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Sustained vs. oscillating expressions of Ngn2, Dll1 and Hes1: A model of neural differentiation of embryonic telencephalon

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HIGHLIGHTS

- We study a model of neural differentiation for two cells consistent with the experimental results.
- The model is robust against fluctuations.
- We introduce the neighbor cells in a self-consistent way.

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ABSTRACT

Neural progenitor cells show oscillatory expression of the Notch ligand Delta-like1 (Dll1), the Notch target Hes1 and the proneural gene Neurogenin 2 (Ngn2) during embryonic development of the mammalian telencephalon. On the other hand, expression of these genes is sustained in postmitotic neurons (upregulated for Ngn2 and Dll1, down regulated for Hes1). These facts suggest that a switch from oscillatory to sustained expression of proneural and other genes is critical in neural fate decisions. Moreover, despite controversies over the role of Numb in determining the neural fate in mammals, there is evidence that inheritance of Numb during neurogenic cell division is involved in neural differentiation. It is also known that mNumb activates Notch1 receptor degradation. The arrest of oscillations in a given cell may be due to increasing degradation of Notch1 brought about by mNumb during neurogenic division. We introduce a modification in a previous model of the gene network for two cells coupled by the Delta-Notch pathway (Wang et al., 2011). We analyze the consequences of an asymmetry between two neighbor cells in the rate of degradation of Notch (mimicking the effect of asymmetric inheritance of mNumb during the neurogenic division). The results show that a slight difference in Notch degradation between the two cells keeps oscillation going in one of them while oscillation stops in the other. Moreover, when Delta-Notch coupling is canceled, both cells show sustained expression (upregulated levels for Ngn2 and Dll1, downregulated for Hes1). We show that the model is stable against parameter variations. Moreover, to take into account the possible influence of the environment on both cells, neighboring cells are included in a mean field approximation. Both, parameter fluctuations and effects of the environment lead to asynchronous oscillations of Hes1/Ngn2 in different progenitor cells.

1. Introduction

The Notch pathway plays a key role in multiple cell fate decisions among initially equivalent cells, in which the adoption of a fate in a cell inhibits its neighbor to adopt the same (lateral inhibition), thereby promoting phenotypic diversity (Alberts et al., 2008).

In the developing nervous system of mammals, neurogenesis requires the expression of basic helix-loop-helix (bHLH) proneural transcription factors such as Mash1 or Neurogenin2 (Ngn2). Among other targets, these proteins activate the synthesis of ligands of Notch as Delta like1 (Dll1), which in turn activates the Notch pathway in the neighboring cells. This activation induces the cleavage of the Notch receptor, generating an intracellular domain (NICD) that translocates to the nucleus, where it forms a complex with the protein RBPj. This complex induces the expression of bHLH repressor such as Hes1 and Hes5, which repress the expression of proneural genes and Notch ligands, inhibiting neural differentiation and maintaining their progenitor state (Kageyama et al., 2008). Beyond interspecific sequence differences and their

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designations, this circuit is conserved in vertebrates and Drosophila (Vetter and Dorsky, 2005).

This circuit allows that, from a population of cells with equivalent levels of expression of proneural genes, some subgroups of them become selected to initiate differentiation, and the remainder stay as neural progenitors cells (NPCs), available to differentiate into different neural types in subsequent stages. The mechanism originally proposed for this selection (based on the neurogenesis of *Drosophila melanogaster* and the development of the vulva in *Ceanorhabditis elegans*) is the following: initially the neuroepithelium of the developing brain is an equivalent cell population (all NPCs have equivalent levels proneural genes and Notch ligands, Dll1). However, due to the stochastic variations, some express slightly higher levels of proneural genes and Dll1 which implies more strong activation of the Notch pathway in neighboring cells, which express higher levels of Hes and consequently a lowering proneural genes and Dll1 (remaining as NPCs) and therefore, decreasing the Notch activity in the first (favoring neural differentiation). Thus, in this scenario, lateral inhibition mediated by the interaction Delta–Notch selects neural progenitors in order to start the differentiation and amplify stochastic variations between neighboring cells (Kageyama et al., 2008). In the developing mammalian brain, the analysis by in situ hybridization and by inmunostaining of gene Hes1 reveals varying levels of expression of the proneural genes and Notch ligands (Hatakeyama et al., 2004; Sommer et al., 1996; Guillemot and Joyner, 1993). These results were interpreted accordingly as neurogenesis in Drosophila: those identifiable cells with increased expression of proneural genes and Notch ligands become immature postmitotic neurons.

However, in the developing dorsal telencephalon, the expression of proneural genes changes dynamically. Indeed, real-time imaging analysis of Ngn2, Dll1 and Hes1 reveals oscillations with a period of 2–3 h in NPCs and sustained levels of Ngn2 and Dll1 (and repressed levels of Hes 1) in postmitotic neurons. Furthermore, immunohistochemical analysis showed that the expression of Hes1 correlates inversely with those of Ngn2 and Dll1, suggesting that fluctuations in Hes1 regulate in an inhibitory way the Ngn2 expression which, in turn, regulates the Dll1 (Shimojo et al., 2008). Furthermore, blockade of the Notch pathway, a condition which induces differentiation (Bertrand et al., 2002), permanently represses the expression of Hes1 and sustainedly activates those of Dll1 and Ngn2. After this series of observations, the authors hypothesize that one function of the Hes1 oscillations is to regulate Ngn2 and Dll1 oscillations (the last by the former), which would keep a set of cells in a state of NPCs by mutually activation of Notch signaling, being the sustained Ngn2 and Dll1 expression and Hes1 repression, a prerequisite for differentiation (Shimojo et al., 2008; Kageyama et al., 2008). This calls into question the role of lateral inhibition mediated by the interaction Delta–Notch amplifying and fixing small stochastic variations (Kageyama et al., 2008) and thus restates the selection mechanism of NPCs to initiate the differentiation: therefore if the Notch-mediated lateral inhibition is necessary for the maintenance of NPCs, but not for the selection of neural fate, which mechanism arrests the described oscillations to initiate the differentiation?

One possible mechanism involves mNumb. Numb is a protein initially identified in Drosophila (dNumb), which is asymmetrically segregated by dividing cells and intervening in specification fate in cell that inheritance, where it is believed, inhibits the action of Notch (Cayoutte and Raff, 2002). This protein has two homologous variants in mammals: mNumb and mNumb-like (Zhong et al., 1996). While mammalian Numb functions are controversial (Gulino et al., 2010), it is known that in isolated NPCs from mouse cortex, the sister cell which preferentially segregates mNumb, initiates the specification of one (in the neurogenic asymmetric division) or both cells (in neurogenic symmetrical division) (Shen et al., 2002). Furthermore, as dNumb, mNumb acts by inhibiting the Notch pathway. Indeed, it has been observed that mNumb promotes Notch1 receptor ubiquitination and degradation of its intracellular domain, with a corresponding loss of transcriptional activity of the Hes1 promoter (Mc Gill and Mc Glade, 2003, 2009).

In summary, if the oscillations in the Notch signaling keep the neuroepithelial cells in a progenitor state and if, moreover, the inheritance of mNumb is critical in neural specification, a possible mechanism to unify both phenomena could be the mNumb inheritance (Shimojo et al., 2011). This hypothesis is supported by the observation that in cultures of mouse NPCs, Hes1 expression is repressed in $Numb^{+}$ cells, which initiate the differentiation while Hes1 expression is maintained in $Numb^{-}$ cells (Ohtsuka et al., 2006).

This paper analyzes the implication of a difference in the value of the parameter that regulates the Notch degradation between two cells coupled by Delta–Notch, mimicking the inheritance of Numb during asymmetric division. Their numerical simulation shows a distinctive feature of mammalian cortical development: the maintaining of a fraction of "NPCs" with oscillations in the expression of Hes1, Ngn2 and Dll1 and sustained levels (upregulated Ngn2 and Dll1 and downregulated Hes1) in "postmitotic neurons".

2. Previous models

Several models have been constructed to analyze the behavior of preserved architecture of the neurogenic network in Drosophila and vertebrates (Momiji and Monk, 2009) and specifically for the study of differentiation in mammals (Kiparissides et al., 2011; Wang et al., 2011).

Momiji and Monk (2009) have analyzed a model of the basic structure of the network of genes (conserved in Drosophila and vertebrates) involved in regulating neural differentiation (neurogenetic network), with delays in the interaction between its components. The motivation of this work is the analysis of a "network motif" common in genetic regulatory networks: a reciprocal inhibition between two regulatory genes that allows multiple systems to function as a "toggle switch", whereby the system can adopt one among two alternative states (in the context of embryonic development, between a differentiated cell and an undifferentiated one). The neurogenetic network is a spatially extended toggle switch formed by reciprocal inhibition between Delta ligands of a cell and Notch receptors in neighboring, embedded in a larger network formed by interaction with the proneural genes and Hes repressors.

The analysis of the model with two coupled cells reveals that the incorporation of explicit delays in the circuit leads, among other dynamic effects, to transient oscillations before sustained states in the expression levels of proneural genes: upregulated in a cell and downregulated in the neighbor. Momiji and Monk (2009) compare this result with the observations of Shimojo et al. (2008): the oscillations temporarily maintain the cells in a state of NPCs before reaching steady levels. However, the model does not capture a fundamental fact of this system: the permanence of a fraction of cells in neural precursor state (oscillations in Hes1, Dll1 and Ngn2) and the differentiation of a fraction of postmitotic neurons (sustained upregulation of Dll1 and Ngn2 and downregulation of Hes1).

Wang et al. (2011) model the Notch signaling, incorporating into the intercellular activation mechanism (trans-activation), the cis-inhibition of Notch exerted by its ligand Delta in the same cell. The model rescues two phenomena of the developing mouse brain: (1) the existence of oscillations in neural progenitors and their arrest in differentiated cells and (2) the asynchrony in these oscillations (Shimojo et al., 2008; Aulehla and Pourquí, 2008). The authors study the behavior of the model in relation to the relative strength of the trans-activation and the cis-inhibition, varying the corresponding parameters.

The study of the model in the parameter space (k_c,k_t) shows two regimes for the stationary dynamics of $H_{C,i}$: an oscillatory regime (high k_c respect to k_t) and a fixed point (high k_t respect to k_c). This observation captures the results obtained by Shimojo et al. (2008) mentioned above, suggesting that the relative intensities of the trans-activation and cis-inhibition could play critical roles in the decision between staying as a NPC or embarking on neural differentiation, respectively.

Beyond the experimental facts that rescue the previous models, a key aspect of the telencephalic neuroepithelium development is not reproduced: the maintenance of undifferentiated stem cells fraction with oscillating levels of expression in the proneural genes (and Hes) and the differentiation of others in postmitotic neurons with sustained levels of expression (upregulated and downregulated, respectively). That is, the models of two coupled cells, so far studied, are symmetrical in their dynamics.

3. The two cell model

One factor that naturally introduces an asymmetry in two coupled cells is the asymmetric inheritance of Numb produced during the cell division.

The introduction of a modified version of the model of Wang et al. (2011) with a small difference in the values of the parameter that regulates the degradation of Notch, mimics the action of Numb, had the interesting consequence of allowing oscillations in the Notch signaling in a cell and simultaneously leads to sustained levels (upregulated of Ngn2 and Dll1 and downregulated of Hes1, respectively) in the "neighbor".

This captures a fundamental fact in cell fate decisions during brain development at the two cells levels: the differentiation of neural progenitor fraction in immature postmitotic neurons and, contemporarily, the maintenance of an undifferentiated progenitors fraction available to differentiate themselves into neural types at subsequent stages. To analyze the effect of a difference in the rate of degradation of Notch between two coupled cells, we made some modifications to the model of Wang et al. (2011):

1. The addition of a new variable (Ngn) that describes the dynamics of Ngn2.
2. An asymmetry in the values of the parameter that regulates the degradation of Notch in each cell.
3. Elimination of cis-inhibition ($k_c \to \infty$) in each cell.

According to the results obtained by Shimojo et al. (2008), the NPCs are characterized by oscillations in antiphase of Ngn2 and Dll1 vs. Hes1. Moreover, the postmitotic neurons exhibit sustained levels of Ngn2 and Dll1 (and repressed Hes1). Therefore, in order to study the possibility of that a difference in the degradation of Notch receptor between two neighbor cells activates the differentiation of one of them, keeping undifferentiated its neighbor, we introduced explicitly Ngn2.

Thus, the model for two coupled cells describes the following: the interaction of Dll1 with Notch receptor induces its cleavage, generating the NICD peptide that translocates to the nucleus, where it activates the transcription of Hes1 gene, whose protein represses the expression of Ngn2 and Dll1 moreover of the expression of itself. In turn, the Ngn2 activates the transcription of Dll1. Finally, mNumb activates the Notch receptor degradation (see Fig. 1).

This circuit can be expressed by seven differential equations describing the dynamics of Notch receptor (N_i), Dll1 (D_i), NICD (S_i), Hes1 mRNA (M_i), cytoplasmatic protein Hes1 (Hc_i), Hes 1 nuclear protein ($H_{N,i}$) and Ngn2 protein (Ngn_i).

$$\frac{dN_i}{dt} = \beta_N - \nu_9 \frac{N_i}{K_9 + N_i} - \frac{N_i \langle D_j \rangle_i}{k_t}, \tag{1}$$

$$\frac{dD_i}{dt} = \beta_D - \nu_8 \frac{D_i}{K_9 + D_i} - \frac{D_i \langle N_j \rangle_i}{k_t} + \nu_7 \frac{K_7^h}{K_7^h + H_{N,i}^h} + \nu_{Ngn} Ngn_i, \tag{2}$$

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

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

$$\frac{dH_{C,i}}{dt} = \nu_3 M_i - \nu_4 \frac{H_{C,i}}{K_4 + H_{C,i}} - \nu_5 H_{C,i}, \tag{5}$$

$$\frac{dH_{N,i}}{dt} = -\nu_6 \frac{H_{N,i}}{K_6 + H_{N,i}} + \nu_5 H_{C,i}, \tag{6}$$

$$\frac{dNgn_i}{dt} = \beta_{Ngn} - \nu_{11} \frac{Ngn_i}{K_{11} + Ngn_i} - \beta_{dec} H_{N,i}, \tag{7}$$

$\langle N_j \rangle$ and $\langle D_j \rangle$ represent concentrations of the Notch receptor and ligand Dll1 in the neighbor cell, respectively.

Except for the new parameters (ν_{Ngn},$\nu_1$1,β_{Ngn},β_{dec} and K_{11}), the parameters were taken from Wang et al. (2011) but to reproduce the period of 2–3 h obtained by Shimojo et al. (2008) we modify the ratios (the parameters ν and β) by multiplying by a constant factor (see Table 1).

4. Results

4.1. The effect of asymmetric division

An asymmetry in the ν_9 value is capable to arrest the oscillation in a cell and keeps its neighbor in an oscillatory state. The cells of the neuroepithelium of the developing telencephalon in the state of NPCs are characterized by oscillations in the expression of Hes1, Ngn2 and Dll1 and immunohistochemical analysis suggests that Hes1 oscillations negatively regulate those of Ngn2 and Dll1 (Shimojo et al., 2008). Consequently, the numerical simulation of the model without the asymmetry in the degradation of Notch, shows such oscillations with a period of 120 min (according to the experimentally observed 2–3 h), being Hes1 oscillations in counterphase on the oscillations of Ngn2 (Fig. 2a).

As mentioned, experimental evidences (Shimojo et al., 2008) suggest that the differentiation of NPCs into postmitotic neurons requires sustained upregulation and downregulation of Ngn2 and Hes1, respectively. On the other hand, it has been argued that a possible mechanism of transition from an oscillating expression of Hes1 (and Ngn2 and Dll1) to sustained expressions (downregulated and upregulated, respectively) could be the heritage of mNumb (Kageyama et al., 2008).

Although mNumb activates the degradation of Notch receptor, which correlates with a lose of the activity of Hes1 promoter (Mc Gill and Mc Glade, 2003), it has not been shown it effectively acts in this way during dorsal telencephalon development.

To verify whether the model can account for this hypothesis, we performed a simulation with a difference between the two cells on the values of the parameter that controls the rate of degradation of Notch (ν_9). Notably, a slight difference (see Table 1) causes an arrest in the oscillations of Ngn2, Hes1 and Dll1 to sustained values (with those of Ngn2 surpassing those of Hes1) in the cell of higher degradation of Notch (greater ν_9), while maintaining oscillations in the neighbor (Fig. 2b). This reproduces qualitatively the experimental results of Shimojo et al. (2008): progenitors with oscillations

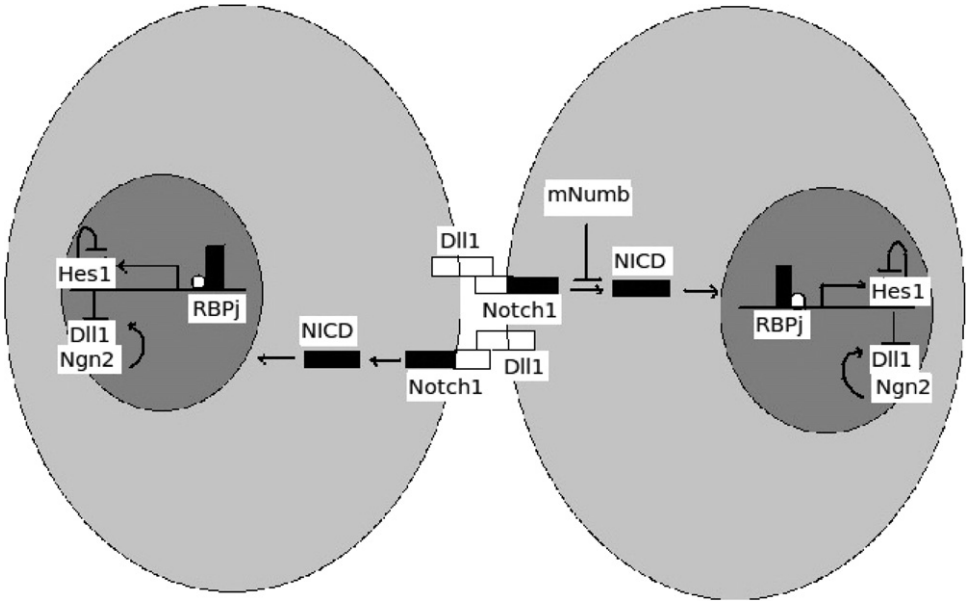

Fig. 1. The interaction between Dll1 and Notch1 of the neighbor cells induces cleavage of the latter, forming the NICD-peptide which translocates to the nucleus where it forms a complex with the protein RBPj. This complex activates the Hes1 transcription, which represses the transcriptions of DLL1, Ngn2 and himself. In turn, Ngn2 can activate transcription of DLL1. The possible presence of mNumb can inhibit the initial step of this pathway by activating the degradation of Notch1.

Table 1
Parameters used in the calculations. These are identical for both cells and the results are shown in Fig. 2a. The results corresponding to Fig. 2b and c, differ only in the rate of degradation of Notch (v_9). The third column corresponds to the standard deviations of the parameters when we introduce their fluctuations.

	Cell 1	Cell 2	σ
v_1	0.064 nM min^{-1}		0.004 nM min^{-1}
v_2	0.064 nM min^{-1}		0.004 nM min^{-1}
v_3	0.184 nM min^{-1}		0.011 nM min^{-1}
v_4	0.2723 nM min^{-1}		0.016 nM min^{-1}
v_5	0.0067 min^{-1}		0.0004 min^{-1}
v_6	0.0518 nM min^{-1}		0.003 nM min^{-1}
v_7	1.28 nM min^{-1}		0.075 nM min^{-1}
v_8	6.4 nM min^{-1}		0.37 nM min^{-1}
v_9	2.72 nM min^{-1}	3.68 nM min^{-1}	0.03 nM min^{-1}
v_{10}	3.2 nM min^{-1}		0.19 nM min^{-1}
v_{11}	0.88 nM min^{-1}		0.009 nM min^{-1}
v_{Ngn}	0.64 min^{-1}		0.004 min^{-1}
K_1	0.157 nM		0.0157 nM
K_2	0.104 nM		0.001 nM
K_4	0.142 nM		0.001 nM
K_6	0.13 nM		0.001 nM
K_d	2 nM		0.02 nM
K_7	0.2 nM		0.002 nM
K_8	0.6 nM		0.006 nM
K_9	0.06 nM		0.0006 nM
K_{10}	10 nM		0.01 nM
K_{11}	0.03 nM		0.0003 nM
k_t	31.25 nM^{-1} min^{-1}		1.81 nM^{-1} min^{-1}
β_D	0.32 nM min^{-1}		0.02 nM min^{-1}
v_c	0.064 nM min^{-1}		0.004 nM min^{-1}
β_N	3.2 nM min^{-1}		0.2 nM min^{-1}
β_{Ngn}	0.8 nM min^{-1}		0.008 nM min^{-1}
β_{dec}	0.9 nM min^{-1}		0.009 nM min^{-1}
h	2		–
n	2		–

in Notch signaling vs. sustained expressions of Ngn2 vs. and Dll1 and Hes1 repressed in postmitotic neurons.

Inhibition of the Notch signaling induces neural differentiation in the embryonic nervous system of vertebrates (Chitnis et al., 1995; Coffman et al., 1993). Consequently, Shimojo et al. (2008) found that inhibition of Notch signaling represses expression of Hes1 and activates Ngn2 and Dll1 expressions.

To test whether the model can account for this result, we cancel the communication between the two cells (by canceling the terms involving the parameter k_t in Eqs. (1)–(3)). Notably, the result of this implementation is an inhibition of the oscillations of Hes1 and sustained expressions of Ngn2 and Dll1 (Fig. 2c, according to the results of Shimojo et al., 2008).

4.2. Model robustness against parameter fluctuations

To test the robustness of the model, we introduced Gaussian fluctuations in the values of the parameters. Dispersions were taken so that the qualitative behavior is the same as the original solution (third column of Table 1). This procedure introduced a dispersion in the period T of the oscillations (Fig. 3) consistent with results of Shimojo et al. (2008). The curve of Fig. 3 was fitted by a Gaussian ($\overline{T}=136.3$ min; $\sigma_T=5.1$ min). Fig. 4 shows the oscillations of Ngn2 for several sets of parameters leading to periods $\overline{T}-\sigma_T<T<\overline{T}+\sigma_T$. As can be seen, there is a marked asynchrony between them.

4.3. The two cells model and their neighboring

To analyze the behavior of the model in a neighborhood of NPCs, we considered the model of two cells (A and B) embedded in an array of eight neighbor NPCs coupled by the Notch pathway (see Fig. 5). The terms of interactions in Eqs. (1)–(3) are

$$\frac{N_A\langle D_j\rangle_i}{k_t}=\frac{N_A(D_B+\sum_{i=1}^{i=5}D_i)}{6k_t}, \tag{8}$$

$$\frac{D_A\langle N_j\rangle_i}{k_t}=\frac{D_A(N_B+\sum_{i=1}^{i=5}N_i)}{6k_t}, \tag{9}$$

for cell A and

$$\frac{N_B\langle D_j\rangle_i}{k_t}=\frac{N_B(D_A+\sum_{i=4}^{i=8}D_i)}{6k_t}, \tag{10}$$

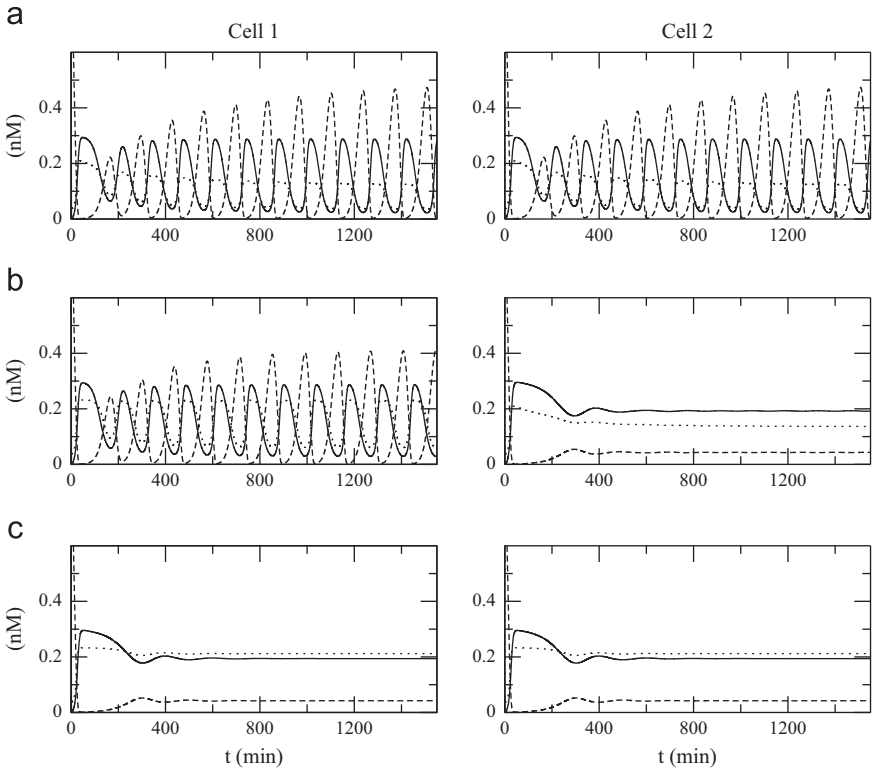

Fig. 2. Concentrations of free Dll1 (pointed line), Hes1 in the nucleus (dashed line) and Neurogenin2 (solid line) as a function of time. (a) The cells are identical (the parameters are the same for both). (b) The cells only differ in the rate v_9. (c) The cells only differ in the rate v_9 but do not interact.

$$\frac{D_B\langle N_j\rangle_i}{k_t} = \frac{D_B(N_A+\sum_{i=4}^{i=8}N_i)}{6k_t}, \tag{11}$$

for cell B.

Given the local nature of each mitosis, we assume that the times of birth of each cell are not related to each other, and that each cell is "born" with a cycle phase relationship Hes1/Ngn2/Dll1 uncorrelated with their neighbors. As the eight surrounding cells are NPCs, to start a self-consistent calculation, we set the values of the variables corresponding to D_i and N_i, as those obtained in Section 4.1 for NPCs: D_o (pointed line in Fig. 2(a)) and N_o. Moreover, given the asynchrony of the oscillations, we introduced a random phase shift between them. That is

$$D_i(t) = D_o(t-\tau_i), \tag{12}$$

$$N_i(t) = N_o(t-\tau_i) \tag{13}$$

where $0<\tau_i<\overline{T}$, $i=1,2,\ldots,8$ are random.

Then, we solved in a self-consistent way the equations for two cases:

1. Both cells (A and B) remain as NPCs ($v_{9A}=v_{9B}=2.72$ nM min^{-1}).
2. A cell (A) remains NPC, while the other (B) becomes postmitotic neuron ($v_{9A}=2.72$ nM min^{-1}; $V_{9B}=3.68$ nM min^{-1}).

For the first case, the self-consistent solution is obtained by reinjecting the solution for D_A (or alternatively D_B) and N_A (or alternatively N_B) in Eq. (13), instead D_o and N_o), with their corresponding phases as new $D_i(t)$ and $N_i(t)$. The calculation is iterated until the D_A, N_A (*or* D_B, N_B) solutions converge (i.e. are the same as those that were reinjected in the last step.)

For the second case, the procedure is similar, but only D_A and N_A are reinjected (as D_B and N_B are not oscillatory). The result is shown in Fig. 6. There it can be seen the Dll1, Hes1 and Ngn2 for cells A and B both in the two cases 1 and 2. The results are not very different

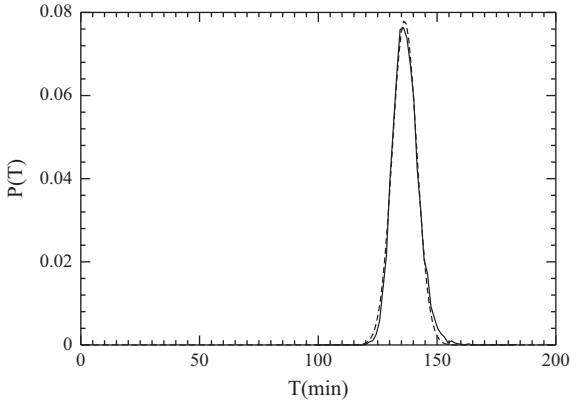

Fig. 3. Distribution (normalized) of periods for the NPCs oscillations introduced by fluctuations of the parameters. The dashed curve corresponds to a Gaussian fit with $\overline{T}=136.3$ min; $\sigma_T=5.1$ min.

from those obtained by completely ignoring the environment which suggests that its influence is perturbative. However, in case 1, it can be seen that the effect of this perturbation leads to a phase difference between the oscillations of the resulting NPCs (A and B) absent if we ignore the environment (the graphs in Fig. 2a are identical for both cells). For case 2, the effect of the environment does not seem to be relevant and the result is qualitatively the same as that obtained in Section 4.1 (graphs in Fig. 2b): persistent oscillations in the cell A and the arrest of these oscillations to the cell B.

5. Discussion and conclusions

The results for two coupled NPCs by Delta–Notch show that a slight difference, introduced by asymmetric division, between the two cells in the parameter values involved in the degradation of Notch

(mimicking a possible action of mNumb) arrests the oscillations of Hes1, Ngn2 and Dll1 to fixed values (downregulated for Hes1 and upregulated for Ngn2 and Dll1). This result reproduces qualitatively the model proposed by Shimojo et al. (2008) and makes plausible the hypothesis of a role of mNumb in specifying neural fate during asymmetric division, differentially affecting Notch receptor degradation in the specified cell. Moreover, the model is consistent with the experimental results that indicate that inhibition of the Notch signaling induces early neural differentiation (Chitnis et al., 1995; Coffman et al., 1993). Indeed, eliminating the coupling terms between neighbor cells ($k_t \to \infty$) in Eqs. (1)–(3), Hes1 oscillations vanish and the expression of Ngn2 and Dll1 reaches sustained levels (mimicking the condition of postmitotic neurons). This result supports the hypothesis that a function of the oscillations of Dll1 (lead by Hes1) is to maintain oscillations in their neighbors, keeping a group of cells as neural NPCs.

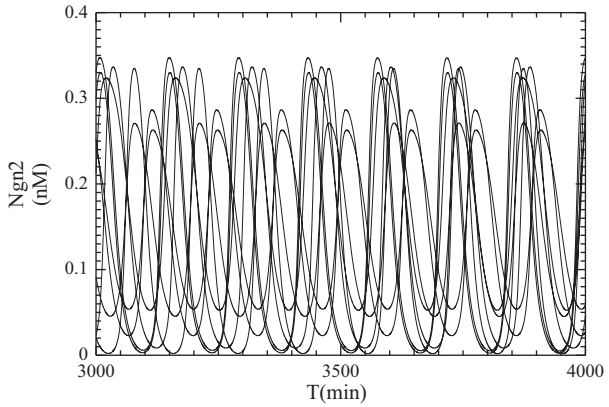

Fig. 4. Oscillations of Ngn2 in NPCs for several sets of parameters leading to periods $\overline{T}-\sigma_T < T < \overline{T}+\sigma_T$.

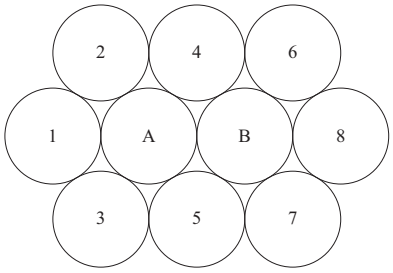

Fig. 5. Cells A and B are the result of mitosis and are surrounded by eight NPCs which are in another stage of the cell cycle. Cells A and B interact with each other and also with the 1–5 and 4–8, respectively.

As mentioned in the Introduction, a common result in the two cells models discussed above, is that they allow both cells to arrest their oscillations (allowing differentiation for two) or both oscillate (remaining both as NPCs). This is not a defect of these models, but a consequence of the questions addressed: the role of delays in the interactions of its components (Momiji and Monk, 2009) or the relative importance of trans-activation and cis-inhibition in Notch signaling (Wang et al., 2011).

The specific contribution of our results is to show that a difference in the rate of degradation of Notch receptor between two coupled cells, allows the maintenance of oscillations in a cell ("NPC") while maintaining a sustained expression of Ngn2 and Dll1 and repressed of Hes1 in the cell of higher rate of degradation of the Notch receptor ("postmitotic neuron"). This rescues the phenomenon of asymmetric neurogenic division and allows us to interpret the results of Shimojo et al. (2008): NPCs with oscillations in Notch signaling vs. postmitotic neurons with sustained expression (upregulated Ngn2 and Dll1 and downregulated Hes1) in terms of differential degradation of the Notch receptor (which in the context of a division, could be caused by asymmetric mNumb inheritance in the specified cell).

Nevertheless the results obtained here, the role of mNumb in neural differentiation in mammals is controversial. While there is evidence of its role in neural specification (Shen et al., 2002) and that it inhibits the Notch signaling by activating the Notch receptor

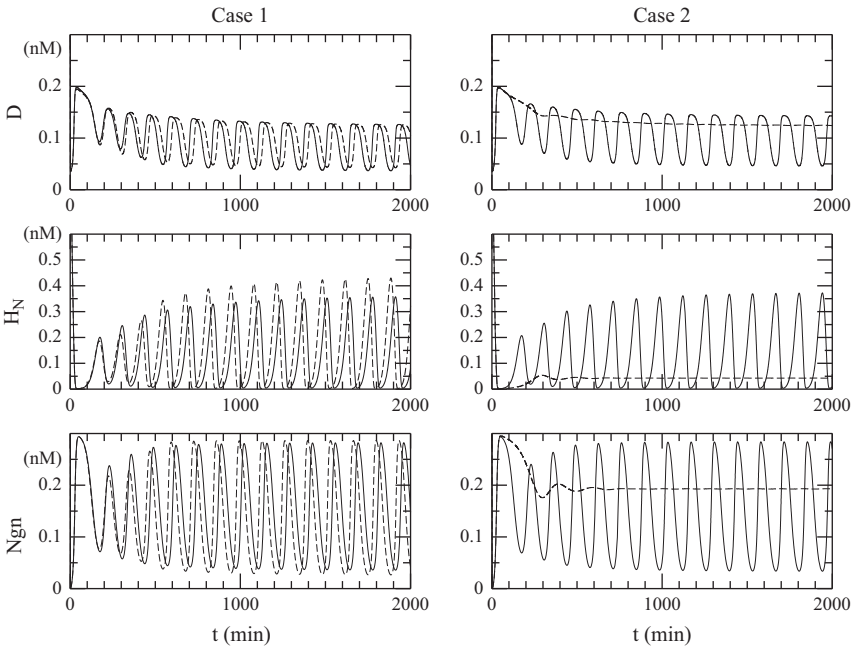

Fig. 6. Results considering the environment in a self-consistent way. The first column corresponds to case 1 in which two cells (*A* and *B*) resulting from mitosis persist as NPCs. We can see that expressions of Dll1, Hes1 and Ngn2 are oscillating for both cells (solid line for *A* and dashed line for *B*). Dll1 and Ngn2 are in phase and both are in antiphase with Hes1 within each cell. A phase shift between the respective expressions for both cells can also be seen. The second column corresponds to the case 2 corresponding to the persistence of *A* as NPC and the differentiation of *B* as postmitotic neuron. The expressions for *A* remain oscillating while *B* is arrested. Dll1 and Ngn2 have high values and Hes1 has a low value.

degradation (Mc Gill and Mc Glade, 2003, 2009), there is also evidence involved in the maintenance of NPCs (Yoon and Gaiano, 2005) and that its inhibitory action on Notch signaling is not their only function (Gulino et al., 2010).

A point made in the literature on the oscillations of the Notch pathway in the NPCs is its asynchronous nature and its difference from the somitogenesis (Aulehla and Pourquí, 2008; Wang et al., 2011; Kageyama et al., 2008): while the somitic mesoderm is a periodic structure in the developing telencephalic neuroepithelium not observed any obvious spatial structure.

In the first case, it has been argued that the coupling Delta–Notch synchronizes oscillations in the expression of genes Hes/Her/cHairy (depending on the species) and the oscillations are arrested at different phases of their cycles in an "arrest front" (established by the minimum concentration FGF4 gradient) located at the anterior end of the PSM (Dubrulle and Pourquiè, 2004). Thus, the oscillations produce a periodic spatial pattern of gene expression, whose frequency sets the boundaries between the somites, where coherent phase relationships at the tissue level, organize the periodical structures that are characteristic of the somitic mesoderm (Aulehla and Pourquí, 2008).

In the developing telencephalon, the situation looks quite different. Indeed, the expression levels of Hes 1, revealed by in situ hybridization, sample a "salt and pepper" pattern: individual cells with varying levels of expression without spatial correlation (Kageyama et al., 2009). As noted in the Introduction, this result was interpreted coming from stochastic variations Hes1 levels, that lateral inhibition mediated by Delta–Notch pathway amplifies to specify groups of cells as postmitotic neurons (high expression of proneural genes; low Hes1) in successive rounds of differentiation.

The discovery of Shimojo et al. (2008) that the expressions of Dll1, Hes1 and Ngn2 are oscillating, has led to reinterpret the salt and pepper pattern as a result of unsynchronized oscillations. This interpretation is supported by the observation that isolated NPCs have variations in the period of the oscillations (2–3 h) (Shimojo et al., 2008).

Attempts have been made to understand the basis of this asynchrony. In the model of Wang et al. (2011), the increase in the cis-inhibition of Notch pathway generates a transition to asynchrony. However, the non-equivalence between cells generated by the lattice structure of the cell array and the imposed boundary conditions can generate asynchronic oscillations regardless of cis-inhibition. In our model, the asynchrony is originated by two sources. As it was showed in Section 4.2, fluctuations in the parameters induce dispersion in the period of the oscillations. Moreover, as it was assumed in Section 4.3, the times of birth of each cell are not related to each other, originating oscillations out of phase. Because the differences between the times in which mitosis occur have no apparent correlation, the cells reach their stationary states asynchronously. Thus, different pairs of NPCs created by mitosis in different sites from the neuroepithelium would have no correlations in the expression levels of Hes1/Ngn2. A phenomenon that is expressed in the observed salt and pepper pattern.

Let us remark that in our model the differentiation process is originated by the asymmetric inheritance and the cell interactions therefore the inclusion of cis-inhibition (an intracellular process) in a symmetric way in both cells would not change the qualitative results. Indeed, we have performed calculations including the cis-inhibition and the result is that the change of ν_9 parameter to lower values is just enough to obtain the showed effects in Fig. 2. We explain this fact as follows: the cis-inhibition contributes to diminish the concentration rate of Notch receptor (Eq. (1)) therefore lower values of the rate degradation of Notch receptor (ν_9) are needed.

In addition to the possible role of mNumb in the arrest of the oscillations discussed here, there are other possible mechanisms to regulate the oscillatory vs. non-oscillatory behavior in this system, which are not treated in this paper. For example, Shimojo et al. (2008) founded that inhibiting Jak2–Stat3 signaling also achieves to cancel the Hes1 oscillations, suggesting that this pathway can also to regulate them. Another possible mechanism is the presence of Id factors (Bai et al., 2007). These helix-loop-helix (HLH) factors are highly expressed in regions of low proliferation and no neurogenesis (boundary regions). In these boundary regions, the Id factors inhibit the autorepressive effect of Hes1, inhibiting their oscillations, unlike what happens in the compartments (for example, the dorsal telencephalon) (Shimojo et al., 2008).

Deserves special attention the finding by Bonev et al. (2012) of a new component of the Hes1 oscillations in NPCs. Indeed, the authors identify a double-negative feedback between a microRNA (miR-9) and Hes1. By means of such feedback, Hes1 represses the transcription of pri-miR-9 (precursor of miR-9) and miR-9 actives the degradation of mRNA Hes1. Moreover Bonev et al. (2012) present evidence that Hes1 shows oscillations for a specific range of degradation rate of mRNA Hes1. In effect, both mutant versions of Hes1 mRNA (miR-9 where the binding is abolished) and overexpression of miR-9 led to reduce the number of cycles before the arrest.

Regarding to this mechanism, the authors propose a model (self-limiting oscillator) where the Hes1 oscillations generate out phase oscillations of pri-miR-9. Moreover, the high miR-9 lifetime would lead to their gradual accumulation until gradually builds up to a threshold concentration capable of arresting the Hes1 oscillations. This mechanistic model is supported by the low rate of degradation of miR-9 relative to that of Hes1 observed by the authors. Subsequent modeling work of this circuit will be needed to establish the extent of Hes1 oscillations can be modulated by miR-9 in the discovered circuit.

Our results provisionally unify two lines in the same experimental framework: the role of Notch signaling oscillations in the maintenance of embryonic NPCs of the telencephalon and the role of mNumb in neural specification. More researches on the role of mNumb on inhibition of Notch signaling in the neural determination are necessary to develop that perspective.

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