Mutual inactivation of Notch and Delta amplifies differences between neighboring cells

David Sprinzak[1], Amit Lakhanpal[1], Lauren LeBon[1], Leah Santat[1], Michelle E. Fontes[1],
Jordi Garcia-Ojalvo[2], Michael B. Elowitz[1]

The Notch-Delta signaling pathway mediates the formation of fine-grained developmental patterns. The Delta ligand has been shown to trans-activate Notch in neighboring cells, as well as to cis-inhibit Notch in its own cell. However, it remains unclear how the Notch pathway integrates these two activities and how this integration facilitates pattern formation. Using quantitative time-lapse microscopy of mammalian cells we show that while the response of Notch to trans-Delta is graded, the response to cis-Delta exhibits a sharp, switch-like response. These observations are explained by a mathematical model, in which mutual inactivation of Notch and Delta generates an ultrasensitive switch. This switch amplifies differences between neighboring cells without additional regulatory interactions and can facilitate boundary formation and lateral inhibition patterning.

***Keywords* — Notch-Delta signaling, cis-inhibition, ultrasensitivity, boundary formation, lateral inhibition.**

During development, cells differentiate in organized patterns, coordinated by direct cell-cell signaling. The Notch-Delta pathway is one of the canonical signaling systems used for this purpose [1]. It is often involved in the formation of 'fine-grained' patterns generating distinct cell fates among groups of initially equivalent neighboring cells and in sharply delineating neighboring regions in developing tissues. Examples include vertebrate inner ear hair cell differentiation [2], as well as sensory organ precursors [3] and wing vein development in *Drosophila* [4] .

Notch and Delta refer to families of single-pass transmembrane proteins that are found in diverse metazoan species. Delta on one cell can bind to and trans-activate Notch in a neighboring cell (trans-activation). Recently, studies in diverse developmental systems have shown that Delta plays a second role, inhibiting Notch activity in its own cell (cis-inhibition)[5]. These observations raise two fundamental questions about how Notch and Delta work together as a signaling system. First, how does Notch activity depend quantitatively on the levels of cis- and trans-Delta? Second, what is the role of this signal integration function in Notch-dependent patterning processes? To address these questions we have applied a new approach to this problem: Rather than dissect the role of Notch in a specific developmental context, we have reconstituted Notch-Delta interactions in a mammalian cell culture system where we can quantitatively manipulate the levels of components and accurately measure system responses over time in individual cells using time-lapse microscopy.

We find that while Notch signaling has a graded (non-cooperative) response to trans-Delta levels, it has a sharp, threshold like, response to cis-Delta. Furthermore, the threshold for cis-Delta is predominantly independent of the level of trans-Delta.

These experimental results, together with a simple mathematical model, provide a clear and unexpected picture of how Delta's two activities are integrated by Notch: We find that mutual inactivation of interacting Notch and Delta proteins results in an ultrasensitive switch that forces cells into reciprocal states of high Delta / low Notch ("sending cells") and low Delta / high Notch ("receiving cells").

At the multi-cellular level, this switch has the effect of amplifying small differences between neighboring cells conveying an essential property of many processes involving Notch signaling: the ability to differentiate neighboring cells into distinct fates. This capability is generated by the interaction between Notch and Delta proteins alone, without additional feedback loops (although such feedbacks can also contribute to the final pattern).

Using our mathematical model we show that this capability dramatically enhances the ability of Notch and Delta to mediate lateral inhibition (e.g. neurogenesis) and to generate sharp boundaries (e.g. fly wing vein). We also show how this new understanding provides explanation for mutant behaviors in these patterning processes.

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[1]Howard Hughes Medical Institute and Departments of Biology and Applied physics, California Institute of Technology. MC114-96 1200 E. California Blvd, Pasadena CA 91125. E-mail: davidsp@caltech.edu

[2]Department de Física i Enginyeria Nuclear, Universitat Politècnica de Catalunya, Colom 11, E-08222 Terrassa, Spain. E-mail: jordi.g.ojalvo@upc.edu

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