Switching Perspectives on Pattern Formation in Biology

Self-organized Notch dynamics generate stereotyped sensory organ patterns in *Drosophila*
Authors: Francis Corson, Lydie Couturier, Hervé Rouault, Khalil Mazouni, and François Schweisguth
Science **356**, eaai7407 (2017)

Recommended with a Commentary by David K. Lubensky, University of Michigan

As they move around, grow, develop, and generally go about their lives, living systems constantly create intricate patterns, ranging from a zebra's stripes to the complex morphologies of bacterial colonies. Scientists have wondered how organisms can generate these patterns—and, moreover, do so reliably and reproducibly—for almost as long as biology has been studied. At least since Turing's classic paper on reaction-diffusion systems [14], theorists have commonly viewed biological pattern formation through the lens of finite-wavelength, linear instabilities of a spatially uniform initial state. In particular, they have often, when confronted with a new pattern, tried to identify systems consisting of a coupled activator and inhibitor to which some version of Turing's ideas can be applied [6]. For a variety of reasons, however, it has proven remarkably difficult to find clear examples of Turing patterns in actual, living organisms [8, 2, 11]. Over the years, a number of alternative pattern formation mechanisms have thus been proposed [15, 4, 10, 1, 9, 7, 13], some of which have been quite successful in explaining observations on particular systems. Whether these alternatives are sufficient to supplant a picture based on Turing-inspired linear instabilities has, however, remained controversial. Now, Corson *et al.* [5] revisit a textbook activator-inhibitor example, the spacing of bristles on the *Drosophila* dorsal notum by Notch-mediated lateral inhibition, using the latest in live imaging technology. With the help of an elegant phenomenological model, they conclude that this system employs a hybrid mechanism to create robust patterns, augmenting templated activation of bistable, switch-like circuits within each cell [1, 9, 7] with a limited capacity for instability-mediated self-organization. They thereby add an important new twist to the ongoing debate on pattern formation in living systems.

Notch-mediated lateral inhibition is a highly-conserved process used to select single, isolated cells to become neurons while ensuring that their neighbors do not assume the neural fate. Fig. 1 gives a caricature of how it works: Cells expressing high levels of neural fate markers (left), indicating they are on the way to becoming neurons, upregulate the ligand molecule Delta (black arrow). This molecule binds to the Notch receptor in neighboring cells, initiating a signaling cascade that ultimately results in the repression of neural fate,

and hence of Delta, in those neighbors (right; blunt-ended black arrow). Thus, once one cell is well on its way to becoming a neuron, it forces those cells that receive its Delta signal not to go down the same path. Depending on which cell "wins" this battle for neural fate, the signals could of course also travel in the opposite direction, but the signaling pathway in this direction is not activated when the right-hand cell has low levels of neural fate markers, as drawn (lighter arrows and Delta and Notch molecules, above). In this simplest form, the Notch-Delta system drives a linear instability from an initial, uniform pattern of Delta expression to a regular pattern of alternating neural and non-neural cells, in a manner very much in the spirit of Turing's original model of diffusing activators and inhibitors (albeit with the important differences that pattern length scales are set by the cell size rather than by diffusion lengths and that self-activation is replaced by the geometric constraint that a cell cannot directly inhibit itself). A large body of recent work, however, has emphasized that the signaling network can in reality be much more complicated; in particular, a variety of molecular mechanisms can have the net effect of introducing positive feedback loops of Delta on its own activity within each cell (orange dashed arrow), which can allow Delta levels to become bistable for certain ranges of Notch signal inputs (see, for example, [12, 1, 7, 3, 5] and references therein).

One classic context where Notch-mediated lateral inhibition is responsible for pattern formation is the stripes of mechanosensory bristles ("microchaetae") on the back of the adult fruit fly. The bristles look like prominent, thick hairs that form a series of parallel rows down the fly's back, with individual bristles having roughly regular spacing along each row (Fig. 2). These patterns of hairs can be traced back to patterns of cells expressing high levels of neural fate markers in the early pupal stage of fly development. (The bristles are associated with neurons that transmit information about mechanical deformations of the hairs back to the fly brain, hence the connection to patterns of neural fate.) In a textbook presentation, the formation of bristle patterns proceeds in two steps: First, neural fate markers are upregulated uniformly in a long "proneural stripe" about 5 or 6 cells wide. What determines the location of this stripe and the spacing between different stripes is often left rather vague; one popular view has been that some sort of prepattern established by the expression of other genes dictates their position. Then, in a second stage, lateral inhibition within each stripe selects individual cells to become neurons while inhibiting neural fate in all of the other cells. (The spacing of the bristles is too large to be consistent with Notch-Delta signaling only between neighboring cells; this is thought to be explained by the presence of long, thin, Delta-rich filopodia that cells extend outwards over distances as large as several cell diameters to signal to cells that are not their immediate neighbors.)

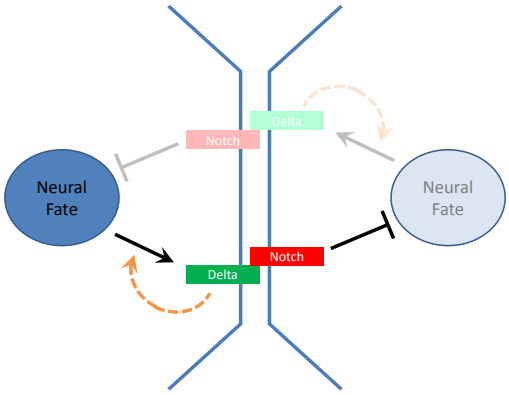

Figure 1: Cartoon of Notch-mediated lateral inhibition. (See text for an explanation of symbols.)

Corson and coworkers set out to determine whether this standard picture is correct. In

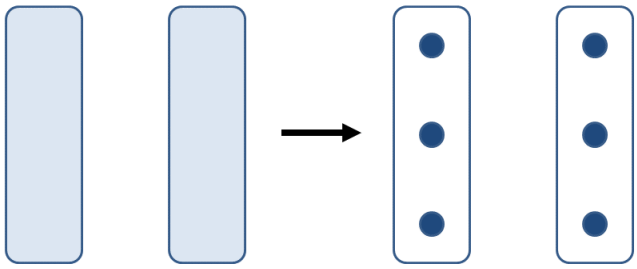

Figure 2: Formation of bristle patterns in *Drosophila*. Initially, all cells have low expression of neural fate markers (not shown). These markers are then upregulated to an intermediate level in stripes of cells (light blue, left). The pattern of neural markers in these stripes then gradually refines, until the markers are expressed at high levels in isolated, regularly spaced cells that will eventually become the bristles (dark blue circles, right), but have been turned off in all other cells. Remarkably, the "winning" bristle precursors are consistently found in the centers of the stripes, an observation that is difficult to reconcile with a pattern-forming mechanism that relies primarily on instabilities of spatially uniform states but that arises naturally within a switch and template model. (Only two stripes and three neural cells per stripe are shown; in reality, both numbers are larger.

particular, they asked: 1) Is the location and spacing of the proneural stripes set entirely by a prepattern, or does self-organization and interaction between the stripes play a role? 2) If self-organization is important at the stripe stage, is it driven by Notch signaling or by some other pathway? 3) Is the selection of individual neural cells from each stripe an entirely self-organized process driven by a linear instability, or do other mechanisms play a role? Their answers, in brief, are 1) There is a pattern of initial Delta expression, but after that stripe location and spacing is driven by interactions between the stripes, not entirely dictated by a rigid prepattern. 2) Thus, stripe formation and stripe resolution to individual cells are both driven by Notch signaling. And 3) The refinement of proneural stripes to a pattern of individual neural cells is not driven by a simple linear instability, but instead by a more involved mechanism in which cell-autonomous bistability plays an important role.

To arrive at these conclusions, Corson *et al.* began by imaging the expression of Delta, of proneural markers, and of other related genes in living flies over the course of about 10 hours early in the pupal stage of development. This remarkable technical feat is central to their paper: If they have been able to understand better how bristles are patterned, this is in large part because they are the first to have been able to watch the evolution of the pattern over time.

To interpret these time courses of gene expression, Corson *et al.* turn to a very simple model. The state of cell j is described by a single variable u_j that varies from 1 for cells committed to become neurons to -1 for cells that definitely will not adopt a neural fate. u_j feeds back positively on itself and is also influenced by an aggregate signal s_j from all neighboring cells, leading to the dynamics

$$\frac{du_j}{dt} = f(u_j - s_j) - u_j \ ,$$

where f is a sigmoidal function encoding the positive feedback and

$$s_j = \sum_k c_{jk} D(u_k)$$

is a weighted sum of inputs from other cells with strength $D(u_k)$, where D is a strongly increasing function of its argument. If the signal s_j is viewed as a bifurcation parameter, then u_j has a classic bistable bifurcation diagram: At high enough values of s_j, u_j always flows to a single, low fixed point, and at low enough s_j it has only a single fixed point at a higher value of u_j. For intermediate s_j, however, both fixed points persist, and u_j is bistable. The author's use of an entirely phenomenological model, in which a great deal of biological complexity is boiled down to a single variable u_j that does not have a straightforward molecular interpretation, is the second key to their ability to make progress on a difficult problem: Previous models have usually felt compelled to use the concentrations of specific molecules as their dynamical variables, and the consequent complexity has often made it hard to analyze the resulting equations cleanly. In contrast, by insisting on the simplest description consistent with the basic experimental facts, Corson *et al.* are able to understand the different possible dynamical regimes in depth and thus to come to clear conclusions about which scenarios are allowed by their data and which are ruled out.

To understand how the Corson *et al.* model behaves, it is useful to think of two limiting cases; Corson and coworkers' full description of the patterning process essentially interpolates between these extremes. One limit is that of a Turing-like linear instability: If u is initially set to an intermediate value in every cell, any small fluctuations in u levels will be amplified, with some cells rising towards the high-u, neural state and others being forced down to the low-u, non-neural state. This limit has the advantage that you are guaranteed that only isolated cells will become neurons, but the disadvantage that instabilities are by their very nature difficult to control, leading to at least some variability in the final pattern. The alternative limit emphasizes the bistable, switch-like nature of u's dynamics. We imagine that u starts low in all cells and that some external signal then gradually causes it to increase (or equivalently, causes s to decrease). Eventually, for small enough s, the low u state will disappear in a saddle-node bifurcation, and cells will begin flowing towards the high u state. Those that get there first will then be in a position to inhibit their neighbors, increasing the s levels they see and forcing them back down to low u. In this mechanism, the timing of initial activation of u essentially determines which cells will eventually become neurons – those that are activated first have a built-in advantage that is difficult to overcome. Thus, the pattern-forming dynamics can refine and extend an initial template or prepattern, but they do not spontaneously break symmetry to create a pattern in the absence of any initiating cues. This has the advantage of allowing a more controlled pattern formation process. In the form in which it was originally proposed [1, 9, 7], however, this "switch and template" mechanism contained no means to turn off cells that had reached the high u fixed point, making it susceptible to characteristic errors in which twinned neural cells appear. The richer description proposed by Corson *et al.*, however, shows how to move away from this extreme limit and ensure that such situations are always unstable. It combines the switch and template advantages of directed, step-wise pattern formation with an instability-driven mechanism's intrinsic error correction ability.

The work of Corson *et al.* hence provides the strongest contribution yet to the growing body of evidence [1, 9, 7, 3] that cell-autonomous positive feedbacks, and the bistable, switch-like behavior they induce, are essential elements of Notch-mediated pattern formation. This mechanism contrasts strongly with one in which everything proceeds through instabilities, but it explains a number of observed features that are otherwise difficult to understand. Foremost among these is the clear, reproducible order in which proneural stripes (and the analogous proneural groups in other related systems) refine: It is almost always seen that the cells at the edges of the stripes turn off first, even though they might naively be thought to receive a weaker inhibitory signal than the cells in the center of the stripe, and that the eventual neural cells are in the center of the stripe. This is difficult to explain with an instability, but makes perfect sense in a switch and template picture when one realizes that the initial activating signal is stronger in the stripe centers than at the edges. At the same time, because all of the cells in the center of the stripe are activated almost simultaneously, a switch-based mechanism cannot easily explain why this center line breaks up into a pattern of single, evenly-spaced neurons. Corson and coworkers solve this problem by allowing an instability to break up groups of cells that the prepattern has been unable to distinguish. Their model thus manages to have the best of both worlds.

References

[1] O. Barad, D. Rosin, E. Hornstein, and N. Barkai. Error minimization in lateral inhibition circuits. *Sci Sign*, 3:ra51, 2010.

[2] T. Biancalani, F. Jafarpour, and N. Goldenfeld. Giant amplification of noise in fluctuation-induced pattern formation. *Phys Rev Lett*, 118:018101, 2017.

[3] K.-Y. Chen, *et al.*. A notch positive feedback in the intestinal stem cell niche is essential for stem cell self-renewal. *Mol Sys Biol*, 13:927, 2017.

[4] J. Cooke and E. C. Zeeman. A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. *J Theor Biol*, 58:455, 1976.

[5] F. Corson, L. Couturier, H. Rouault, K. Mazouni, and F. Schweisguth. Self-organized notch dynamics generate stereotyped sensory organ patterns in drosophila. *Science*, 356:eaai7407, 2017.

[6] A. J. Koch and H. Meinhardt. Biological pattern formation: from basic mechanisms to complex structures. *Rev Mod Phys*, 66:1481, 1994.

[7] D. K. Lubensky, M. W. Pennington, B. I. Shraiman, and N. E. Baker. A dynamical model of ommatidial crystal formation. *Proc Nat'l Acad Sci USA*, 108:11145, 2011.

[8] P. K. Maini, T. E. Woolley, R. E. Baker, E. A. Gaffney, and S. S. Lee. Turing's model for biological pattern formation and the robustness problem. *Interface Focus*, 2:487, 2012.

[9] M. W. Pennington and D. K. Lubensky. Switch and template pattern formation in a discrete reaction-diffusion system inspired by the drosophila eye. *European Phys J E*, 33:129, 2010.

[10] S. Y. Shvartsman, C. B. Muratov, and D. A. Lauffenburger. Modeling and computational analysis of egf receptor-mediated cell communication in drosophila oogenesis. *Development*, 129:2577, 2002.

[11] E. D. Siggia. A geometric model of stripe refinement. *Dev Cell*, 41:225, 2017.

[12] D. Sprinzak, A. Lakhanpal, L. Lebon, L. A. Santat, M. E. Fontes, G. A. Anderson, J. Garcia-Ojalvo, and M. B. Elowitz. Cis-interactions between notch and delta generate mutually exclusive signalling states. *Nature*, 465:86, 2010.

[13] D. Thalmeier, J. Halatek, and E. Frey. Geometry-induced protein pattern formation. *Proc Nat'l Acad Sci USA*, 113:548, 2016.

[14] A. M. Turing. The chemical basis of morphogenesis. *Phil Trans Royal Soc B*, 237:37, 1952.

[15] L. Wolpert. Positional information and the spatial pattern of cellular differentiation. *J Theor Biol*, 25:1, 1969.