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

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ABSTRACT

Motivation: In the developing nervous system, the expression of proneural genes, i.e., Hes1, Neurogenin-2 (Ngn2) and Delta-like-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 intracellular 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.

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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 Alamo 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 (Ozbudak 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 (Ozbudak 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 (Ozbudak 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

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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 patterning 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 and potential implications of cis-inhibition for neural fate decisions are less clear. The model presented here involves several aspects. First, Notch of concentration $N_i$ in cell $i$ binds to extracellular Dll1 of concentration $D_{j,i}$, i.e. the average Dll1 level of all neighbors $j$ of $i$, leading to the release of the Notch intracellular domain $S_i$, and degradation of its extracellular domain. Similarly, Notch in neighboring cells, $N_{j,i}$, can bind to Dll1. Second, Notch can also bind to Dll1 within the same cell, causing inactivation of Notch. Third, the Notch intracellular domain $S_i$ 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 $N_i$, $D_{j,i}$, $S_i$, $H_{E_i}$, and Hes1 protein in the nucleus, in cell $i$ ($i = 1, \ldots, n$):

\[
\begin{align*}
\frac{dN_i}{dt} & = f_N - v_N \frac{N_i}{K_{N} + N_i} \frac{D_{D_i}}{N_i} \frac{N_i(N_{j,i})}{k_i} \\
\frac{dD_{j,i}}{dt} & = f_D - v_D \frac{D_{k,j,i} + D_{k,j}}{k_c} - \frac{D_{k,j} + D_{k,j,i}}{k_t} \\
\frac{dS_i}{dt} & = -v_S \frac{S_i}{K_{S} + S_i} + \frac{v_t}{K_{t} + H_{E_i}} \\
\frac{dH_{E_i}}{dt} & = v_E \frac{H_{E_i}}{K_{E} + H_{E_i}} - v_S \\
\end{align*}
\]
According to the parameter sensitivity analysis, we increase for the Hes1 autoregulation, we adopt parameter values used even 3D lattices, can be similarly discussed.

In Lewis (2003); Uriu et al. (2010). The notations \( (D_i^j) \) and \( (N_i^j) \) refer to the average Dll1 and Notch levels of all neighbors \( j \) of \( i \), respectively. In particular,

\[
(D_i^j) = \sum P_{ij} D_j \quad \text{and} \quad (N_i^j) = \sum P_{ij} N_j,
\]

where \( P \) is the connectivity matrix of a 2D lattice in which \( P_{ij} = 1/6 \) 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 \( \sim 25–35 \) min. However, the period of oscillations in neural progenitors is \( \sim 2–3 \) 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 \( \sim 2–3 \) h because the period increases as \( v_1 \) increases, as shown in Figure 4b. In particular, the following parameter values are used as standard values unless otherwise indicated: \( v_1 = 1.0 \text{nM min}^{-1} \), \( v_2 = 0.2 \text{nM min}^{-1} \), \( v_5 = 0.575 \text{ min}^{-1} \), \( v_6 = 0.85 \text{nM min}^{-1} \), \( v_9 = 0.021 \text{ min}^{-1} \), \( v_{10} = 0.162 \text{nM min}^{-1} \), \( v_7 = 10 \text{nM min}^{-1} \), \( v_{11} = 2 \text{nM min}^{-1} \), \( v_{12} = 10 \text{nM min}^{-1} \), \( v_{13} = 8.5 \text{nM min}^{-1} \), \( v_{14} = 5 \text{nM min}^{-1} \), \( K_1 = 0.157 \text{nM} \), \( K_2 = 0.104 \text{nM} \), \( K_3 = 0.142 \text{nM} \), \( K_4 = 0.13 \text{nM} \), \( K_5 = 2 \text{nM} \), \( K_6 = 4.72 \text{nM} \), \( h = 0.8 \), \( K_0 = 0.06 \text{ nM} \), \( K_0 = 10 \text{nM} \), \( K_0 = 2 \text{nM} \), \( K_0 = 4.72 \text{nM} \), \( h = 2 \). 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\textsuperscript{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\textsuperscript{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 \( k_c \) and \( k_t \) is shown in Figure 2a. Oscillations disappear and the system evolves toward a stable steady state in the
These results indicate that Hes1 can regulate neural fate decisions and all cells remain as progenitors. These results explain why cells ectopically express Dll1 or Dll1\textsuperscript{cis}, 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 $k_v$ versus $k_c$ at lower $v_7$, i.e. $v_7 = 2 \text{nM min}^{-1}$, and higher $v_7$, i.e. $v_7 = 16 \text{nM min}^{-1}$, are shown in Figure 3c and d, respectively. They have qualitatively similar tendency, i.e. the larger $k_v$ becomes, the larger $k_c$ is needed to generate oscillations, due to the opposite roles played by the cis-inhibition and trans-activation, except that relatively larger $k_v$ is needed to generate oscillations at relatively larger $v_7$. These results also indicate that the balance between cis-inhibition and

![Fig. 3. Bifurcation properties. (a) A bifurcation set in a parameter space of $v_7$ and $v_1$ at $k_c = 6.0 \text{nM min}^{-1}$. The larger $v_1$ becomes, the smaller the $v_7$ 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 combinatorial regulation of negative autoregulation and intercellular coupling. (b) A bifurcation set in a parameter space of $v_7$ versus $k_v$ at $v_1 = 0.2 \text{nM min}^{-1}$ (c) A bifurcation set in a parameter space of $k_v$ versus $k_c$ at $v_1 = 16 \text{nM min}^{-1}$ trans-activation exists for both cases so as to regulate the fate decisions.](image)

### 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 (Ozbudak 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 $v_7 = 0$. Hes1 signaling has been extensively studied and several computational models have been presented, in particular for the segmentation clock (Urui et al., 2010b). It has been shown that negative autoregulation with delays (Lewis, 2003), interaction with Wnt and Notch signaling...
The three-variable model for a single cell has the form of the dependent on each other. As shown in Figure 3a, the larger delayed somitogenesis model (Lewis, 2003). Such multiple sources generated by intercellular Notch signaling alone at Notch signaling alone, i.e. eliminating direct autoregulation 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 the Goodwin oscillator (Goodwin, 1965), but uses the MM kinetics. Goodwin oscillator (Goodwin, 1965), but uses the MM kinetics. Oscillations generated by negative autoregulation or Notch signaling alone in two interacting cells at time (min)

Fig. 4. Oscillations generated by negative autoregulation or Notch-signaling alone to generate oscillations. Therefore, when

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., 2005) and segmentation clocks (Uriu et al., 2010a, b). 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 \( \Delta \) 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 \( \Delta \) is released. Finally, a sufficiently large cis-inhibition strength induces less of synchronization, as shown in Figure 5a. To characterize the transition to asynchrony, a quantity
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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. $k_c = 4 \text{nM}^{-1} \text{min}^{-1}$, 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 $10 \text{nM}^{-1} \text{min}^{-1}$, 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. $v_c = 0.5 \text{nM}^{-1} \text{min}^{-1}$, a small enough cis-inhibition strength, e.g. $k_c > 8 \text{nM}^{-1} \text{min}^{-1}$, 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. $\beta_D$ and $k_S$, 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 $\beta_D$ and degradation rate $\nu_D$, and Hes1 mRNA, as indicated in its basal transcription rate $\nu_1$ and degradation rate $\nu_2$. 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 $\nu_1$, $\nu_2$ and $K_s$, all of which are related to the autoregulation of Hes1. The system is very insensitive to perturbation of all other parameters, e.g. $\beta_D$, as shown Figure 8a and other figures shown in the Supplementary.

Fig. 5. In the unsynchronized regime, $R$ denoted the time average, and the dynamics of $C_1$ for increasing the cis-inhibition strength, i.e. small enough $k_c$.

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 $v_c$.

$R$ is defined as

$$R = \frac{\left(\frac{\sum_{i=1}^{n} (H_{C_1}^\gamma_i)}{n}\right)^2 - \left(\frac{\sum_{i=1}^{n} (H_{C_1}^\gamma_i)}{n}\right)\cdot \left(\frac{\sum_{i=1}^{n} H_{C_1}^\gamma_i}{n}\right)}{\text{Var}(U) / \text{Mean}(\text{Var}(H_{C_1}^\gamma))},$$

where $U(t) = (1/n)\sum_{i=1}^{n} H_{C_1}^\gamma_i(t)$ is the average over all cells, $\gamma$ denotes the time average, and the dynamics of $H_{C_1}$ 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.
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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 be not be unique and the oscillations are perhaps produced by combinatorial 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 Ozbudak 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 combinatorial 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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Conflict of Interest: none declared.

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