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From Notch signaling to fine-grained patterning: Modeling meets experiments

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Notch signaling is the canonical signaling pathway between neighboring cells. It plays an important role in fine-grained patterning processes such as the formation of checkerboard-like differentiation patterns and sharp boundaries between developing tissues. While detailed information about many of the genes and proteins involved have been identified, we still lack a quantitative mechanistic understanding of these processes. Here we discuss several recent studies that provide novel insights into Notch-dependent patterning by combining mathematical models with quantitative experimental results. Such approaches allow identification of mechanisms and design principles controlling how patterns are generated in a reproducible and robust manner.

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Introduction

The effort to understand how patterns of differentiation are generated during development has been divided for many years into two main approaches. On one hand, abstract mathematical models provided conceptual frameworks but lacked information about molecular details. On the other hand, genetic approaches provided detailed molecular information but typically lacked quantitative systemic understating. In recent years, successful combination of experimental and modeling approaches provided new, systems-level understanding of various developmental processes [1,2] bridging the gap between these two approaches.

In this review, we focus on a large class of developmental patterning processes, termed fine-grained patterning, in which neighboring cells adopt distinct fates. Fine-grained developmental processes such as lateral inhibition and boundary formation occur repeatedly during development, often employing similar genes, signaling pathways, and circuit architectures. The key component in these processes is the Notch signaling pathway, which is the canonical signaling pathway between neighboring cells in metazoans [3]. Several recent works used a combination of modeling and experiments to reveal general design principles underlying Notch-dependent fine-grained patterning. These provided deeper understanding of the role of post-translational regulation, complex cell morphology, and dynamic behaviors in such processes.

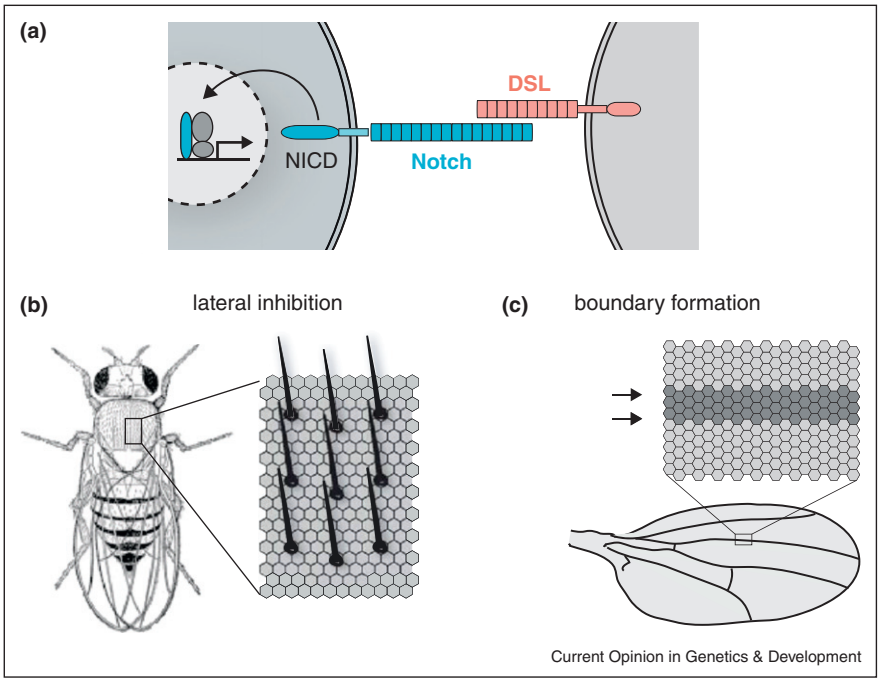
This review is organized as follows. After a short overview of the Notch signaling pathway, we review several of the most significant works in the field, addressing the following aspects of Notch-dependent patterning. Firstly, how the Notch signaling pathway converts graded input into a sharp switch. Secondly, how robust lateral inhibition patterns are formed. Thirdly, how lateral inhibition spacing is determined. And finally, the emergence of dynamic lateral inhibition phenomena. Due to space limitations, several modeling-based studies are not directly covered in this review [4–9].

Overview of Notch signaling and Notch-dependent patterning

Notch signaling is mediated by the interaction between Notch receptors and Delta/Serrate/LAG-2 (DSL) ligands, which are highly conserved across metazoans [10]. Upon interaction between a Notch receptor in one cell and a DSL ligand in a neighboring cell, the Notch intracellular domain (NICD) is cleaved, translocates to the nucleus and co-activates downstream transcriptional targets (Figure 1a).

One of the classical Notch-dependent patterning processes is lateral inhibition, which is a general mechanism for the formation of alternating, checkerboard-like, differentiation patterns [11]. This mechanism is based on the competition between an initially equivalent group of cells, with one or more cells ''winning the race'' by inhibiting the differentiation of its neighbors. The winner is determined by amplification of small initial, often stochastic, differences between the cells [12] (see Box 1 for a mathematical description of this process). Examples of lateral inhibition include the AC/VU differentiation in *Caenorhabditis elegans* [13], bristle patterning in flies [14–16] (Figure 1b), neurogenesis in flies and vertebrates [17–19], and inner ear patterning in vertebrates [20–22].

Figure 1



Notch signaling and Notch-dependent patterning. **(a)** Notch signaling is mediated by the interaction of Notch receptors and Delta/Serrate/LAG-2 (DSL) ligands. This interaction is followed by the cleavage of Notch intracellular domain (NICD) which then translocates to the nucleus and co-activates downstream transcriptional targets. **(b)** Lateral inhibition process underlies the robust bristle patterning on the fly notum. **(c)** Notch signaling is essential for defining sharp boundaries in the formation of wing veins in the fly.

Notch signaling is also commonly used for defining boundaries between developing tissues, for example the formation of wing veins (Figure 1c) [23,24] and margin boundaries [25–28] in the developing fly and the rhombomere boundaries in vertebrate hindbrain [29]. Notch has a role in various other processes such as synchronizing cells during somitogenesis [30], asymmetric cell division [31,32], and neuronal plasticity [33,34].

Generating a sharp switch

Notch signaling is often used for enhancing differences and generating a switch-like behavior during cell fate determination of neighboring cells. For example, wing vein boundaries in *Drosophila* are sharply defined by a process involving Notch signaling [23,24]. Sprinzak *et al.* [35••] have recently shown that inhibitory interactions between Notch and DSL within the same cell, termed *cis*-inhibition (Figure 2a), might bring such a sharp switch about. *Cis*-inhibition between Notch and DSL ligands has been previously shown to occur in various developmental contexts but its physiological role has been controversial [23,36–43].

In this study, time-lapse microscopy of individual mammalian cells was used to quantitatively monitor the Notch signaling response to Delta ligands in *cis* and in *trans*. The key feature which stood out from the experimental data was that Notch signaling response exhibited a sharp switch-like behavior with respect to *cis*-Delta levels and a graded response with respect to *trans*-Delta. How can signaling response be so different for ligands presented in *cis* and in *trans*?

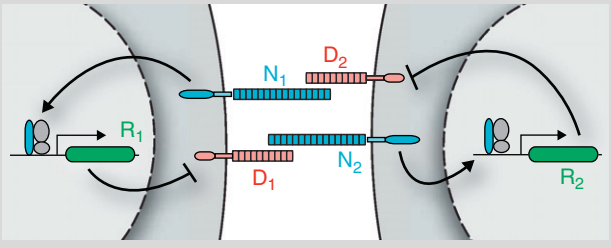
Using a mathematical model Sprinzak *et al.* [35••] showed that such behavior could be explained if Notch and Delta in the same cell bind and mutually inactivate each other. In this case, the relative levels of Notch and Delta determine the cell's signaling state: If a cell has more Notch than Delta it becomes a 'receiver' (the cell can receive but not send, Figure 2b, left). Conversely, if a cell has more Delta than Notch then it becomes a 'sender' cell (the cell can send but not receive; Figure 2b, right). For strong *cis*-interactions the transition between these two states is extremely sharp which accounts for the observed switch-like behavior.

This model provided insight into how sharp wing vein boundaries in *Drosophila* are formed. In that case, the sharp boundary is formed when a switch between 'senders' and 'receivers' occurs along a gradient of Delta expression (Figure 2b) transverse to the vein axis. Interestingly, this model explains a peculiar mutant behavior where a double heterozygous mutant of Notch and Delta

Box 1 Modeling lateral inhibition

Lateral inhibition is one of the most studied developmental patterning processes. The concept of lateral inhibition was initially proposed by Wigglesworth [65] who noticed that new bristles in the developing kissing bug (*Rhodnius prolixus*) emerged at positions, which are the most distant from existing bristles. He hypothesized that existing bristles inhibit the formation of new ones by draining ''an essential element'' from their surroundings. It took additional 50 years to realize that Notch signaling is the inhibitory signal involved in this process [14]. Mathematical models of Notch-dependent lateral inhibition have emerged soon after [12] (although reaction–diffusion-based lateral inhibition was treated earlier [66]). Here, we describe a simplified model of lateral inhibition and explain some of the modifications to the base model that have been proposed in the works described in this review.

Base model: Each cell (labeled by index i) is characterized by the concentrations of Notch (N_i), Delta (D_i) and a repressor (R_i) whose interactions are depicted in the diagram below.



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This set of interactions can be described by the following differential equations:

$$\frac{dN_i}{dt} = \beta_N - N_i \quad (1)$$

$$\frac{dD_i}{dt} = \beta_D\, f_{dec}(R_i) - D_i \quad (2)$$

$$\frac{dR_i}{dt} = \beta_R g_{inc}(S_{ij}) - R_i \quad (3)$$

where β_N, β_D, and β_R are the normalized production rates of Notch, Delta, and the repressor. f_{dec} is a decreasing sigmoidal (Hill) function corresponding to the repressions of Delta by the repressor. g_{inc} is an increasing sigmoidal (Hill) function corresponding to the activation of the repressor by the signal from neighboring cells. And, S_{ij} is the total signal cell i receives from its neighbors, which is given by: $S_{ij} = \sum_j s_j \propto \sum_j N_i D_j$. Here, the sum is over the signals, s_j, from all the neighbors j of cells i. This model can produce lateral inhibition patterning such as the one showed in Figure 2a. Various versions of this model have been analyzed by several groups [12,67–71].

***Cis*-inhibition**: *Cis*-inhibition can be added to the base model by introducing a binding reaction between Notch and Delta in the same cell that leads to an inactivated complex: $N_i + D_i \rightleftharpoons [N_i D_i] \rightarrow \phi$. In the limit of strong *cis*-inhibition [35••], the resulting equations for Notch and Delta are

$$\frac{dN_i}{dt} = \beta_N - N_i - k_c N_i D_i \quad (4)$$

$$\frac{dD_i}{dt} = \beta_D\, f_{dec}(R_i) - D_i - k_c N_i D_i \quad (5)$$

where the parameter, k_c, describes the binding strength between Notch and Delta in *cis*. The two additional terms give rise to the sharp switch-like behavior described in the text.

Time delay: The regulatory steps in the base model actually lump together several processes such as transcription, translation, and translocation, which are not explicitly described by the model. These effects can be described mathematically via introduction of time delayed variables [46••,58,59]. For example, the expression of the repressor at time t may be a function of the signal at an earlier time, $(t-\tau)$. In that case, the signal, S_{ij}, in Eq. (3) will be replaced by $S_{ij}(t-\tau)$.

Number of cells in contact: Number of cells in contact may also be an important parameter in the system [47••] and may dynamically vary over time. Mathematically, the signal a cell receives is summed over all the cells it contacts which can be tuned to the geometry considered.

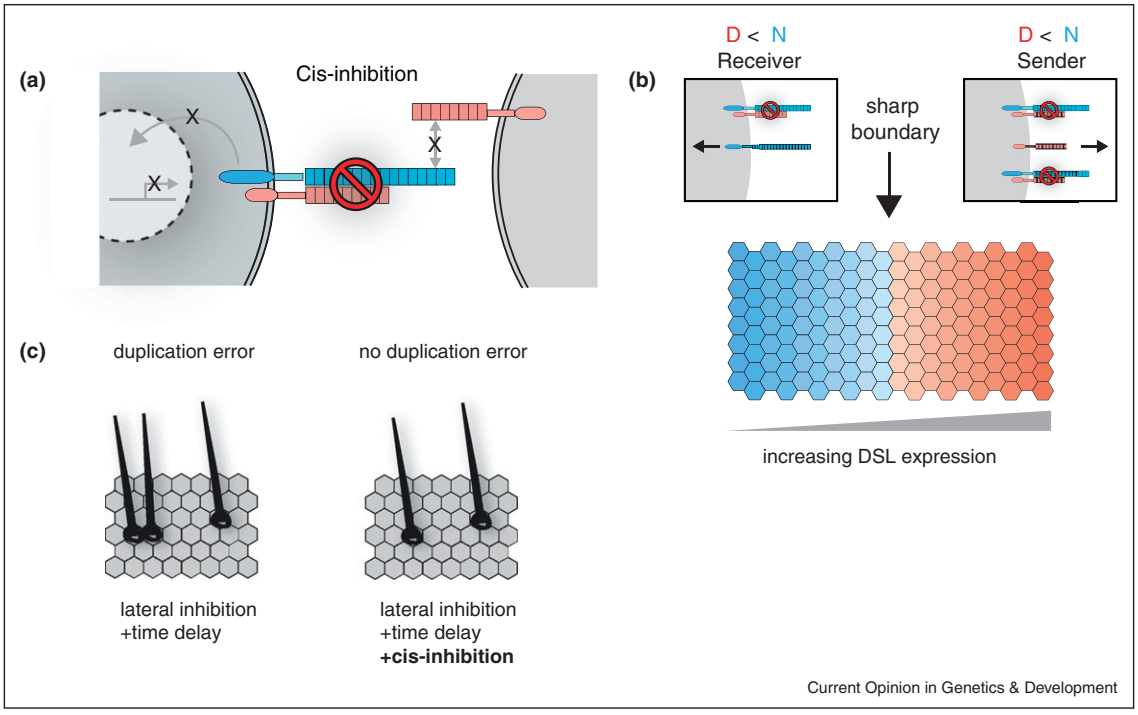
(single gene copy of both) exhibits no phenotype, while single heterozygous mutant, (either Notch or Delta) exhibit thick veins phenotypes [44]. Further analysis of this model revealed that the *cis*-inhibition switch can facilitate the robust formation of both sharp boundaries and lateral inhibition patterning over a broader set of parameters compared to classical models [45•].

Error minimization in patterning

An additional, surprising, role for *cis*-inhibition in the formation of bristle patterning in Drosophila has recently been proposed by Barad *et al.* [46••]. The pattern of sensory bristles in flies is determined through a process of lateral inhibition, where sensory organ precursor cells (SOP) are selected from a group of cells called the proneural clusters [14–16]. The patterns observed in wild-type flies are highly reproducible; however, in rare cases a bristle duplication error is observed (i.e. formation of two adjacent bristles, see Figure 2c). The authors used a probabilistic model to show that the classical model of lateral inhibition is expected to produce a much higher duplication error rate if realistic time delays due to transcription and translation (a few minutes) are introduced into it. The duplication errors in this case are formed because neighboring cells can become nonresponsive to each other's inhibitory signals during the time delay. How then, such a low error rate is observed in wild-type flies?

Barad *et al.* hypothesized that strong *cis*-interactions will produce a threshold response in each cell where signaling is initiated only after Delta accumulates enough to deplete the free Notch from the cell. Once the prospective SOPs (the one with highest Delta) cross this threshold, it will start signaling its neighbors and at the same time become unresponsive to their signals. The neighboring cells, being below threshold level at this

Figure 2



Cis inhibition and error minimization. **(a)** Interaction between Notch and DSL ligand in the same cell leads to inhibition of signaling. Notch receptors and DSL ligands form complexes which prevent their binding to receptors and ligands from the neighboring cells. **(b)** When a cell expresses more Notch than DSL, most of the DSL ligand is bound to Notch. This results in excess of free Notch leading to a 'receiver' state (left). Conversely, when a cell expresses more DSL than Notch, most of the Notch is bound to DSL, resulting in a 'sender' state (right). A sharp boundary is formed when a switch between 'senders' and 'receivers' occurs along a gradient of Delta expression. **(c)** Models of SOP selection with time delays may introduce frequent errors in lateral inhibition patterning resulting in two adjacent SOPs being selected. Models which also include *cis*-inhibition reduce the effective time delays and thus the error rate.

stage, are receptive to the inhibitory signals. This type of response is much faster than transcriptional feedback and was estimated to reduce delays to less than 1 min. Such speedup of patterning due to *cis*-inhibition was also observed in comprehensive simulations across a broad range of parameters [45•]. The model was experimentally validated by checking its prediction that reduction in the abundance of both Notch and its ligands will retain wild-type error rate. Note that this behavior is similar to the double heterozygous mutant behavior observed in wing veins as discussed above.

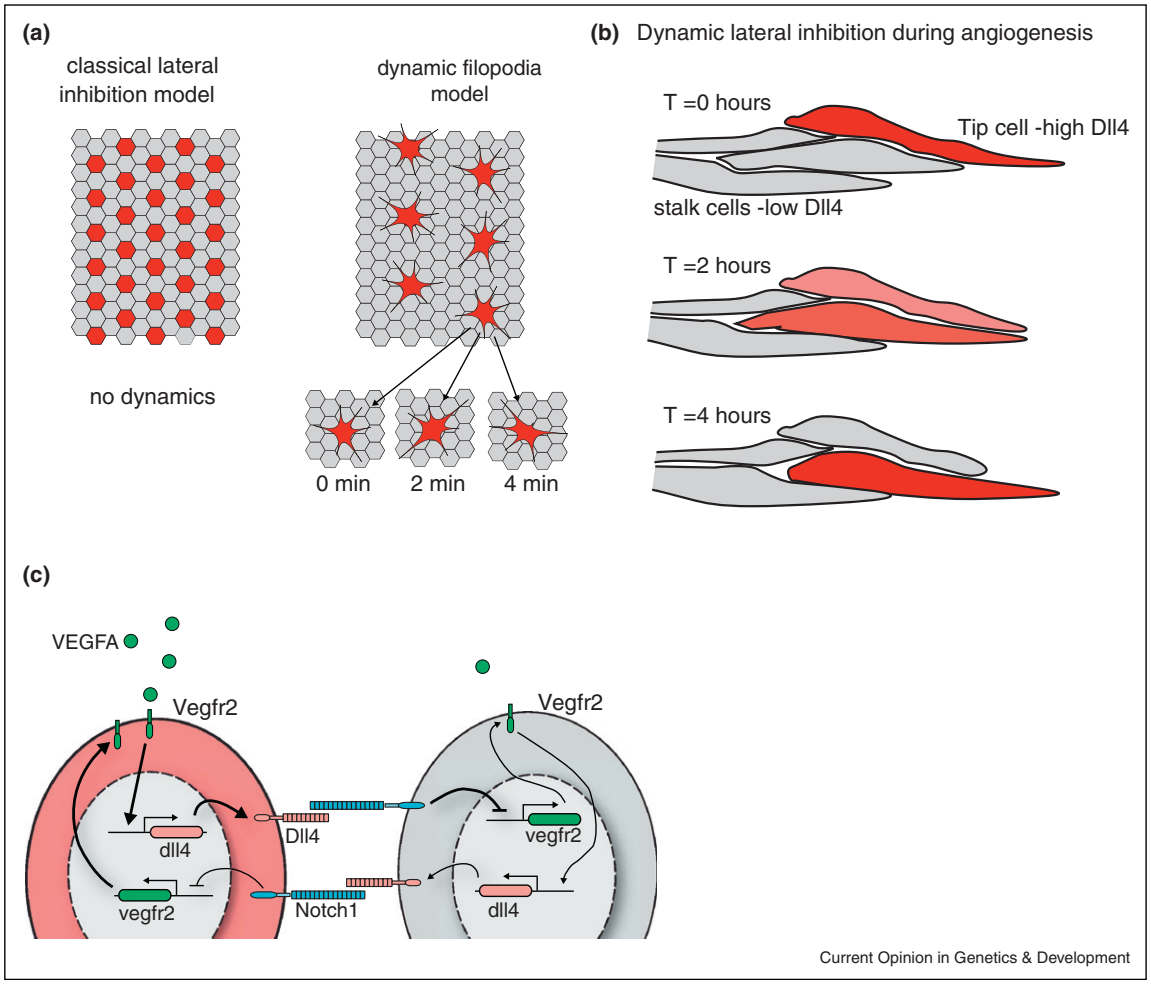
Producing the right spacing

While bristle patterning has long been considered as the canonical example for lateral inhibition, the observed patterning has an unexpected feature: The spacing between SOP cells is typically 4-5 cells. This is much higher than expected from classical lateral inhibition (Figure 3a, left) since all non-SOP cells must be inhibited by a direct contact with a SOP [12]. So how can such large spacing between SOP cells be achieved?

According to a recent paper by Cohen *et al.* [47••], the answer lies with filopodia which extend beyond their nearest neighbors at the basal side of epithelial cells [48]. When these filopodia were examined using time-lapse microscopy, they were found to be extending and retracting dynamically (average lifetime ~500 s) forming transient contacts with cells located several cell diameters away (Figure 3a, right). They also noticed that the pattern goes through a refinement process, where an initially unorganized pattern (i.e. more variable spacing) becomes more organized possibly via cell movement and apoptosis. To test their hypothesis the authors incorporated filopodia mediated signaling into a mathematical model of lateral inhibition. Interestingly, the dynamic filopodia model allows the system to probe alternative patterns and reach an optimal state with large and uniform inter-bristle distances.

To validate their hypothesis *in vivo*, the authors used experimental perturbations to inhibit the filopodia dynamics. The lengths and dynamics measured were then inserted as data into the model. Both model and experiment yielded a reduction in precursor cell spacing induced by the loss of filopodia. In addition, a noticeable increase in precursor cell disorder and a decrease in pattern refinement were observed. It is interesting to

Figure 3



Dynamic lateral inhibition. **(a)** The spacing between SOPs in the lateral inhibition models is limited to one or two cells; however, the observed spacing is larger. Dynamic filopodia model, in which cells send inhibitory signals to more distant neighbors through basal cytoplasmic extensions can account for this observation. Dynamic extension and retraction of filopodia (bottom) leads to a refinement process generating relatively large, but uniform inter-cell spacing. **(b)** Tip cell sprouting during angiogenesis is controlled by the VEGF and Notch signaling pathways. Cells, selected to become tip cells, express higher levels of Dll4 and inhibit their neighbors from becoming tip cells themselves. In this system, the inhibition is transient: Cells dynamically compete for the 'tip' position in a shuffling process regulated by Notch activity. **(c)** Suggested circuit diagram controlling tip cell differentiation. Here, cells responding to higher VEGFA signal would activate Dll4, which in turn inhibits the Vegfr2 expression in the neighboring cell preventing it from responding to VEGFA.

note that the refinement mechanism may be an additional way to reduce errors on top of the mechanism suggested by Barad *et al.* [46••] described in the previous section.

Dynamic lateral inhibition in angiogenesis and beyond

Angiogenesis is the growth process of new blood vessels from pre-existing ones, and is known to be regulated by two signaling pathways simultaneously, vascular endothelial growth factors (VEGF) and Notch [49]. VEGF induces sprouting of existing endothelial stalk cells into 'tip' cells, which spearhead branching of new blood vessels. Delta-like 4 (Dll4)-Notch1 signaling regulate the selection of 'tip' cells through what seems like lateral inhibition: Cells selected to become tip cells are the ones that have higher levels of Dll4 [50•,51,52]. These cells inhibit their neighbors from becoming tip cells and as a result, branching is limited to the desired level.

A recent study of sprouting angiogenesis by Jakobsson *et al.* [53••] has found a fascinating new phenomenon occurring during this process: The cells dynamically compete for the 'tip' position in a shuffling process regulated by Notch activity. Live-cell imaging of sprouting angiogenesis (both *in vivo* and *in vitro*) has shown that the 'tip' cell is dynamically replaced by migrating 'stalk' cells (Figure 3b). What determines which cell eventually becomes a tip cell and at what time? The authors co-cultured wild type and

mutant cells and measured the relative frequency of each cell type in the 'tip' position. By comparing the results to a computational model [50•,54], the authors were able to determine the role of Notch signaling in the shuffling process. They showed that tip cell competition is mediated by Notch-dependent downregulation of VEGF receptor 2 (Vegfr2, see Figure 3c); however, this downregulation was not sufficient to prevent stalk cells from competing with tip cells at later times.

The authors suggest an intriguing hypothesis for the purpose of this cell shuffling during sprouting angiogenesis. Since the competition for tip cell position is affected by the relative Vegfr levels, they speculate that the position exchange ensures that the leading cell is ideally equipped for responding to a gradient of VEGF at any time point. It will be interesting to see if this hypothesis can be proved experimentally.

Dynamic processes of lateral inhibition have also been observed in the developing nervous system, in which, Notch signaling regulates neural progenitors' differentiation. Dynamic time lapse imaging of neural precursors in the developing mouse brain [55••,56•,57] showed that Dll1 ligands, the Hes-Her Notch target genes, and the proneural gene neurogenin-2, all exhibit oscillatory expression levels with an average period of 2–3 hours. A new lateral inhibition paradigm was suggested to underlie these oscillations in which Notch signaling coordinates out of phase oscillations between cell-autonomous clocks. Interestingly, Monk *et al.* [58] showed that time-delayed lateral inhibition models can produce oscillatory behavior, typically in-phase. However, more recent models that take into account internal clocks showed both in-of-phase and out-of-phase oscillations [59]. What is the role for these oscillations? Kageyama *et al.* hypothesized that this behavior is required for maintaining cells in an undifferentiated state over extended periods, yet allows quick differentiation when required [56•].

Finally, Notch signaling has been implicated in synchronizing the oscillations of cells in the presomitic mesoderm during somitogenesis [60•,61•,62,63]. Unlike the other processes described above, Notch signaling in this case mediates long range coordination rather than short range differentiation. This system is nicely reviewed elsewhere [2,30].

Summary

The works presented here expand our understating of Notch-dependent pattern formation in several important ways. First, post-translational regulation in the form of *cis*-interactions plays a crucial role in generating patterns in a robust manner. Second, changes in cell morphology (filopodia) occurring concurrently with differentiation are also an important determinant of patterning through their effect on signaling. And third, differentiation processes may involve dynamic changes in cellular states.

Several future directions in this field may come to mind. On the experimental side, improved techniques for the collection of quantitative dynamic data are needed. For example, quantitative *in vivo* imaging of Notch pathway components and its regulators will allow development of better, more accurate models. Such imaging approaches are expected to benefit also from the recent development in optogenetic techniques [64], which could be used to genetically perturb the system in a controlled localized manner. On the modeling side, novel-modeling approaches that can deal with cell mechanics (e.g. forces and tensions), cell morphology (e.g. filopodia), and cellular signaling may be necessary for understanding complex differentiation processes.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lewis J: **From signals to patterns: space, time, and mathematics in developmental biology**. *Science* 2008, **322(5900)**:399-403.
2. Oates AC, Gorfinkiel N, Gonzalez-Gaitan M, Heisenberg CP: **Quantitative approaches in developmental biology**. *Nat Rev Genet* 2009, **10(8)**:517-530.
3. Artavanis-Tsakonas S, Muskavitch MA: **Notch: the past, the present, and the future**. *Curr Top Dev Biol* 2010, **92**:1-29.
4. Buceta J, Herranz H, Canela-Xandri O, Reigada R, Sagues F, Milan M: **Robustness and stability of the gene regulatory network involved in dv boundary formation in the drosophila wing**. *PLoS One* 2007, **2(7)**:e602.
5. Agrawal S, Archer C, Schaffer DV: **Computational models of the notch network elucidate mechanisms of context-dependent signaling**. *PLoS Comput Biol* 2009, **5(5)**:e1000390.
6. Buske P, Galle J, Barker N, Aust G, Clevers H, Loeffler M: **A comprehensive model of the spatio-temporal stem cell and tissue organisation in the intestinal crypt**. *PLoS Comput Biol* 2011, **7(1)**:e1001045.
7. Cohen M, Baum B, Miodownik M: **The importance of structured noise in the generation of self-organizing tissue patterns through contact-mediated cell–cell signalling**. *J R Soc Interface* 2011, **8(59)**:787-798.
8. Formosa-Jordan P, Ibanes M: **Diffusible ligand and lateral inhibition dynamics for pattern formation**. *J Stat Mech-Theory E* 2009.
9. Canela-Xandri O, Sagues F, Reigada R, Buceta J: **A spatial toggle switch drives boundary formation in development**. *Biophys J* 2008, **95(11)**:5111-5120.
10. Bray S: **Notch signalling: a simple pathway becomes complex**. *Nat Rev Mol Cell Biol* 2006, **7(9)**:678-689.
11. Chitnis AB: **The role of notch in lateral inhibition and cell fate specification**. *Mol Cell Neurosci* 1995, **6(6)**:311-321.

12. Collier JR, Monk NA, Maini PK, Lewis JH: **Pattern formation by lateral inhibition with feedback: a mathematical model of delta–notch intercellular signalling**. *J Theor Biol* 1996, **183(4)**:429-446.

13. Seydoux G, Greenwald I: **Cell autonomy of lin-12 function in a cell fate decision in *C. elegans***. *Cell* 1989, **57(7)**:1237-1245.

14. Heitzler P, Simpson P: **The choice of cell fate in the epidermis of drosophila**. *Cell* 1991, **64(6)**:1083-1092.

15. Heitzler P, Simpson P: **Altered epidermal growth factor-like sequences provide evidence for a role of notch as a receptor in cell fate decisions**. *Development* 1993, **117(3)**:1113-1123.

16. Simpson P: **Notch signalling in development: on equivalence groups and asymmetric developmental potential**. *Curr Opin Genet Dev* 1997, **7(4)**:537-542.

17. Seugnet L, Simpson P, Haenlin M: **Transcriptional regulation of notch and delta: requirement for neuroblast segregation in drosophila**. *Development* 1997, **124(10)**:2015-2025.

18. Appel B, Givan LA, Eisen JS: **Delta–notch signaling and lateral inhibition in zebrafish spinal cord development**. *BMC Dev Biol* 2001, **1**:13.

19. Pierfelice T, Alberi L, Gaiano N: **Notch in the vertebrate nervous system: an old dog with new tricks**. *Neuron* 2011, **69(5)**:840-855.

20. Goodyear R, Richardson G: **Pattern formation in the basilar papilla: evidence for cell rearrangement**. *J Neurosci* 1997, **17(16)**:6289-6301.

21. Daudet N, Ariza-McNaughton L, Lewis J: **Notch signalling is needed to maintain, but not to initiate, the formation of prosensory patches in the chick inner ear**. *Development* 2007, **134(12)**:2369-2378.

22. Daudet N, Lewis J: **Two contrasting roles for notch activity in chick inner ear development: specification of prosensory patches and lateral inhibition of hair-cell differentiation**. *Development* 2005, **132(3)**:541-551.

23. de Celis JF, Bray S: **Feed-back mechanisms affecting notch activation at the dorsoventral boundary in the drosophila wing**. *Development* 1997, **124(17)**:3241-3251.

24. Huppert S, Jacobsen T, Muskavitch M: **Feedback regulation is central to delta–notch signalling required for drosophila wing vein morphogenesis**. *Development* 1997, **124(17)**:3283-3291.

25. de Celis J, Garcia-Bellido A: **Roles of the notch gene in drosophila wing morphogenesis**. *Mech Dev* 1994, **46(2)**:109-122.

26. Diaz-Benjumea FJ, Cohen SM: **Serrate signals through notch to establish a wingless-dependent organizer at the dorsal/ventral compartment boundary of the drosophila wing**. *Development* 1995, **121(12)**:4215-4225.

27. Kim J, Irvine KD, Carroll SB: **Cell recognition, signal induction, and symmetrical gene activation at the dorsal–ventral boundary of the developing drosophila wing**. *Cell* 1995, **82(5)**:795-802.

28. Rulifson EJ, Blair SS: **Notch regulates wingless expression and is not required for reception of the paracrine wingless signal during wing margin neurogenesis in drosophila**. *Development* 1995, **121(9)**:2813-2824.

29. Cheng YC, Amoyel M, Qiu X, Jiang YJ, Xu Q, Wilkinson DG: **Notch activation regulates the segregation and differentiation of rhombomere boundary cells in the zebrafish hindbrain**. *Dev cell* 2004, **6(4)**:539-550.

30. Dequeant ML, Pourquie O: **Segmental patterning of the vertebrate embryonic axis**. *Nat Rev Genet* 2008, **9(5)**:370-382.

31. Roegiers F, Jan YN: **Asymmetric cell division**. *Curr Opin Cell Biol* 2004, **16(2)**:195-205.

32. Bardin AJ, Le Borgne R, Schweisguth F: **Asymmetric localization and function of cell-fate determinants: a fly's view**. *Curr Opin Neurobiol* 2004, **14(1)**:6-14.

33. Lieber T, Kidd S, Struhl G: **Dsl-notch signaling in the drosophila brain in response to olfactory stimulation**. *Neuron* 2011, **69(3)**:468-481.

34. Alberi L, Liu S, Wang Y, Badie R, Smith-Hicks C, Wu J, Pierfelice TJ, Abazyan B, Mattson MP, Kuhl D *et al.*: **Activity-induced notch signaling in neurons requires arc/arg3.1 and is essential for synaptic plasticity in hippocampal networks**. *Neuron* 2011, **69(3)**:437-444.

35. Sprinzak D, Lakhanpal A, LeBon L, Santat LA, Fontes ME,
•• Anderson GA, Garcia-Ojalvo J, Elowitz MB: **Cis-interactions between notch and delta generate mutually exclusive signalling states**. *Nature* 2010, **465(7294)**:86-95.
This paper together with Ref. [45•] show the important role of *cis*-inhibition in generating sharp developmental switches using combined experimental and theoretical approaches, and how this switch facilitates boundary formation and lateral inhibition.

36. Klein T, Brennan K, Arias AM: **An intrinsic dominant negative activity of serrate that is modulated during wing development in drosophila**. *Dev Biol* 1997, **189(1)**:123-134.

37. Micchelli CA, Rulifson EJ, Blair SS: **The function and regulation of cut expression on the wing margin of drosophila: Notch, wingless and a dominant negative role for delta and serrate**. *Development* 1997, **124(8)**:1485-1495.

38. Miller AC, Lyons EL, Herman TG: **Cis-inhibition of notch by endogenous delta biases the outcome of lateral inhibition**. *Curr Biol* 2009, **19(16)**:1378-1383.

39. Matsuda M, Chitnis AB: **Interaction with notch determines endocytosis of specific delta ligands in zebrafish neural tissue**. *Development* 2009, **136(2)**:197-206.

40. Li Y, Baker N: **The roles of cis-inactivation by notch ligands and of neuralized during eye and bristle patterning in drosophila**. *BMC Dev Biol* 2004, **4(5.)**.

41. Jacobsen TL, Brennan K, Arias AM, Muskavitch MA: **Cis-interactions between delta and notch modulate neurogenic signalling in drosophila**. *Development* 1998, **125(22)**:4531-4540.

42. Becam I, Fiuza UM, Arias AM, Milan M: **A role of receptor notch in ligand cis-inhibition in drosophila**. *Curr Biol* 2010, **20(6)**:554-560.

43. del Alamo D, Rouault H, Schweisguth F: **Mechanism and significance of cis-inhibition in notch signalling**. *Curr Biol* 2011, **21(1)**:R40-R47.

44. de Celis JF, Bray SJ: **The abruptex domain of notch regulates negative interactions between notch, its ligands and fringe**. *Development* 2000, **127(6)**:1291-1302.

45. Sprinzak D, Lakhanpal A, Lebon L, Garcia-Ojalvo J, Elowitz MB:
• **Mutual inactivation of notch receptors and ligands facilitates developmental patterning**. *PLoS Comput Biol* 2011, **7(6)**:e1002069.
See the annotation to [35••].

46. Barad O, Rosin D, Hornstein E, Barkai N: **Error minimization in
•• lateral inhibition circuits**. *Sci Signal* 2010, **3(129)**:ra51.
Measurements of duplication-errors in SOP patterning are combined with a probabilistic model to show how *cis*-inhibition reduces the error rate by shortening time delays in the regulatory system.

47. Cohen M, Georgiou M, Stevenson NL, Miodownik M, Baum B:
•• **Dynamic filopodia transmit intermittent delta–notch signaling to drive pattern refinement during lateral inhibition**. *Dev Cell* 2010, **19(1)**:78-89.
The effect of long-distance Notch–Delta signaling via dynamic filopodia is experimentally characterized and mathematically analyzed. It is shown to account for the observed large spacing between SOPs on the fly's notum.

48. De Joussineau C, Soule J, Martin M, Anguille C, Montcourrier P, Alexandre D: **Delta-promoted filopodia mediate long-range lateral inhibition in drosophila**. *Nature* 2003, **426(6966)**:555-559.

49. Phng LK, Gerhardt H: **Angiogenesis: a team effort coordinated by notch**. *Dev cell* 2009, **16(2)**:196-208.

50. Bentley K, Gerhardt H, Bates PA: **Agent-based simulation of
• notch-mediated tip cell selection in angiogenic sprout initialisation**. *J Theor Biol* 2008, **250(1)**:25-36.
See the annotation to [53••].

51. Hellstrom M, Phng LK, Gerhardt H: **Vegf and notch signaling: the yin and yang of angiogenic sprouting**. *Cell Adhes Migration* 2007, **1(3)**:133-136.

52. Dufraine J, Funahashi Y, Kitajewski J: **Notch signaling regulates tumor angiogenesis by diverse mechanisms**. *Oncogene* 2008, **27(38)**:5132-5137.

53. •• Jakobsson L, Franco CA, Bentley K, Collins RT, Ponsioen B, Aspalter IM, Rosewell I, Busse M, Thurston G, Medvinsky A *et al.*: **Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting**. *Nat Cell Biol* 2010, **12(10)**:943-953.
Live-cell imaging and mathematical modeling of sprouting angiogenesis shows that the cells dynamically compete for the 'tip' position in a shuffling process regulated by Notch activity (mathematical model is discussed in [50•]).

54. Bentley K, Mariggi G, Gerhardt H, Bates PA: **Tipping the balance: robustness of tip cell selection, migration and fusion in angiogenesis**. *PLoS Comput Biol* 2009, **5(10)**:e1000549.

55. •• Shimojo H, Ohtsuka T, Kageyama R: **Oscillations in notch signaling regulate maintenance of neural progenitors**. *Neuron* 2008, **58(1)**:52-64.
See the annotation to [56•].

56. • Kageyama R, Ohtsuka T, Shimojo H, Imayoshi I: **Dynamic notch signaling in neural progenitor cells and a revised view of lateral inhibition**. *Nat Neurosci* 2008, **11(11)**:1247-1251.
Time-lapse imaging is used to show oscillatory expression levels in Dll1 ligands, the Hes-Her Notch target genes, and proneural genes [55••] in neural progenitors *in vivo*. This suggests a new paradigm of dynamic lateral inhibition [56•].

57. Kageyama R, Ohtsuka T, Shimojo H, Imayoshi I: **Dynamic regulation of notch signaling in neural progenitor cells**. *Curr Opin Cell Biol* 2009, **21(6)**:733-740.

58. Veflingstad SR, Plahte E, Monk NAM: **Effect of time delay on pattern formation: competition between homogenisation and patterning**. *Physica D* 2005, **207(3–4)**:254-271.

59. Momiji H, Monk NA: **Oscillatory notch-pathway activity in a delay model of neuronal differentiation**. *Phys Rev E: Stat Nonlinear Soft Matter Phys* 2009, **80(2 Pt 1)**:021930.

60. • Ozbudak EM, Lewis J: **Notch signalling synchronizes the zebrafish segmentation clock but is not needed to create somite boundaries**. *PLoS Genet* 2008, **4(2)**:e15.
See the annotation to [61•].

61. • Riedel-Kruse IH, Muller C, Oates AC: **Synchrony dynamics during initiation, failure, and rescue of the segmentation clock**. *Science* 2007, **317(5846)**:1911-1915.
The paper analyzes the synchronization of oscillations in the presomitic mesoderm during somitogenesis. While this topic is not directly covered here, it is certainly an excellent example of combing experiments and modeling.

62. Jiang YJ, Aerne BL, Smithers L, Haddon C, Ish-Horowicz D, Lewis J: **Notch signalling and the synchronization of the somite segmentation clock**. *Nature* 2000, **408(6811)**:475-479.

63. Lewis J: **Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator**. *Curr Biol: CB* 2003, **13(16)**:1398-1408.

64. Fenno L, Yizhar O, Deisseroth K: **The development and application of optogenetics**. *Annu Rev Neurosci* 2011, **34**:389-412.

65. Wigglesworth VB: **Local and general factors in the development of ''pattern'' in *Rhodnius prolixus* (hemiptera)**. *J Exp Biol* 1940, **17**:180-201.

66. Meinhardt H, Gierer A: **Applications of a theory of biological pattern formation based on lateral inhibition**. *J Cell Sci* 1974, **15(2)**:321-346.

67. Meir E, von Dassow G, Munro E, Odell GM: **Robustness, flexibility, and the role of lateral inhibition in the neurogenic network**. *Curr Biol: CB* 2002, **12(10)**:778-786.

68. Plahte E: **Pattern formation in discrete cell lattices**. *J Math Biol* 2001, **43(5)**:411-445.

69. Martens H, Veflingstad SR, Plahte E, Martens M, Bertrand D, Omholt SW: **The genotype-phenotype relationship in multicellular pattern-generating models — the neglected role of pattern descriptors**. *BMC Syst Biol* 2009:3.

70. Podgorski GJ, Bansal M, Flann NS: **Regular mosaic pattern development: a study of the interplay between lateral inhibition, apoptosis and differential adhesion**. *Theor Biol Med Model* 2007, **4(43)**.

71. Wearing HJ, Owen MR, Sherratt JA: **Mathematical modelling of juxtacrine patterning**. *B Math Biol* 2000, **62(2)**:293-320.