.
18. Oster, G. F., Shubin, N., Murray, J. D. & Alberch, P. (1988) *Evolution (Lawrence, Kans.)* **45,** 862–884.
19. Asai, R., Taguchi, E., Kume, Y., Saito, M. & Kondo, S. (1999) *Mech. Dev.* **89,** 87–92.
20. Ito, S. (2003) *Pigment Cell Res.* **16,** 230–236.
21. Oyehaug, L., Plahte, E., Vage, D. I. & Omholt, S. W. (2002) *J. Theor. Biol.* **215,** 449–468.
22. Kipling, R. (1902) *Just So Stories* (Doubleday, New York), reprinted (1994) (Puffin, New York).