Neural Fate Decisions Mediated by Notch-Delta Signaling

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***Abstract*—In the developing nervous system, the expression of proneural genes, i.e., *Hes1*, *Neurogenin-2* (*Ngn2*), and *Deltalike-1* (*Dll1*), oscillates in neural progenitors with a period of 2–3 h, but is persistent in postmitotic neurons. In this paper, we present a computational model for neural fate decisions based on intertwined Notch-Delta signaling involving the Hes1, Notch, and Dll1 proteins. In agreement with experimental observations, the model predicts that Notch-Delta signaling plays critical roles in regulating the choice between remaining as a progenitor and embarking on neural differentiation.**

I. Introduction

In the developing mammalian nervous system, it has been shown that the expression of proneural genes, i.e., *Hes1*, *Ngn2*, and *Dll1*, oscillates in neural progenitors. However, in immature postmitotic neurons, *Hes1* is downregulated, but *Ngn2* and *Dll1* are upregulated in a sustained manner, suggesting that oscillatory versus sustained expression of proneural genes is critical for neural fate decisions [1], [2]. Notch-Delta signaling is involved in a wide variety of processes and generally controls binary fate decisions between neighboring cells [3]. Ligand–receptor *trans*-interactions, i.e., interactions between neighboring cells, results in *trans*-activation and release of the Notch intracellular domain. Ligand–receptor interactions also take place within the same cell, i.e., *cis*-interaction, which induces the inactivation of Notch by a process called *cis*-inhibition. Both *trans*-activation and *cis*-inhibition have emerged as key regulatory mechanisms in both vertebrates and invertebrates [4]. *Trans*-activation has been extensively investigated both experimentally and theoretically, especially in the control of vertebrate neurogenesis [1], [2] and somite formation [5], [6]. In contrast, the operating mechanisms and potential implications of *cis*-inhibition are less clear and need to be further investigated. Analyzing cell fate decisions based on Notch-Delta signaling may have a broad impact on our system-level understanding of Notch signaling and will be an important topic for future exploration [7].

The purpose of this paper is to present a computational model for Notch-Delta signaling involving the Hes1, Notch, and Dll1 proteins. In agreement with experimental observations, the model presented here can account for both the oscillations observed in neural progenitors, and the persistency observed in neurons, depending on cooperation between *trans*-activation and *cis*-inhibition. Analysis of a two-cell system uncovers a possible mechanism of neural fate decisions, making the model a good candidate for providing the first qualitative example of neural fate decisions mediated by Notch-Delta signaling.

II. The Model

The model, which describes the regulatory processes between the products of proneural genes, *trans*-activation, and *cis*-inhibition in neural progenitors, is schematized in Figure 1(a). The regulatory processes can be expressed by a set of ordinary differential equations for the concentrations of free Notch, N_i, free Dll1, D_i, the Notch intracellular domain, S_i, *Hes1* mRNA, M_i, Hes1 protein in the cytoplasm, $H_{C,i}$, and Hes1 protein in the nucleus, $H_{N,i}$, in cell i ($i = 1, \ldots, n$):

$$\frac{dN_i}{dt} = \beta_N - v_9\frac{N_i}{K_9+N_i} - \frac{D_iN_i}{k_c} - \frac{N_i\langle D_j\rangle_i}{k_t}, \quad (1)$$

$$\frac{dD_i}{dt} = \beta_D - v_8\frac{D_i}{K_8+D_i} - \frac{D_iN_i}{k_c} - \frac{D_i\langle N_j\rangle_i}{k_t} + v_7\frac{K_7^h}{K_7^h+H_{N,i}^h}, \quad (2)$$

$$\frac{dS_i}{dt} = \frac{N_i\langle D_j\rangle_i}{k_t} - v_{10}\frac{S_i}{K_{10}+S_i}, \quad (3)$$

$$\frac{dM_i}{dt} = (v_1 + v_c\frac{S_i}{K_d+S_i})\frac{K_1^n}{K_1^n+H_{N,i}^n} - v_2\frac{M_i}{K_2+M_i}, \quad (4)$$

$$\frac{dH_{C,i}}{dt} = v_3M_i - v_4\frac{H_{C,i}}{K_4+H_{C,i}} - v_5H_{C,i}, \quad (5)$$

$$\frac{dH_{N,i}}{dt} = v_5H_{C,i} - v_6\frac{H_{N,i}}{K_6+H_{N,i}}, \quad (6)$$

where β_N and β_D denote the production rates of Notch and Dll1, respectively; k_c and k_t denote the strengths of *cis*-inhibition and *trans*-activation, respectively. The degradation rates of all the components are assumed to obey the Michaelian–Menten (MM) kinetics. The last term in Equation 2 represents repression of Dll1 directly by Hes1 and indirectly through Ngn2, which is assumed to obey the MM kinetics. In Equation 4, v_1 is the basal transcription rate of *Hes1* mRNA and v_c is the activation rate by the Notch

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intracellular domain S. Equation 4 means that the production of *Hes1* mRNA is negatively regulated by Hes1 in the nucleus and positively regulated by the Notch intracellular domain S [6], [8], [9]. The translation from *Hes1* mRNA to the Hes1 protein in the cytoplasm and the transport of the Hes1 protein from the cytoplasm to the nucleus in Equations 5–6 are assumed to be linear [10]. The notations $\langle D_j \rangle_i$ and $\langle N_j \rangle_i$ refer to the average Dll1 and Notch levels of all neighbors j of i, respectively. In particular,

$$\langle D_j \rangle_i = \Sigma_j M_{ij} D_j, \quad and \quad \langle N_j \rangle_i = \Sigma_j N_{ij} N_j, \qquad (7)$$

where M is the connectivity matrix of a two-dimensional lattice in which M_{ij} is 1/6 if i and j are neighbors and 0 otherwise [3], as shown in Figure 1(b). To illustrate the analysis, we just consider the case of two cells ($n = 2$), as in [6], [11]. More realistic cases can be similarly discussed.

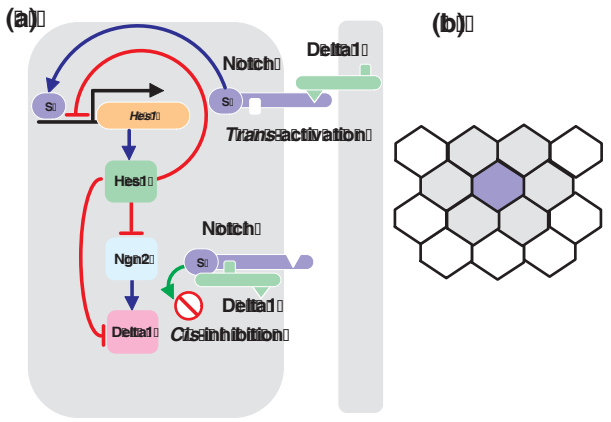

Fig. 1. Schematic descriptions of Notch signaling with *cis*-inhibition and *trans*-activation, and of the lattice structure. (a) The proneural gene *Ngn2* induces expression of the Notch ligand, Dll1, which *trans*-activates Notch in neighboring cells. On activation, the Notch intracellular domain S is released from the transmembrane region and transferred to the nucleus, where it induces *Hes1* expression. The Hes1 protein represses expression of its own gene *Hes1* and the gene *Dll1*. Notch can also be *cis*-inhibited by Dll1 within the same cell. (b) The lattice structure, in which each cell is in direct contact with six neighboring cells.

The following parameter values are used as standard values unless otherwise indicated: $v_1 = 0.2$ nM min^{-1}, $v_2 = 0.2$ nM min^{-1}, $v_3 = 0.575$ min^{-1}, $v_4 = 0.851$ nM min^{-1}, $v_5 = 0.021$ min^{-1}, $v_6 = 0.162$ nM min^{-1}, $v_7 = 10$ nM min^{-1}, $v_8 = 20$ nM min^{-1}, $v_9 = 8.5$ nM min^{-1}, $v_{10} = 10$ nM min^{-1}, $K_1 = 0.157$ nM, $K_2 = 0.104$ nM, $K_4 = 0.142$ nM, $K_6 = 0.13$ nM, $K_d = 2$ nM, $K_7 = 2$ nM, $K_8 = 4.72$ nM, $h = 2$, $K_9 = 0.06$ nM, $K_{10} = 10$ nM, $n = 2$, $k_t = 10.0$ nM^{-1} min^{-1}, $\beta_D = 1$ nM min^{-1}, $v_c = 0.2$ min^{-1}, and $\beta_N = 10$ nM min^{-1}. The model will be evaluated to see how the choice between remaining as a progenitor and embarking on neural differentiation is mediated by both *trans*-activation and *cis*-inhibition.

III. Results

A. Notch signaling with both cis-inhibition and trans-activation regulates neural fate decisions

It has been shown experimentally that cells can misexpress Dll1 or a dominant-negative derivative of Dll1, Dll1^{dn}, thereby activating or blocking Notch signaling [12], [13]. Normally, only the nascent neurons, scattered among the dividing progenitors, express Dll1. When cells are forced to express Dll1, neurogenesis is suppressed and all cells remain as progenitors. Conversely, when cells are forced to express Dll1^{dn}, they differentiate prematurely as neurons and no dividing progenitors remain. Notch signaling with both *cis*-inhibition and *trans*-activation is therefore the mechanism that regulates the choice between remaining as a progenitor and embarking on differentiation.

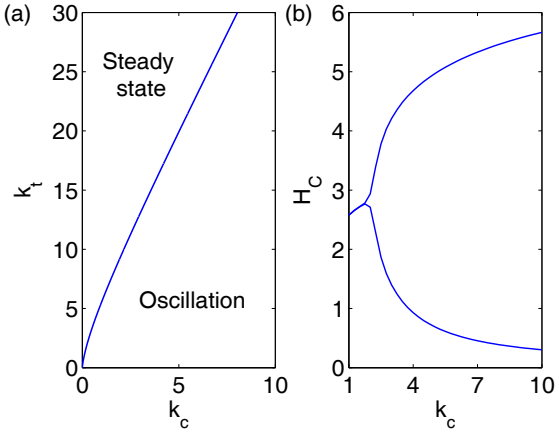

Fig. 2. Bifurcation properties. (a) A bifurcation set in a parameter space of *trans*-activation strength k_t and *cis*-inhibition strength k_t. (b) A bifurcation set in a parameter space of k_c at $k_t = 10$ nM^{-1} min^{-1}. The variable H_C at the steady state or at the minimum and maximum of the oscillations is plotted.

The expression of Hes1 oscillates in neural progenitors, but it is persistent in postmitotic neurons [2]. The precise mechanism of the regulation of oscillatory versus persistent Hes1 expression remains to be determined. However, it is known that inhibition of Notch signaling may induce neural differentiation. To decide whether the model can account for such a phenomenon, we may determine whether oscillations still occur when preventing Notch signaling, by increasing the *cis*-inhibition strength k_c or decreasing the *trans*-activation strength k_t. The bifurcation set in a parameter space of k_t and k_c is shown in Figure 2(a). Oscillations disappear and the system evolves toward a stable steady-state in the upper-left region, corresponding to the case of blocked intercellular Notch signaling, as shown in Figure 2(b). Such a phenomenon is consistent with experimental observations that the expression of Hes1 is downregulated and that of Dll1 is upregulated in a sustained manner in postmitotic neurons, and no dividing progenitors remain [2]. In contrast, in the lower-right region, where efficient intercellular Notch signaling can

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be realized, expression of Hes1 and Dll1 becomes oscillatory, corresponding to suppressed neurogenesis, and all cells remain as progenitors. These results explain why cells need to mis-express Dll1 or Dll1^{dn}, depending on the need for activation or inhibition of the Notch signaling. Both *cis*-inhibition and *trans*-activation therefore play critical roles in regulating the choice between remaining as a progenitor or embarking on neural differentiation.

B. Hes1 regulates neural fate decisions via Dll1, which can either cis-inhibit or trans-activate Notch signaling, depending on its concentration

Phenotypes of sustained Hes1 expression and those of Notch inactivation seem to be similar to each other because each of them induces neural differentiation. Hes1 functions as both a regulator and an effector of Notch signaling. In cells with persistently low Hes1 expression, Notch signaling is kept inactive, and thus the function of Hes1 as an inhibitor seems to be dominant. However, low Hes1 expression induces high Dll1 expression because of the repression of the gene *Dll1* by Hes1 and further activation of Notch signaling via *trans*-activation. One possible explanation for this contradiction is that the main function of Dll1 at high concentrations is to *cis*-inhibit rather than to *trans*-activate Notch signaling.

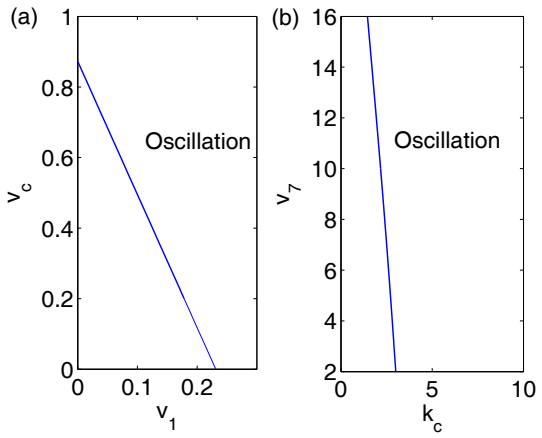

Fig. 3. Bifurcation properties. (a) A bifurcation set in a parameter space of v_c and v_1. (b) A bifurcation set in a parameter space of v_7 versus k_c.

The bifurcation set in a parameter space of v_7 and k_c is shown in Figure 3(b); this reflects the relationship between the repression of Dll1 by Hes1 and the *cis*-inhibition. For a larger v_7, a smaller k_c is needed to produce persistently low Hes1 expression. A larger v_7 corresponds to higher Dll1 expression, and a smaller k_c corresponds to greater *cis*-inhibition strength. The main function of high Dll1 expression induced by persistently low Hes1, in which Hes1 acts as an inhibitor to inactivate Notch signaling, is therefore to *cis*-inhibit Notch signaling and thus induce neural differentiation. These results are in agreement with experimental observations, i.e., Dll1 can exert an inhibitory effect on Notch signaling in a concentration-dependent manner: high Dll1 expression induces the *cis*-inhibition effect, whereas when lower Dll1 expression is present, only the *trans*-activation effect is observed [7], [14]. In contrast, after activation of Notch signaling, oscillatory Hes1 expression seems to be induced as an effector. An increased mean level of Hes1 will induce less mean Dll1 expression. Under such conditions, only the *trans*-activation effect of Dll1 is observed. These results indicate that Hes1 can regulate neural fate decisions by controlling Dll1; Dll1 can *cis*-inhibit or *trans*-activate Notch signaling, depending on its concentration.

C. Oscillatory versus persistent Hes1 depends on both cis-inhibition and trans-activation

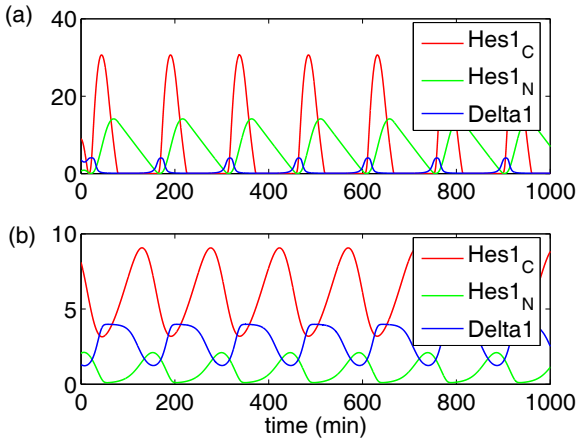

Fig. 4. Oscillations generated by negative autoregulation or Notch signaling alone in two interacting cells. (a) $v_1 = 1$ nM min^{-1}, $v_c = 0$ min^{-1}, and $k_c = 10$ nM^{-1} min^{-1}. (b) $v_1 = 0$ nM min^{-1}, $v_c = 0.8$ min^{-1}, $k_c = 5$ nM^{-1} min^{-1}, and $H^n_{N,i} = 0$ in Figure 4.

The existence of intertwined negative autoregulation, intercellular communication, and competition between *trans*-activation and *cis*-inhibition raises the possibility that the mechanism producing the oscillations may not be unique. Negative autoregulation may generate oscillations. We may determine whether oscillations still occur when intercellular communication is prevented, i.e., by letting $v_c = 0$. This model is closely related to those for the circadian clock in *Neurospora* and can generate oscillations, as shown in Figure 4(a).

As well as negative autoregulation, intercellular Notch signaling also forms an additional feedback loop, i.e., Notch$_1$ $\rightarrow$ Hes1$_1$ $\dashv$ Dll1$_1$ $\rightarrow$ Notch$_2$ $\rightarrow$ Hes1$_2$ $\dashv$ Dll1$_2$ $\rightarrow$ Notch$_1$, which is capable of generating oscillations. The oscillation generated by intercellular Notch signaling alone, i.e., eliminating direct autoregulation by letting $v_1 = 0$ and $H^n_{N,i} = 0$ in Equation 4, is shown in Figure 4(b). Intercellular coupling-induced oscillations are also observed in the delayed somitogenesis model [11]. Such multiple sources of oscillations may reflect complex and often combinatory regulation in Notch signaling.

When Notch signaling is inactive, Hes1 expression is persistent in neurons, but active Notch signaling leads to oscillatory Hes1 expression in neural progenitors, suggesting that the

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oscillations depend on intercellular Notch signaling. Although either negative autoregulation or intercellular coupling alone can generate oscillations, they are not mutually exclusive and, in principle, depend on each other. As shown in Figure 3(a), the larger v_1 becomes, the smaller the v_c needed to generate oscillations. The oscillations of Hes1 are therefore perhaps produced by combinatory regulation of negative autoregulation and intercellular coupling so as to make the oscillations more robust against various perturbations.

IV. Conclusion

In contrast to the results of the substantial studies on *trans*-activation between Dll1 and Notch, the operating mechanisms and potential implications of *cis*-inhibition are less clear. In this paper, we present a computational model for neural fate decisions based on intertwined Notch-Delta signaling involving the Hes1, Notch, and Dll1 proteins. In good agreement with experimental observations, the model predicts that Notch-Delta signaling plays critical roles in neural fate decisions. More precisely, *trans*-activation is essential for the generation of oscillations, and *cis*-inhibition is important for the asynchrony between them, indicating that the asynchronous oscillations in neural progenitors depend on cooperation between *trans*-activation and *cis*-inhibition. In contrast, *cis*-inhibition plays more critical roles in embarking on neural differentiation. All these results indicate that our model provides a good framework for the theoretical analysis of the mechanisms underlying neural fate decisions mediated by both *trans*-activation and *cis*-inhibition.

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