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Development: Painting Flowers with MYBs

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Color patterns influence how attractive flowers are to bees, butterflies, and birds. By combining experiments and theory, a new study shows how a pair of MYB transcription factors orchestrates the formation of pigmentation patterns on monkeyflowers.

An insightful drawing by Alan Turing's mother shows her son — the founding father of computer science — as a schoolboy engaged in 'watching the daisies grow' rather than joining the ongoing hockey game next to him. Alan Turing kept a keen interest in the structures and patterns of plants and other organisms throughout his life [1], which in 1952 culminated in his famous pattern formation theory [2]. The basic idea of this self-organizing reaction–diffusion theory was that virtually any pattern — say spots or stripes — could be generated by mobile interacting molecules, which would function as a blueprint to regulate the patterning of color or structure [2]. Twenty years later, Hans Meinhardt and Alfred Gierer realized that one of the simplest ways to implement such self-organizing interactions is to couple a self-promoting short-range activator to an activator-induced long-range inhibitor [3–5]. In the absence of the activator no pattern is made, whereas in the absence of the inhibitor the activator uniformly fills the field. Only the interaction between the short-range activator and the long-range inhibitor generates spatial patterns. Strikingly, even this simple system can create an enormous diversity of patterns that are also observed in nature — from leopard spots to zebra stripes [6]. In this issue of *Current Biology*, Ding, Patterson, Holalu *et al.* [7] report that in monkeyflowers (*Mimulus* spp.) two transcription factors of the MYB family implement the simple activator–inhibitor system envisioned by Turing, Meinhardt, and Gierer (Figure 1A). This system can produce a large diversity of spot patterns, which the authors show to be important for proper plant–pollinator interactions.

Mimulus flowers display a conspicuous pigmentation pattern of red spots on a yellow background called nectar guides (Figure 1B). Spot formation had previously been shown to be under control of the self-promoting and pigment-activating MYB transcription factor NEGAN. Mutants deficient for NEGAN lack all red spots (Figure 1C), whereas ectopic NEGAN can activate uniform pigmentation and induce endogenous NEGAN expression [8]. To identify additional regulators of nectar-guide pigmentation, Ding, Patterson, Holalu *et al.* mutagenized *Mimulus* plants and recovered mutant offspring — coined RED TONGUE (RTO) — in which the normally spotted area was now nearly homogeneously red (Figure 1C). The authors hypothesized that the patterning phenotypes induced in NEGAN and RTO mutants can be interpreted within the Turing-Meinhardt-Gierer activator–inhibitor framework, in which NEGAN plays the role of the self-promoting activator and RTO acts as an inhibitor of spot patterning. However, it was not clear whether these putative activator and inhibitor molecules indeed fulfill the essential conditions needed to form spatial patterns. Is the inhibitor expressed under the control of the activator, and does the inhibitor act over a longer spatial range than the activator?

Using whole-genome sequencing, they mapped the RTO mutation to a transcription factor homolog that belongs to the same MYB family as the pigmentation activator NEGAN [9,10]. When the authors experimentally disrupted the inhibitor RTO by RNAi or CRISPR/Cas9-mediated mutagenesis, the coloring recapitulated the phenotype observed in the original RTO mutants — red-tongued flowers (Figure 1C). Importantly, overexpression of RTO abolished the red spots, demonstrating that RTO acts as an inhibitor in the spot patterning system (Figure 1C).

How exactly does the inhibitor (RTO) block the activator (NEGAN)? The authors noted that the normal RTO protein lacks DNA-binding and activation domains and thus cannot elicit a positive effect on transcription. The inhibitor RTO belongs to the R3 subclass of MYB proteins, whereas the pigmentation activator NEGAN is an R2R3 MYB transcription factor. It had previously been shown in other flowering plants that R3-MYB proteins block R2R3-MYB transcription factors by disrupting their interactions with important binding partners [8,11,12]. Thus, the inhibitor RTO likely acts as a decoy that competes with the activator for proteins necessary to induce transcription. Importantly, the authors also showed that production of the inhibitor RTO is under the control of the activator NEGAN, as predicted by theory.

Another fundamental tenet of the classical activator–inhibitor system is that the inhibitor must act at a longer range than the activator: Spatial patterns arise because activator-induced inhibitor molecules travel farther and suppress further activator production in the surrounding area, leading to non-uniform activator distribution, for example, as seen in spots. But since RTO is a transcription factor homolog, would it be able to move from cell to cell? The short answer is yes. To address this question, and to test the theoretical differential-range prediction, the authors generated a fluorescently tagged RTO and followed its

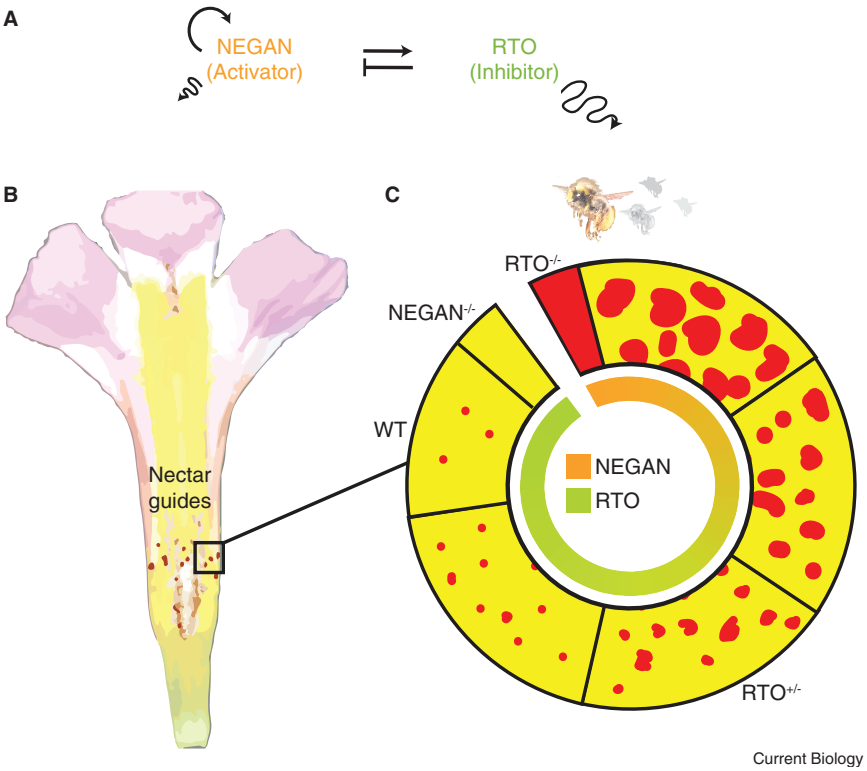

Figure 1. The pigmentation of monkeyflowers is controlled by an activator inhibitor system. (A) In a simple reaction diffusion model, a poorly mobile activator (NEGAN) induces the production of a more mobile inhibitor (RTO). (B,C) The spot pattern inside a monkeyflower's yellow tongue (flower image modified based on data from http://mimubase.org/strains) is predicted by the relative activator and inhibitor strengths in a gradual fashion, as indicated by the counterclockwise change of spot sizes (C). Pollinators, such as bumblebees, can be attracted to specific flower coloring and patterns.

distribution from producing cells using live imaging. In combination with careful observation of the pigments in the microscopic images, they indeed found that the inhibitor can travel much farther away from the producing cells than the activator, consistent with the requirements of classical self-organizing activator–inhibitor models.

Mimulus flowers naturally vary in their nectar guide patterns. To understand the potential ecological impact of the pigmentation changes, the authors turned to two naturally occurring red-tongued *Mimulus* varieties and found that these phenotypes are also caused by sequence changes in RTO. Interestingly, under laboratory conditions, bumblebees preferred to visit variants with red-tongued flowers (Figure 1C). These results suggest that parameters of the reaction–diffusion system, such as inhibitor levels, might affect the fitness of natural populations. It is tempting to speculate that this insight might be exploited in the future to grow crops more effectively by shifting pollinator attraction.

Taken together, this study used a powerful combination of genetics, computational modeling, and behavioral analyses to identify a new activator–inhibitor pair in plants, adding to the growing list of regulatory molecules that implement reaction–diffusion systems [6,13–15]. Several exciting questions remain about the *Mimulus* patterning system. First, concerning the emergence of the spotted patterns: How do the spots evolve during flower development, how does flower growth influence the patterns, and do spot densities and spacing proportionately adjust with flower size? Second, concerning the different ranges of activator and inhibitor: Do the activator and inhibitor proteins freely diffuse between cells, or do they require a dedicated transport system, similar to the plant morphogen auxin [16]? Furthermore, are the different ranges due to differences in mobility or protein stability, and are the range differences indeed important for pattern formation? Recent studies have shown that the requirement for differential diffusion in simple two-component activator–inhibitor systems can be relaxed in multi-component systems with mobile and immobile cell-autonomous factors [17,18]. It is thus possible that the different mobilities of NEGAN and RTO might act to tune the quality, spacing and robustness of the pattern rather than serving as a pattern formation condition *per se*. Finally, can the system be tuned to produce stripes rather than spots on the nectar guides of monkeyflowers, perhaps by saturating the production of the activator as predicted by theory [6]? To address these questions, it may be useful to follow the lead of the young Turing — to watch the monkeyflowers grow and thereby fully unravel the mechanism by which MYB proteins paint their flowers.

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