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Neural fate decisions mediated by *trans*-activation and *cis*-inhibition in Notch signaling

Ruiqi Wang[1,*], Kaihui Liu[1], Luonan Chen[1,2,3] and Kazuyuki Aihara[3]

[1]Institute of Systems Biology, Shanghai University, Shanghai 200444, [2]Key Laboratory of Systems Biology, SIBS-Novo Nordisk Translational Research Centre for PreDiabetes, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China and [3]Collaborative Research Center for Innovative Mathematical Modelling, Institute of Industrial Science, University of Tokyo, Tokyo 153-8505, Japan

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ABSTRACT

Motivation: 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 post-mitotic neurons. Unlike the synchronization of segmentation clocks, oscillations in neural progenitors are asynchronous between cells. It is known that Notch signaling, in which Notch in a cell can be activated by Dll1 in neighboring cells (*trans*-activation) and can also be inhibited by Dll1 within the same cell (*cis*-inhibition), is important for neural fate decisions. There have been extensive studies of *trans*-activation, but the operating mechanisms and potential implications of *cis*-inhibition are less clear and need to be further investigated.

Results: In this article, we present a computational model for neural fate decisions based on intertwined dynamics with *trans*-activation and *cis*-inhibition involving the Hes1, Notch and Dll1 proteins. In agreement with experimental observations, the model predicts that both *trans*-activation and *cis*-inhibition play critical roles in regulating the choice between remaining as a progenitor and embarking on neural differentiation. In particular, *trans*-activation is essential for generation of oscillations in neural progenitors, and *cis*-inhibition is important for the asynchrony between adjacent cells, 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 by inactivating intercellular Notch signaling. The model presented here might be a good candidate for providing the first qualitative mechanism of neural fate decisions mediated by both *trans*-activation and *cis*-inhibition.

Contact: rqwang@shu.edu.cn

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1 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 post-mitotic 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 (Kageyama *et al*., 2008; Shimojo *et al*., 2008). Additionally, Notch signaling via intercellular communication also plays important roles in neural fate decisions, i.e. activation of Notch signaling prevents cells from differentiating and plays a crucial role in maintaining neural progenitor populations during development (Matsuda and Chitnis, 2009). Intercellular communication via Notch signaling is involved in a wide variety of processes and generally controls binary fate decisions between neighboring cells (Sprinzak *et al*., 2010). 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 (del Álamo *et al*., 2011). *Trans*-activation has been extensively investigated both experimentally and theoretically, especially in the control of vertebrate neurogenesis (Kageyama *et al*., 2008; Shimojo *et al*., 2008) and somite formation (Özbudak and Lewis, 2008; Uriu *et al*., 2010a). 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 both *trans*-activation and *cis*-inhibition may have a broad impact on our system-level understanding of Notch signaling and will be an important topic for future exploration (Fiuza *et al*., 2010).

Both oscillation and synchronization of clock genes induced by intercellular Notch signaling are necessary for normal segmentation (Özbudak and Lewis, 2008; Uriu *et al*., 2010a, b). However, oscillations in neural progenitors are asynchronous between neighboring cells. Progenitor cells tend to asynchronously differentiate into diverse cell types so that they can respond differently to the same environmental condition and thereby contribute to generation of diversity in the developing brain (Aulehla and Pourquié, 2008; Kageyama *et al*., 2009; Shimojo *et al*., 2008). Both experiments and computational models have shown that intercellular Notch signaling can successfully lead to synchronized segmentation clocks (Özbudak and Lewis, 2008; Uriu *et al*., 2010a, b). However, synchronization cannot be realized, despite the existence of intercellular Notch signaling, in neural progenitors (Henrique *et al*., 1997). It has been speculated that

*To whom correspondence should be addressed.

the stochastic nature of various biochemical processes can result in loss of synchronization (Özbudak and Lewis, 2008). However, other studies show that stochasticity may play constructive roles in producing synchronization (Chen *et al*., 2005), and oscillators with noise can still be synchronized (Garcia-Ojalvo *et al*., 2004; Horikawa *et al*., 2006). Whether or not stochasticity is the main reason for asynchrony has therefore still to be explored, and we need to examine more carefully how synchrony and asynchrony between neighboring cells can be realized by intercellular Notch signaling in different developmental processes.

The purpose of this article is to present a computational model for Notch signaling in neural progenitors based on intertwined dynamics with *cis*-inhibition and *trans*-activation involving the Hes1, Notch and Dll1 proteins. Not surprisingly, mathematical models of Notch signaling, with different levels of sophistication, have been proposed for virtually every organism for which sufficient knowledge of molecular biology exists [see e.g. Collier *et al*. (1996); Sprinzak *et al*. (2010); Uriu *et al*. (2010a)]. All these models can produce sustained oscillations but are not sufficient in several important respects. First, most of the previous models do not include an essential characteristic of Notch signaling, i.e. *cis*-inhibition (Collier *et al*., 1996; Özbudak and Lewis, 2008; Uriu *et al*., 2010a, b). Second, even when *cis*-inhibition is incorporated, its link to proneural genes and its effects on neural fate decisions have not been well considered (Sprinzak *et al*., 2010). Most models focus on how oscillations occur and how synchronization is realized in somite patenting through intercellular Notch signaling, but asynchrony in neural progenitors has not been investigated theoretically. Thus, a new model is needed which incorporates both *cis*-inhibition and *trans*-activation, and which can account for their mechanisms, relevance and potential implications in neural fate decisions.

In agreement with experimental observations, the model presented here can account for both the asynchronous 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 and an *n*-cell system in a 2D lattice 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 both *trans*-activation and *cis*-inhibition.

2 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 1a. The expression of proneural genes in neural progenitors oscillates asynchronously between neighboring cells. Blockade of Notch signaling, a condition known to induce neural differentiation, represses Hes1 expression and upregulates *Ngn2* and *Dll1* expression persistently at higher levels (Kageyama *et al*., 2008). Notch signaling, in which Notch in a cell can be *cis*-inhibited by Dll1 within the same cell, as well as *trans*-activated by Dll1 in neighboring cells, as shown in Figure 1a, thus integrating both *cis*- and *trans*-Dll1, as shown in Figure 1c, plays important roles in neural fate decisions.

Mathematical models of Notch signaling have previously been proposed in insect neurogenesis (Collier *et al*., 1996) and vertebrate somitogenesis (Sprinzak *et al*., 2010; Uriu *et al*., 2010a), mainly including the *trans*-activation process, but the operating mechanisms

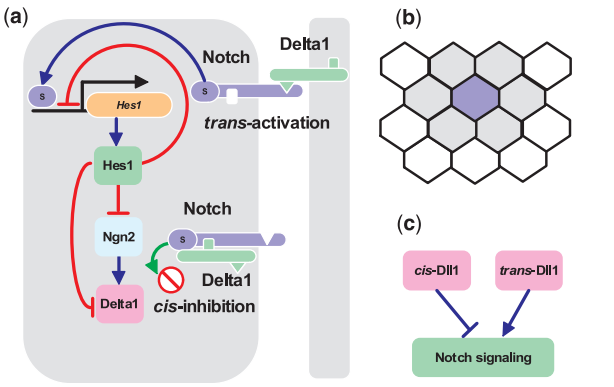

Fig. 1. Schematic descriptions of Notch signaling with *cis*-inhibition and *trans*-activation, and of the lattice structure. (**a**) The proneural gene *Ngn2* induces expression of the Notch ligand, Dll1, which *trans*-activates Notch in neighboring cells. On activation, the Notch intracellular domain *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. (**c**) Notch activity integrates both *cis*- and *trans*-Dll1.

and potential implications of *cis*-inhibition for neural fate decisions are less clear. The model presented here involves several aspects. First, Notch of concentration N in cell i binds to extracellular Dll1 of concentration $\langle D_j\rangle_i$, i.e. the average Dll1 level of all neighbors j of i, leading to release of the Notch intracellular domain S, and degradation of its extracellular domain. Similarly, Notch in neighboring cells, $\langle N_j\rangle_i$ can bind to Dll1. Second, Notch can also bind to Dll1 within the same cell, causing inactivation of Notch. Third, the Notch intracellular domain S induces expression of the gene *Hes1* in the nucleus. Fourth, the protein Hes1 represses the transcription of its own gene *Hes1* and gene *Dll1*.

These 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 ($i=1,\ldots,n$):

$$\frac{dN_i}{dt} = \beta_N - \nu_9\frac{N_i}{K_9+N_i} - \frac{D_iN_i}{k_c} - \frac{N_i\langle D_j\rangle_i}{k_t}, \quad (1)$$

$$\frac{dD_i}{dt} = \beta_D - \nu_8\frac{D_i}{K_8+D_i} - \frac{D_iN_i}{k_c} - \frac{D_i\langle N_j\rangle_i}{k_t} + \nu_7\frac{K_7^h}{K_7^h+H_{N,i}^h}, \quad (2)$$

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

$$\frac{dM_i}{dt} = \left(\nu_1+\nu_c\frac{S_i}{K_d+S_i}\right)\frac{K_1^n}{K_1^n+H_{N,i}^n} - \nu_2\frac{M_i}{K_2+M_i}, \quad (4)$$

$$\frac{dH_{C,i}}{dt} = v_3 M_i - v_4 \frac{H_{C,i}}{K_4 + H_{C,i}} - v_5 H_{C,i}, \quad (5)$$

$$\frac{dH_{N,i}}{dt} = v_5 H_{C,i} - v_6 \frac{H_{N,i}}{K_6 + H_{N,i}}. \quad (6)$$

The degradation rates of all the components are assumed to obey the Michaelis–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. 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* (Rodríguez-González *et al*., 2007; Uriu *et al*., 2010a, b). 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 (Chen *et al*., 2010). 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,

$$\langle D_j \rangle_i = \Sigma_j P_{ij} D_j \quad \text{and} \quad \langle N_j \rangle_i = \Sigma_j P_{ij} N_j, \quad (7)$$

where P is the connectivity matrix of a 2D lattice in which P_{ij} is 1/6 if i and j are neighbors and 0 otherwise (Sprinzak *et al*., 2010), as shown in Figure 1b. The case of Neumann neighborhood in which each cell has four nearest neighbors, as in Uriu *et al*. (2010b), can be similarly discussed. Because of the high computational and memory costs of simulating a system comprising many oscillators with six variables each, only a very small lattice can be studied. To illustrate the analysis, we only consider the case of two cells, i.e. $n = 2$, as in Lewis (2003); Uriu *et al*. (2010a), except when asynchrony is analyzed in Section 3.4, where a larger lattice with zero boundary conditions, i.e. $n = 14$, will be chosen, as shown in Figure 1b. More realistic cases, e.g. periodic boundary conditions, larger lattices or even 3D lattices, can be similarly discussed.

Because the kinetic parameters are not reliably known from experimental data, we adopt parameter values within the ranges given in Sprinzak *et al*. (2010) for the Notch signaling. While for the Hes1 autoregulation, we adopt parameter values used in Uriu *et al*. (2010a, b) except the basal transcription rate of *Hes1* mRNA, v_1. The range of v_1 in Uriu *et al*. (2010a) is 0.001–0.01 nM min^{-1} and the period of oscillations is ∼25–35 min. However, the period of oscillations in neural progenitors is ∼2–3 h. According to the parameter sensitivity analysis, we increase v_1 to 0.15–1 nM min^{-1} so that the period of oscillations becomes ∼2–3 h because the period increases as v_1 increases, as shown in Figure 8b. In particular, the following parameter values are used as standard values unless otherwise indicated: $v_1 = 1.0$ 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}, $k_c = 4.0$ nM^{-1} min^{-1}, $\beta_D = 1$ nM min^{-1}, $v_c = 0.2$ min^{-1} and $\beta_N = 10$ nM min^{-1}. The definition of each parameter can be found in Supplementary Table S1. 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.

3 RESULTS

3.1 Notch signaling with both *cis*-inhibition and *trans*-activation regulates neural fate decisions

It has been shown experimentally that cells can ectopically express Dll1 or a dominant-negative derivative of Dll1, Dll1^{dn}, thereby activating or blocking Notch signaling (Austin *et al*., 1995; Henrique *et al*., 1997). 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.

The expression of Hes1 oscillates in neural progenitors, but it is persistent in post-mitotic neurons (Shimojo *et al*., 2008). 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 2a. Oscillations disappear and the system evolves toward a stable steady state in the

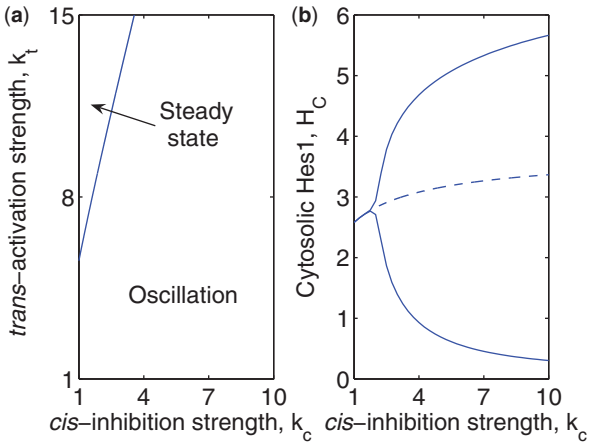

Fig. 2. Bifurcation properties. (**a**) A bifurcation set in a parameter space of *trans*-activation strength k_t and *cis*-inhibition strength k_c at $v_1 = 0.2$ nM min^{-1}. In the upper-left region, strong *cis*-inhibition and weak *trans*-activation induce inactivation of intercellular Notch signaling and neural differentiation, corresponding to the persistently low Hes1 expression, as shown in shown in Figure 1c. In contrast, in the lower-right region, weak *cis*-inhibition and strong *trans*-activation cause activation of intercellular Notch signaling, corresponding to the maintenance of neural progenitors and oscillatory expression of Hes1 and Dll1. (**b**) A bifurcation set in a parameter space of k_c at $k_t = 10$ nM^{-1} min^{-1} and at $v_1 = 0.2$ nM min^{-1}. The variable H_C at the stable steady state or at the minimum and maximum of the oscillations is plotted. A supercritical Hopf bifurcation occurs, resulting in the stability loss of steady states (dashed line) and appearance of a stable branch of limit cycles. Increasing the *cis*-inhibition strength (decreasing k_c) induces inactivation of intercellular Notch signaling and further neural differentiation with persistently low Hes1 expression.

upper-left region, corresponding to the case of blocked intercellular Notch signaling, as shown in Figure 2b. 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 post-mitotic neurons, and no dividing progenitors remain (Shimojo *et al*., 2008). In contrast, in the lower-right region, where efficient intercellular Notch signaling can be realized, expression of Hes1 and Dll1 becomes oscillatory, corresponding to suppressed neurogenesis, and all cells remain as progenitors. These results explain why cells ectopically 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.

3.2 Dll1 regulates Notch signaling in a concentration-dependent manner

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 (Shimojo *et al*., 2008). 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.

The bifurcation set in a parameter space of v_7 and k_c is shown in Figure 3b, which 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 the 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 (Fiuza *et al*., 2010; Jacobsen *et al*., 1998). In contrast, after activation of Notch signaling, oscillatory Hes1 expression seems to be induced as an effector. An increased mean level of Hes1 reduces 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.

The bifurcation sets in a parameter space of k_t versus k_c at lower v_7, i.e. $v_7 = 2\,\mathrm{nM\,min^{-1}}$, and higher v_7, i.e. $v_7 = 16\,\mathrm{nM\,min^{-1}}$, are shown in Figure 3c and d, respectively. They have qualitatively similar tendency, i.e. the larger k_c becomes, the larger k_t is needed to generate oscillations, due to the opposite roles played by the *cis*-inhibition and *trans*-activation, except that relatively larger k_t is needed to generate oscillations at relatively larger v_7. These results also indicate that the balance between *cis*-inhibition and *trans*-activation exists for both cases so as to regulate the fate decisions.

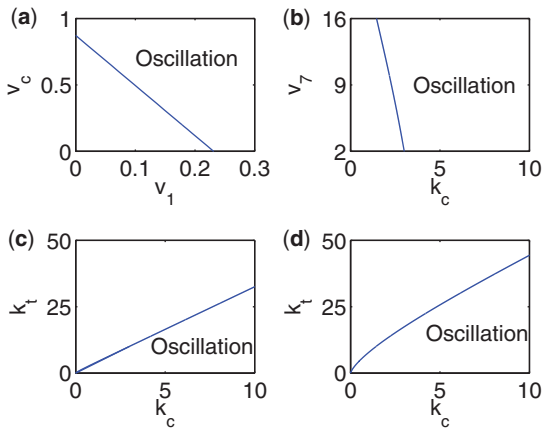

Fig. 3. Bifurcation properties. (**a**) A bifurcation set in a parameter space of v_c and v_1 at $k_c = 6.0\,\mathrm{nM^{-1}\,min^{-1}}$. The larger v_1 becomes, the smaller the v_c is needed to generate oscillations and vice versa, which means that two sources of oscillation can co-compensate the inefficiency of each other to generate oscillations, showing that the oscillations of Hes1 are perhaps produced by combinatory regulation of negative autoregulation and intercellular coupling. (**b**) A bifurcation set in a parameter space of v_7 versus k_c at $v_1 = 0.2\,\mathrm{nM\,min^{-1}}$. It reflects the relationship between the repression of Dll1 by Hes1 and the *cis*-inhibition. (**c**) A bifurcation set in a parameter space of k_t versus k_c at $v_7 = 2\,\mathrm{nM\,min^{-1}}$. (**d**) A bifurcation set in a parameter space of k_t versus k_c at $v_7 = 16\,\mathrm{nM\,min^{-1}}$.

3.3 Oscillatory versus persistent Hes1 depends on both *cis*-inhibition and *trans*-activation

Although Hes1 is oscillatory in neural progenitors and persistent in neurons, the precise mechanism of the regulation of persistent versus oscillatory Hes1 is not known. In other words, it is not clear if Notch signaling comprises a part of the oscillator mechanism or if Notch signaling simply coordinates the activity of the oscillators between neighboring cells. It has been shown that Notch signaling serves to maintain synchrony in zebrafish presomitic mesoderm (PSM) but is not necessary for oscillations of individual cells (Özbudak and Lewis, 2008). In contrast to what happens in zebrafish, elimination of all Notch activity abolishes oscillatory gene expression and somite formation in mouse PSM (Ferjentsik *et al*., 2009). The different roles that Notch signaling seems to play in mouse and zebrafish somitogenesis could be the result of different degrees of complexity of the segmentation clock mechanism in these two species.

The existence of intertwined negative autoregulation, intercellular communication, and competition between *trans*-activation and *cis*-inhibition raises the possibility that several mechanisms are involved, independently or cooperatively, to produce the oscillations. Negative autoregulation may generate oscillations. We may determine whether oscillations still occur when intercellular communication is prevented, i.e. by letting $v_c = 0$. Hes1 signaling has been extensively studied and several computational models have been presented, in particular for the segmentation clock (Uriu *et al*., 2010b). It has been shown that negative autoregulation with delays (Lewis, 2003), interaction with Wnt and Notch signaling

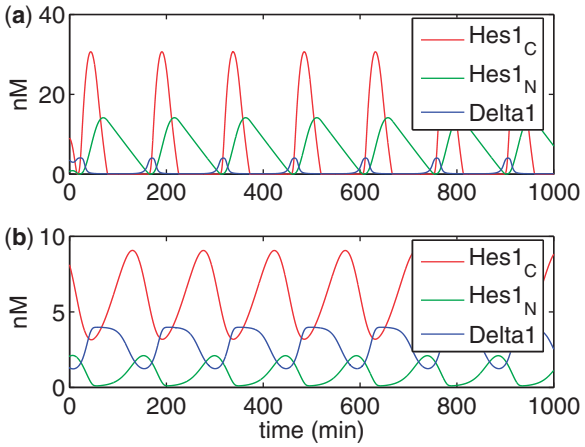

Fig. 4. Oscillations generated by negative autoregulation or Notch signaling alone in two interacting cells at $k_c = 5\ \text{nM}^{-1}\ \text{min}^{-1}$. (**a**) Oscillations generated by negative autoregulation alone at $v_1 = 1\ \text{nM}\,\text{min}^{-1}$ by blocking the intercellular Notch signaling, i.e. letting $v_c = 0\ \text{min}^{-1}$. (**b**) Oscillations generated by intercellular Notch signaling alone at $v_c = 0.8\ \text{min}^{-1}$ by eliminating direct autoregulation, i.e. letting $v_1 = 0\,\text{nM}\,\text{min}^{-1}$ and $H_{N,i} = 0\,\text{nM}$ in Equation (4).

(Rodríguez-González *et al*., 2007) or interaction with Jak-Stat signaling (Shimojo *et al*., 2008) can generate Hes1 oscillations. The three-variable model for a single cell has the form of the Goodwin oscillator (Goodwin, 1965), but uses the MM kinetics for the degradation steps. Protein degradation is controlled by phosphorylation, ubiquitination and proteasomal degradation, and thus it is reasonable to assume the MM kinetics. This model is closely related to those for the circadian clock in *Neurospora* (Leloup *et al*., 1999; Ruoff *et al*., 2001) and can generate oscillations, as shown in Figure 4a.

As well as negative autoregulation, intercellular Notch signaling also forms an additional feedback loop, i.e. $\text{Notch}_1 \rightarrow \text{Hes1}_1 \dashv \text{Dll1}_1 \rightarrow \text{Notch}_2 \rightarrow \text{Hes1}_2 \dashv \text{Dll1}_2 \rightarrow \text{Notch}_1$, 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 Figure 4, is shown in Figure 4b. Intercellular coupling-induced oscillations are also observed in the delayed somitogenesis model (Lewis, 2003). 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 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 3a, the larger v_1 becomes, the smaller the v_c needed to generate oscillations. Therefore, when intercellular Notch signaling is insufficient to produce oscillations, negative autoregulation can compensate for the inefficiency and vice versa. In other words, the existence of autoregulation reduces the requirement of coupling strength for 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.

Oscillatory or sustained Hes1 expression also depends on cooperation between *trans*-activation and *cis*-inhibition. When the *cis*-inhibition strength is increased (reduced k_c), more Notch will be *cis*-inhibited and less S can be released; thus, intercellular Notch signaling will be blocked and oscillations will disappear. In other words, increased *cis*-inhibition reduces the oscillation capability; this facilitates the cessation of oscillations and embarkment on neural differentiation. In contrast, increased *trans*-activation will be helpful for generating oscillations and making cells remain as progenitors. These results indicate that both *trans*-activation and *cis*-inhibition play differential roles in neural fate decisions and can account for the experimental observations, e.g. inhibition of Notch signaling can induce neuronal differentiation (Austin *et al*., 1995; Matsuda and Chitnis, 2009) and Hes1 regulates neural fate decisions via Dll1, which can either *cis*-inhibit or *trans*-activate Notch signaling, depending on its concentration (Fiuza *et al*., 2010; Henrique *et al*., 1997; Jacobsen *et al*., 1998). These results show that the interplay between *trans*-activation and *cis*-inhibition can generate different states (Sprinzak *et al*., 2010) and modulate neurogenic signaling (Jacobsen *et al*., 1998).

3.4 *Cis*-inhibition induces asynchrony between adjacent cells

Synchronization of multicellular systems has been extensively studied, e.g. coupled repressilators (Garcia-Ojalvo *et al*., 2004), circadian oscillators (Gonze *et al*., 2005) and segmentation clocks (Uriu *et al*., 2010a, b). In contrast to synchronization of segmentation clocks, oscillations in neural progenitors are asynchronous among cells (Aulehla and Pourquié, 2008; Kageyama *et al*., 2009; Shimojo *et al*., 2008). A model by Lewis proposed a way by which DeltaC synchronizes oscillations between neighboring cells (Lewis, 2003). The model postulates that the intracellular oscillations of *her1* and *her7* genes are coupled to an intercellular oscillator involving Delta ligands. In fact, Her1 and Her7 negatively regulate deltaC, influencing Notch activity in the neighboring cells and finally their own intracellular oscillations. This intercellular coupling mechanism results in synchronization. Similar models based on *trans*-activation have also been proposed for the synchronization of segmentation clocks (Uriu *et al*., 2010a, b). Intercellular Notch signaling exists in neural progenitors as well as in vertebrate somitogenesis. One natural question then arises: why is intercellular Notch signaling unable to induce synchronization in neural progenitors as it does in somitogenesis?

It has been shown that segmentation clocks based on *trans*-activation can be easily synchronized (Lewis, 2003; Uriu *et al*., 2010a, b). In contrast, the model presented here incorporates both *trans*-activation and *cis*-inhibition. When there is no *cis*-inhibition or its strength is small, i.e. k_c is large, the release of the Notch intracellular domain S through *trans*-activation provides a mechanism for intercellular coupling. Here, we just consider the case of lattice structure with $n = 14$. When the coupling strength v_c is large enough, synchronized oscillations can be observed, as shown in Figure 5b. As the *cis*-inhibition strength is increased, more Notch is *cis*-inhibited and less S is released. Finally, a sufficiently large *cis*-inhibition strength induces loss of synchronization, as shown in Figure 5a. To characterize the transition to asynchrony, a quantity

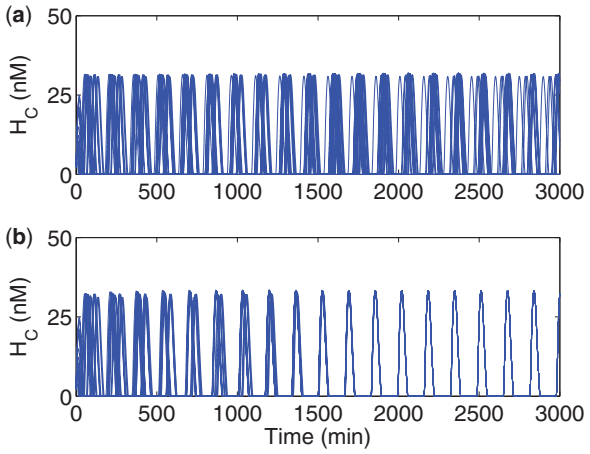

Fig. 5. Asynchrony induced by *cis*-inhibition. A sufficiently strong *cis*-inhibition strength, i.e. small enough k_c, can cause asynchrony. (**a**) $k_c = 4\,\mathrm{nM}^{-1}\,\mathrm{min}^{-1}$. (**b**) $k_c = 10\,\mathrm{nM}^{-1}\,\mathrm{min}^{-1}$.

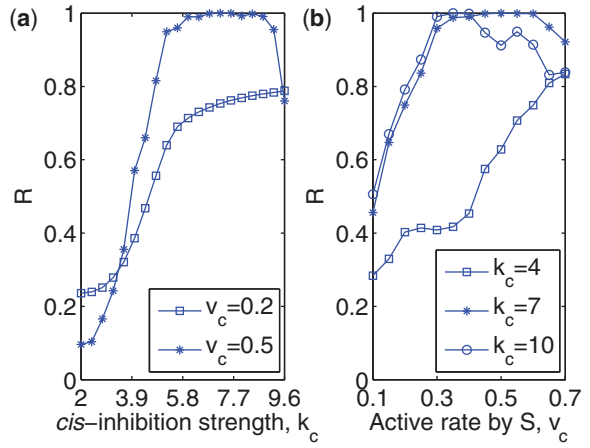

Fig. 6. Asynchrony induced by *cis*-inhibition. (**a**) Asynchrony transition for increasing the *cis*-inhibition strength, i.e. decreasing k_c, for different v_c. (**b**) Asynchrony induced by *cis*-inhibition for different k_c.

R is defined as

$$R = \frac{\langle U^2\rangle - \langle U\rangle^2}{\frac{1}{n}\sum_{i=1}^{n}(\langle H_{C,i}^2\rangle - \langle H_{C,i}\rangle^2)} = \frac{\mathrm{Var}_t(U)}{\mathrm{Mean}_i(\mathrm{Var}_t(H_{C,i}))}, \qquad (8)$$

where $U(t) = (1/n)\sum_{i=0}^{n} H_{C,i}(t)$ is the average over all cells, $\langle\cdot\rangle$ denotes the time average, and the dynamics of $H_{C,i}$ is defined by Figure 5. In the unsynchronized regime, $R << 1$, whereas $R \approx 1$ in the synchronized regime (Garcia-Ojalvo *et al.*, 2004; Gonze *et al.*, 2005). The dependence of R on the *cis*-inhibition strength k_c for two different v_c values is shown in Figure 6a. The results indicate that a transition from a synchronized to an unsynchronized regime exists as the *cis*-inhibition strength increases. Furthermore, the larger v_c is, the larger the asynchrony threshold, i.e. the smaller k_c is, because of their opposite effects in inducing asynchrony.

How asynchrony is realized is further shown in Figure 6b. Generally, synchronization is realized as the coupling strength increases (Garcia-Ojalvo *et al.*, 2004; Gonze *et al.*, 2005). However, for a sufficiently large *cis*-inhibition strength, e.g. $k_c = 4\,\mathrm{nM}^{-1}\,\mathrm{min}^{-1}$, increasing v_c is not enough to induce synchronization; this indicates that *cis*-inhibition plays a critical role in regulating asynchrony. When we decrease the *cis*-inhibition strength, e.g. $k_c = 7$ or $10\,\mathrm{nM}^{-1}\,\mathrm{min}^{-1}$, increasing the coupling strength can induce synchronization. Interestingly, if we further increase the *cis*-inhibition strength, loss of synchronization can be observed, as shown in Figure 6b. Such a phenomenon can also be observed in Figure 6a. For a given coupling strength, e.g. $v_c = 0.5\,\mathrm{nM}\,\mathrm{min}^{-1}$, a small enough *cis*-inhibition strength, e.g. $k_c > 8\,\mathrm{nM}^{-1}\,\mathrm{min}^{-1}$, cannot always achieve synchronization. There are therefore at least two sources which can induce asynchrony in neural progenitors: intermediate *cis*-inhibition and excessively strong coupling strength. The first mechanism is evident because strong *cis*-inhibition causes less release of the Notch intracellular domain S and insufficient coupling, which induces loss of synchronization. However, loss of synchronization can also be observed when the coupling is too strong. The mechanism may be that variations in v_c significantly change the system dynamics and thus make synchronization impossible; this needs to be further studied. These results suggest that *trans*-activation is essential for the generation of oscillations, and *cis*-inhibition is important for asynchrony between oscillations, indicating that the asynchronous oscillations in neural progenitors depend on cooperation between *trans*-activation and *cis*-inhibition.

3.5 Parameter sensitivity analysis

Robustness characterizes the ability to maintain performance in the face of perturbation of system parameters and is one of the essential features of cellular systems (von Dassow *et al.*, 2000). Parameter sensitivity analysis is a method frequently used to quantify robustness. A higher sensitivity of a parameter implies a lower robustness of the corresponding element. Two types of sensitivity analysis are performed. One is the state sensitivity which can be applied to steady regime. The other is period sensitivity and can be applied to operating regime of oscillations.

To perform the state sensitivity, each parameter increased and decreased its value 10% off its base value. The relative changes in their steady states are shown in Figure 7. The simulations show that the model in the steady regime is maintained over a relatively wide range of parameter values. In fact, for some of the parameters, e.g. β_D and k_7, the effect of perturbations is negligible, showing good robustness of the system. These observations indicate that the most important factors determining the differentiated state are Notch, as indicated in its production rate β_N and degradation rate v_9, and *Hes1* mRNA, as indicated in its basal transcription rate v_1 and degradation rate v_2. These observations highlight the central role of Notch and autoregulation of Hes1 in determining the dynamics of the network.

How each parameter influences the period in the asynchronous but oscillatory regime at the base parameter values is shown in Figure 8. The most sensitive parameters are v_1, v_2 and K_1, all of which are related to the autoregulation of Hes1. The system is very insensitive to perturbation of all other parameters, e.g. β_N, as shown Figure 8a and other figures shown in the Supplementary

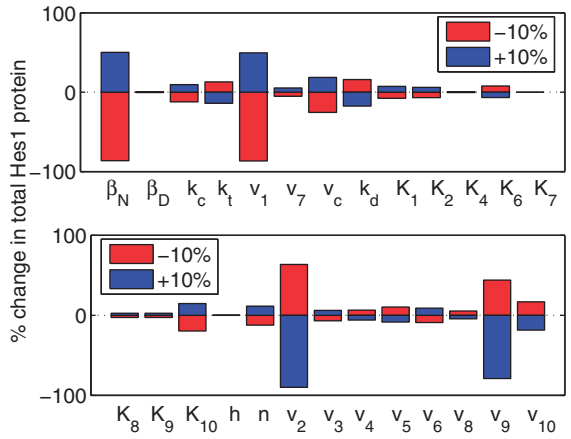

Fig. 7. Parameter sensitivity analysis. Relative changes in the steady states of the total Hes1 proteins, i.e. Hes1 in nucleus and cytoplasm, with respect to their value in the stable regime at $v_1 = 0.15\,\text{nM min}^{-1}$ when each parameter is increased and decreased by 10% over its standard value.

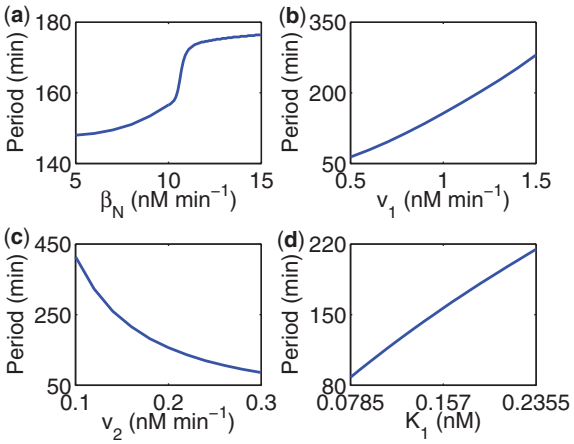

Fig. 8. Dependence of the period on (**a**) production rate of Notch, β_N; (**b**) basal transcription rate of *Hes1* mRNA, v_1; (**c**) maximum degradation rate of *Hes1* mRNA, v_2; and (**d**) threshold constant for the suppression of *Hes1* mRNA transcription by Hes1 protein, K_1.

Material. These observations indicate that the most important factor determining the period of the system is the autoregulation of Hes1.

4 DISCUSSION

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 article, we present a computational model for neural fate decisions based on intertwined dynamics with *trans*-activation and *cis*-inhibition involving the Hes1, Notch and Dll1 proteins. The model predicts that both *trans*-activation and *cis*-inhibition play critical roles in neural fate decisions and therefore provides a good framework for the theoretical analysis of the mechanisms underlying neural fate decisions mediated by both *trans*-activation and *cis*-inhibition.

Consistent with known properties of the Notch signaling, the model shows that: (i) inhibition of Notch signaling, e.g. by *cis*-inhibition, can induce neuronal differentiation and (ii) Hes1 regulates neural fate decisions via Dll1, which can either *cis*-inhibit or *trans*-activate Notch signaling, depending on its concentration. In addition, the model makes a number of testable predictions: (i) *trans*-activation is essential for the generation of oscillations and *cis*-inhibition is critical for the asynchrony between them, indicating that the asynchronous oscillations in neural progenitors depend on cooperation between *trans*-activation and *cis*-inhibition; (ii) in contrast, *cis*-inhibition plays more critical roles in embarking on neural differentiation; and (iii) the mechanism producing the oscillations may not be unique and the oscillations are perhaps produced by combinatory regulation of negative autoregulation and intercellular coupling so as to make the oscillations more robust against various perturbations.

Several experiments can be performed to test the model predictions. One of the principal predictions of the model is that *cis*-inhibition is critical for the asynchrony between adjacent cells. It might be detected by constructing cell lines which allows us to modulate the concentrations of *cis*- and *trans*-Dll1 independently, as in Sprinzak *et al*. (2010), and then observing temporal pattern of individual oscillations, as in Özbudak and Lewis (2008), to see if asynchrony can be realized when the concentration of *cis*-Dll1 is high enough. Other predictions, e.g. the interplay between *trans*-activation and *cis*-inhibition, can be similarly detected by modulating the concentrations of *cis*- and *trans*-Dll1 independently.

Some other models proposed for the gene *Hes1* assume explicit time delays (Lewis, 2003). Similar to the model presented in Uriu *et al*. (2010a, b), we modeled the Hes1 protein in the cytoplasm and the nucleus, and the model can generate oscillations without any time delays. Ultradian oscillatory networks in neural progenitors are probably more complicated. For example, Jak-Stat signaling regulates Hes1 oscillations in neural progenitors (Shimojo *et al*., 2008). However, it is not clear if Notch signaling comprises a part of the oscillator mechanism or simply coordinates the activities of the oscillators among neighboring cells. The results here suggest that multiple sources for oscillations are not mutually exclusive and, in principle, may depend on each other. The existence of autoregulation reduces the requirement for the coupling strength for oscillations and vice versa; this indicates that Hes1 oscillations are perhaps produced by combinatory regulation of negative autoregulation and intercellular coupling so as to make the oscillations more robust against various perturbations.

We have omitted some known components in the Hes1 circuit, such as Ngn2 and Mash1. Although their contributions to neural fate decisions may be important, at this stage, we are more confident considering only Hes1 as the primary regulator of Notch. Other components that are parts of the Hes1 circuit, such as Stat, which negatively regulates Hes1 and is related to neural fate decisions (Foshay and Gallicano, 2008), have not been included in our model because detailed regulatory information is still scarce. Although the model presented here is unlikely to be correct in all its details, it provides a theoretical framework for understanding the neural fate decisions mediated by both *cis*-inhibition and *trans*-activation.

The MM kinetics and different logic gates between components are assumed due to a lack of information on detailed regulatory processes. The model can be further improved when more information is available. We speculate that similar results can be

obtained when other kinetics and logic gates are used because the network topology may play crucial roles in determining the nonlinear dynamics, as in adaptations defined by network topologies (Ma *et al*., 2009). It seems that the operating mechanisms and biological implications uncovered by the model are also closely related to the network topology. For example, *cis*-inhibition may induce asynchrony simply because more Notch is *cis*-inhibited and less Notch intracellular domain can be released, so insufficient coupling is induced; this mechanism is not constrained to detailed parameter values. A concentration-dependent *cis*-inhibition process can also be obtained from the network topology but not from detailed parameter values. We therefore believe that our model captures the main features of neural fate decisions mediated by both *cis*-inhibition and *trans*-activation.

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