SYNTHETIC BIOLOGY

Reconstituting tissue patterning

A synthetic morphogen reveals quantitative principles of tissue patterning

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Multicellular organisms develop through a sequence of patterning events, in which cells adopt distinct cell fates. In many instances, patterns are established by morphogen gradients that determine cell fates according to the position of cells within a uniform field. On pages 327 and 321 of this issue, Toda *et al.* (*1*) and Stapornwongkul *et al.* (*2*), respectively, use synthetic approaches to study morphogen gradients. Why are synthetic approaches helpful? Patterning systems operate in complex biological settings, and synthetic reconstitution isolates and defines the key players. Because the features of such systems depend directly on quantitative parameters, synthetic approaches allow reconstituting systems in which parameters can be precisely tuned and their effects measured with precision. Additionally, the regulation of patterning systems relies on different feedback loops, and synthetic rewiring highlights the logic of the critical circuits. Overall, the key parameters and players can be examined from different angles.

The concept of morphogen gradients as mediators of tissue patterning was proposed by Wolpert in 1969, coined as the "French-Flag" model (*3*). Morphogens are molecules that can trigger cells to adopt different fates, depending on the morphogen concentration the cells encounter. Localized production of a morphogen at a restricted source creates a concentration gradient that accordingly confers a complex, position-dependent patterning of the receiving cells (*4*). Experiments over the past three decades confirmed the involvement of morphogen gradients in a large number of patterning events, such as specification of distinct neural fates within the spinal cord or positioning of veins in the fly wing (*5*–*7*). Yet, these experiments also revealed that the actual establishment and utilization of morphogens are considerably more complex than initially envisioned.

Questions arose as to whether the movement of morphogen across the field depends on passive diffusion in the extracellular milieu or if more active processes involving transport between cells through specialized mechanisms play a role (*8*).

Moreover, in all systems studied, morphogen distribution depends on a myriad of feedback loops regulating its movement, degradation, production, or downstream activity. Perhaps surprisingly, although systems utilizing patterning by morphogens are continuously discovered, the number of identified morphogens remains small: Systems representing different contexts or organisms repeatedly employ the same few morphogen molecules.

The potential of synthetic systems to disentangle the complexity of morphogen systems and reveal their design principles was demonstrated with the Hedgehog (HH) morphogen (*9*). The HH pathway is distinct
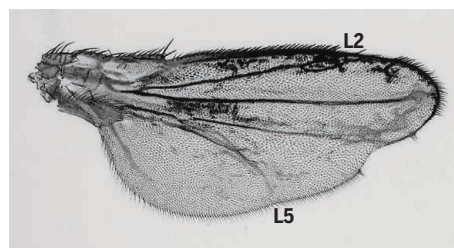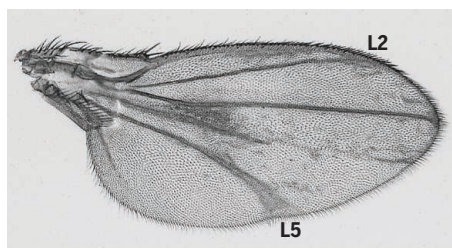

An engineered green fluorescent protein (GFP) response reconstitutes patterning in the *Drosophila melanogaster* wing pouch. Stapornwongkul *et al.* reconstituted wing patterning in a *dpp* (decapentaplegic) mutant background. Secreted GFP was expressed from a stripe of cells at the disc center. All cells in the wing pouch expressed chimeric receptors carrying the GFP nanobody on the extracellular domain (left panel). Additional expression of an anchoring protein displaying a GFP nanobody led to more accurate patterning (right panel). The nested expression of *sal* (homeotic spalt-major) and *omb* (optomotor-blind) target genes was restored, as reflected in proper localization of L2 and L5 longitudinal wing veins.

from most morphogen signaling pathways because it uses a "bifunctional-negative" strategy within the same molecule: The HH receptor Patched (PTCH) inhibits downstream HH signaling and also sequesters external HH. Binding of HH to PTCH relieves the PTCH inhibitory activity and induces the expression of PTCH as one of its downstream targets. What could be the consequence of such a design? Motivated by a mathematical model and using the ability to control parameters within a synthetic system, it was revealed that the double-negative design promotes reliability: It accelerates the approach to steady state and provides robustness to variation in ligand production rates

Synthetic systems therefore enable flexible control of parameters such as cell density or availability of different molecules. When using the normal signaling compo-

nents in heterologous settings, however, the synthetic approach is still limited by the properties and complexity of the selected pathway. This restricts the ability to fully test or construct artificial morphogen-based mechanisms. The studies by Toda *et al.* and Stapornwongkul *et al.* have overcome these hurdles by converting an inert molecule—green fluorescent protein (GFP)—into a morphogen. This allowed tight control of the morphogen parameters and a broad examination of morphogen-based mechanisms.

Toda *et al.* converted a secreted GFP into a morphogen by adapting a paradigm they previously established (*10*). In this system, a synthetic Notch receptor carrying a GFP-binding protein is activated to induce target genes of interest when presented with GFP that is anchored to a neighboring cell membrane. To follow the distribution of secreted GFP, the authors mixed receiving cells, carrying the synthetic Notch receptor, with cells carrying a transmembrane protein capable of binding GFP and anchoring it. GFP is then made to act as a morphogen: Diffusible GFP is released from a localized source of secreting cells into a field composed of a mixture of receiving cells carrying the synthetic Notch receptor, and anchoring cells that capture the diffusible GFP and present it to the receiving cells.

This system provides easy control of different morphogen parameters. For example, changing the density of the anchor cells or the affinity by which they bind GFP modulated the range of GFP diffusion. Expression of a secreted inhibitory molecule from an opposite pole provided another means for reducing the range and level of the gradient. Additionally, a positive feedback loop can be generated by making secreted GFP

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a target gene for the synthetic receptor. Combining these manipulations, Toda *et al.* generated a propagating wave of patterning, akin to the movement of the morphogenetic furrow in the developing *Drosophila melanogaster* (fruitfly) eye. Conversely, an engineered negative feedback response to the pathway dampened the response amplitude and accelerated the approach to steady state, which is the state at which most morphogen gradients function.

With this toolkit in hand, it was possible to generate elaborate patterns. In their final demonstration, the authors combined one pole that secreted the red fluorescent protein mCherry as the morphogen, and an opposite pole that secreted the inhibitor. The cells between the poles expressed synthetic receptors for either mCherry or GFP and carried the positive feedback to express secreted GFP. With this setting, three domains of gene expression could be identified, mimicking an expected morphogen response (see the figure).

Stapornwongkul *et al.* took on the additional challenge of examining whether a secreted GFP can act as a morphogen within the context of the whole organism. They focused on the well-characterized Dpp (decapentaplegic) gradient in the wing imaginal disc of *D. melanogaster* (*5, 6*), investigating whether a circuit can be engineered that will imitate Dpp function in disc patterning. They tested diffusion properties of secreted GFP by releasing it from the stripe of cells normally secreting Dpp in vivo. Having established the properties of GFP distribution, the authors investigated whether GFP distribution can replace the function of Dpp in patterning the wing disc. They engineered the normal Dpp receptors Thickveins (Tkv) and Punt (Put), to bind GFP. The assumption was that a GFP dimer would juxtapose the two receptors, leading to their activation. This was indeed the case, although, surprisingly, even a monomeric GFP was sufficient for pathway activation. A nested expression of the target genes *sal* (homeotic spalt-major) and *omb* (optomotor-blind) could be detected, but with a profile that differed from normal. However, when a nonsignaling membrane-tethered protein that facilitated GFP trapping was also expressed, the resulting gene expression pattern was markedly similar to the wild-type profile (see the images).

Generating a synthetic morphogen

Toda *et al.* created a source of secreted green fluorescent protein (GFP). The response was marked by transcription factor (TF)–mediated induction of mCherry expression. When the cells carried a positive feedback loop by induction of GFP expression, the response was propagated. When the cells harbored a negative feedback loop, the response was attenuated and steady state was reached faster.
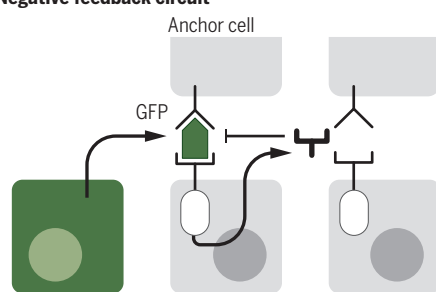
Morphogen feedback circuit
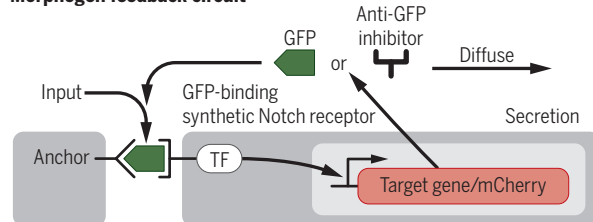
Positive feedback circuit
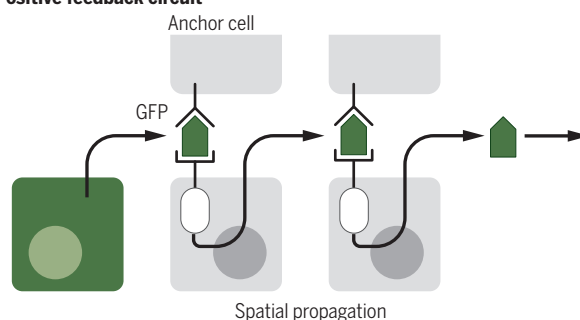
Negative feedback circuit

Nonsignaling receptors allow more stable trapping of the diffusible ligand, increasing the concentration of ligands presented to the signaling receptor. Such traps, however, also limit the diffusion of the morphogen. This explains the need for fine-tuning their expression, or alternatively, to allow nonsignaling receptors to dissociate from the membrane and traffic the associated morphogen further.

The generation of synthetic morphogen gradients with different regulatory modules represents an impressive technological feat. In addition to confirming previous ideas that were based on genetic manipulations, it allows detailed analysis of quantitative parameters that are not approachable with endogenous systems. Particularly informative is the ability to monitor, in time and space, the dynamics in which the system reaches steady state. The dynamics provides

essential clues regarding the generation and maintenance of a stable morphogen gradient. Such properties render these systems promising for addressing long-standing issues that are seminal to morphogen patterning. For example, an important question is how sharp borders of target-gene expression are created between adjacent groups of cells. Although morphogen gradients are continuous, sharp gene expression borders ensue, suggesting that cells can distinguish small differences in morphogen concentration and translate them to a "yes or no" decision regarding gene expression. The systems described by Toda *et al.* did not give rise to such sharp borders, suggesting that additional mechanisms are required.

Another intriguing topic involves the ability of developmental systems to buffer morphogen gradients against fluctuations (robustness) and adjust the gradient with tissue size (scaling). Several models have been proposed to explain robustness and scaling—for example, self-regulated production of a diffusible molecule that impinges on the global distribution of the morphogen. When expression of such a molecule depends on the morphogen, it allows measurement of the morphogen concentration at the edge of the gradient, and adjusts the entire profile accordingly (*11*). It will be exciting to investigate whether synthetic approaches could test such models rigorously in the future. In the long term, synthetic approaches may facilitate tissue engineering. With the capacity to manipulate the production, diffusion, and response to engineered proteins and pathways, it may be easier to execute in culture developmental processes that will allow the formation of artificial tissues. ∎

REFERENCES AND NOTES

1. S. Toda *et al.*, *Science* **370**, 327 (2020).
2. K. S. Stapornwongkul *et al.*, *Science* **370**, 321 (2020).
3. L. Wolpert, *J. Theor. Biol.* **25**, 1 (1969).
4. K. W. Rogers, A. F. Schier, *Annu. Rev. Cell Dev. Biol.* **27**, 377 (2011).
5. T. Lecuit *et al.*, *Nature* **381**, 387 (1996).
6. D. Nellen *et al.*, *Cell* **85**, 357 (1996).
7. A. Sagner, J. Briscoe, *Development* **146**, dev182154 (2019).
8. P. Müller *et al.*, *Development* **140**, 1621 (2013).
9. P. Li *et al.*, *Science* **360**, 543 (2018).
10. L. Morsut *et al.*, *Cell* **164**, 780 (2016).
11. B. Z. Shilo, N. Barkai, *Dev. Cell* **40**, 429 (2017).

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