Neural fate decisions mediated by oscillatory and sustained Hes1

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***Abstract*—During central nervous system (CNS) developing, Hes1 shows short period oscillations in progenitor cells, while stable low levels in neurons. The reason why diverse expression modes of Hes1 exist remains unknown. Here, we develop a mathematical model involving Hes1 and BM88, with the aim of understanding the complex molecular mechanism that orchestrates the processes of neural fate decision. Our simple but fundamental model can account for both Hes1 oscillations observed in neural progenitors and Hes1 regulation to BM88 in differentiation progress. Our results suggest that a relatively simple network is capable of accounting for some fundamental principles in progenitor maintenance and differentiation.**

I. Introduction

During central nervous system (CNS) developing, neural progenitor cells (NPCs) undergo self-renewal, and they are responsible for generating different cell lineages that build the nervous system [1]. A key regulator of these processes is Hes1 [2]. Hes1 is found to automatically oscillate with a period of about 2-3 h in NPCs, and the oscillations are necessary for efficient cell proliferation [3]. On the other hand, the best studied target genes for Hes1 are proneural bHLH genes such as Ascl1 (also called Mash1) [4]. They are also expressed in NPCs but exhibit an inverse correlation to Hes1. Hes1 oscillations induce oscillatory expression of Ascl1 by periodic repression, and the oscillatory pattern enable the maintenance of NPCs [5]. However, diverse expression modes of Hes1 exist during CNS developing [6], [7], [8]. Amount of experimental findings suggest that oscillatory versus sustained expression patterns of Hes1 are important for NPC proliferation versus neuronal differentiation [9]. To further explore the mechanisms underlie, more and more studies have focused on the roles of oscillatory expression of Hes1 in neural progenitors.

Recent findings indicate that multipotent state correlates with oscillatory expression of bHLH factors such as Ascl1 and Hes1 [10], [11]. At the crest of Ascl1 oscillations whereas Hes1 is low, cells have higher potential for neuronal differentiation. However, this potential is not decisive. Accumulation of Ascl1 during G1 phase is the only decisive signal for differentiation [5], [12], [13]. The oscillations of Hes1 just work to maintain cells in NPCs state by repressing other cell fate determination factors [14]. This sounds a waste of energy. Indeed, experimental findings suggest that sustained expression of Hes1 inhibits the proliferation of NPCs [8]. But the mechanism is less clear and need to be further analyzed. In addition, Hes1 oscillations induce the oscillatory expression of Ascl1 which most likely cannot induce neuronal differentiation, probably because many target genes downstream do not respond to Ascl1 oscillations. Only rapidly responding genes such as Dll1 can be induced to express in oscillatory pattern and subsequently activate Notch signaling for NPCs maintenance [9], [15], [16]. Moreover, Hes1 oscillations might control the timing of transition from proliferation to differentiation. The possible mechanism is that the rhythmic expression of Ascl1 in neural progenitors can lead to a step-wise accumulation of downstream factors like BM88 (Cend1). Sufficient amount of BM88 can promote asymmetric or symmetric neurogenic division [17]. In this process, a possible role of Hes1 oscillations is to act as a cellular clock, but still need to be determined.

BM88 acts as an neuronal-lineage specific modular in coupling cell cycle exit with neuronal differentiation [18]. It is controlled by proneural genes during embryonic and postnatal neurogenesis [19]. BM88 is dynamically expressed during CNS development [20], [21], [22]. It is possible that increase in BM88 may function to measure the time for cell proliferation and cell cycle exit. Meanwhile, because proneural genes are transiently expressed in progenitors, and their potential to induce neuronal differentiation relies on downstream genes that can accumulate over time and subsequently promote differentiation. BM88 is a strong candidate for performing this function, though the exact mechanism has still to be explored [17].

The purpose of this article is to present a mathematical model involving Hes1 and BM88. Not surprisingly, a lot of models involving Hes1 have been proposed but can not give a suitable explain for Hes1 oscillations in NPCs. In addition, current evidences suggest that the balance between cell cycle control and neuronal differentiation is essential for generation of appropriate number of neurons [18]. Both Hes1 and BM88 participate in these cellular events, but the complex machanism that orchestrates these processes remains unclear. To explore the molecular mechanisms, a new model is needed which incorporates both Hes1 and BM88. Because both of them interact with Ascl1, we speculate that Hes1 can indirectly repress the expression of BM88. Our simple but fundamental model present here can account for both

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Hes1 oscillations observed in neural progenitors and Hes1 regulation to BM88 in differentiation progress.

II. METHODS

A. A mathematical model

A mathematical model concerning Hes1 oscillations has previously been constructed, and it mainly focuses on Notch signaling in regulating Hes1 oscillatory behavior [23]. Here, we develop a model by integrating BM88 into Hes1 oscillatory model (Fig. 1(a)). The activities of *Hes1* mRNA, Hes1 protein in cytoplasm, and Hes1 protein in nucleus are dynamic variables that follow ordinary differential equations (ODEs), and they are from the study of [23].

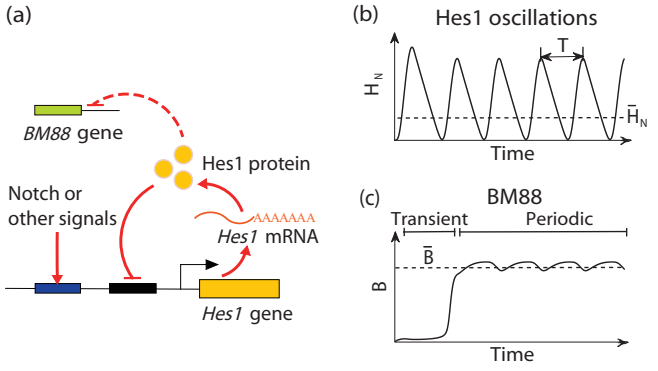

Fig. 1. Simplified roles of Hes1 in BM88 regulation. (a) Schematic descriptions of the network involving Hes1 and BM88. We denote direct effects with red arrowed lines and indirect effects with dotted arrowed lines. Inhibitory effects are shown as blunted red arrows, and active effects are shown as red arrows. (b) Hes1 protein oscillations have period T and average $\bar{H}_N$. (c) After an initial transient, BM88 performs an oscillatory expression for the oscillatory input of Hes1 shown in (b), and the average value of BM88 reaches the steady value of $\bar{B}$.

$$\frac{dM}{dt} = v_1 \frac{K_1^n}{K_1^n + H_N^n} - v_2 \frac{M}{K_2 + M}, \quad (1)$$

$$\frac{dH_C}{dt} = v_3 M - v_4 \frac{H_C}{K_4 + H_C} - v_5 H_C, \quad (2)$$

$$\frac{dH_N}{dt} = v_5 H_C - v_6 \frac{H_N}{K_6 + H_N}, \quad (3)$$

It has been reported that BM88 is a marker of NPCs that will progress towards neuronal differentiation. It expresses at a low level in neuronal precursor, but a high level in differentiated neuron [20]. The mode of BM88 expression in precursors and neurons leads to a speculation that BM88 can perform as a late molecular switch for neurogenesis. We assume that BM88 works as a switch and has two stable steady states. It is known that bistability can be generated by a positive feedback loop [24], so we suppose BM88 is self-promoted. Noting that BM88 can form a dimer [25], the Hill coefficient is therefore set to 2. Different from the degradation of Hes1, we adopt the simple form of degradation on BM88 due to lack of information about BM88. The expression level of BM88 yields the following ODEs:

$$\frac{dB}{dt} = v_7 + v_8 \frac{B^2}{1 + K_7 B^2 + K_8 H_N^2} - v_9 B, \quad (4)$$

Standard parameters are used in all simulations unless noted. These values are: $v_1 = 1.0$ 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}, $v_7 = 0.05$ nM min^{-1}, $v_8 = 1$ nM min^{-1}, $v_9 = 0.4$ min^{-1}, $K_1 = 0.157$ nM, $K_2 = 0.104$ nM, $K_4 = 0.142$ nM, $K_6 = 0.13$ nM, $K_7 = 0.5$, $K_8 = 0.05$.

B. The mean activity of Hes1 and BM88

In our model, we consider two input species of Hes1, with two corresponding output species of BM88. For constant Hes1 signal, the concentration of BM88 is constant in time; While, for the oscillatory pattern of Hes1, the output of BM88 oscillates in time. The states of Hes1 oscillations are characterized by period T and expression level H_N (Fig. 1(b)). For simplicity, the initial value of Hes1 is set to zero. The states of oscillatory Hes1 are encoded in the mean level of H_N, denote as $\bar{H}_N$, which can be described by function

$$\bar{H}_{N,i} = \frac{1}{T} \int_{iT}^{(i+1)T} H_N(t) dt, \quad (5)$$

$\bar{H}_N = \lim_{i \to \infty} \bar{H}_{N,i}$.

We assume that the expression of BM88 is at a low level before cell differentiation. The response of BM88 to the Hes1 oscillations goes through an initial transient state before reaching to the periodic state (Fig. 1(c)). The mean value of BM88, $\bar{B}_i$, during the i^{th} oscillation cycle is

$$\bar{B}_i = \frac{1}{T} \int_{iT}^{(i+1)T} B(t) dt, \quad (6)$$

After some algebra, the stationary value $\bar{B} = \lim_{i \to \infty} \bar{B}_i$.

III. RESULTS

A. Regulation of BM88 by Hes1

To evaluate the role of Hes1 relative to BM88 expression in neuronal lineage progress, we track BM88 dynamics, taking into account the indirect repression by Hes1 protein regardless of Hes1 oscillations. A saddle-node bifurcation arises from changing Hes1 levels. Bistability is a characteristic feature of saddle-node bifurcation, in which BM88 expression jumps from low stable steady state to high stable steady state as Hes1 is continuously decreased, but jumps from high stable steady state back to low stable steady state as Hes1 continuously increased. The values of Hes1 and BM88 at the bifurcation points are calculated and shown in Fig. 2(a). Due to the low expression of BM88 in NPCs, BM88 is remained at a low level as Hes1 declines unless H_N is small enough to cross the bifurcation point. This result implies that BM88 expression is persistently remained at quite a low level in a wide range of Hes1 expression.

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In fact, it is known that Hes1 expression oscillates autonomously and depends on negative autoregulation in NPCs. We therefore need to determine the condition that allows for Hes1 oscillations maintenance. In this case, since parameter v_5, the cytoplasm-nucleus transportation rate of Hes1 protein, represents the level of Hes1 protein in nucleus, we perform the bifurcation set in a parameter space of v_5. A hopf bifurcation occurs at $\bar{v}_5 = 0.5568$. It delineates the boundary between oscillatory and sustained Hes1 expression in the single parameter space. When $v_5 < \bar{v}_5$, Hes1 oscillations can be maintained, and it is necessary to keep a population of cells in the progenitor state.

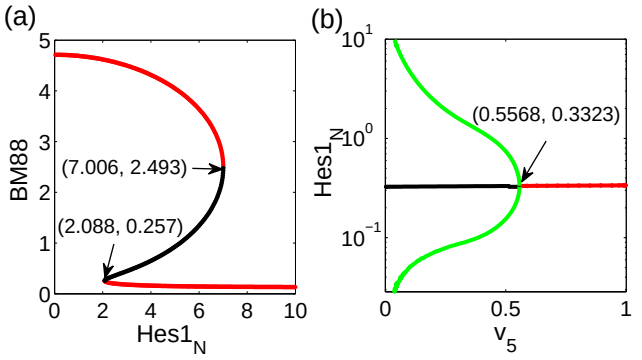

Fig. 2. Regulation of BM88 by Hes1 protein. (a) A bifurcation set in a parameter space of Hes1. (b) A bifurcation set in a parameter space of v_5 shows the boundary between oscillatory and sustained Hes1 expression.

In summary, we evaluate how sensitively BM88 responds to changes in Hes1 levels. Sustained signal from Hes1 can result in two stable steady states of BM88. So a question arises, why Hes1 needs to oscillate in NPCs. In order to get an explanation, we fix the range of v_5 to less than 0.55 to maintain Hes1 in oscillatory state for further study.

B. The effects of different signals on BM88

Given a certain amount of Hes1, what is the difference between sustained and oscillatory signals in regulating BM88? To observe the degree of BM88 depending on Hes1 oscillatory signal, we measure the average BM88 concentration $\bar{B}$. This method has been used in the study of gene responses to calcium oscillatory signal [26]. We compare BM88 responses achieved with constant signal and oscillatory signal of the same average Hes1 $\bar{H}_N$ (Fig. 3). In order to keep Hes1 in oscillatory pattern, the value of parameter v_5 is set to less than 0.55.

The result for sustained signal has already been shown in Fig. 2(a), and also been shown in Fig. 3 as red and black curves. Different from sustained signal, BM88 expression only has one state for oscillatory signal regardless of the initial value, and $\bar{B}$ is enhanced as $\bar{H}_N$ decreases. In particular, $\bar{B}$ sharply rises at a certain threshold level of $\bar{H}_N$ (Fig. 3). Hence, Hes1 oscillations can efficiently drive changes in BM88 expression in a certain range. Importantly,

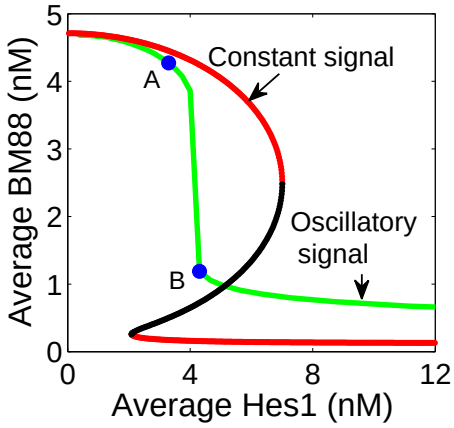

Fig. 3. BM88 expression in response to constant and oscillatory Hes1 signals. For constant signal, a saddle-node bifurcation occurs as Hes1 varies, shown as red and black curves. For oscillatory signal, the average BM88 $\bar{B}$ is increased by decreasing the average Hes1 $\bar{H}_N$, shown as green curve. The two points of A and B represent the sharp change of BM88 expression, shown as blue dots.

given a certain amount of Hes1, the level of $\bar{B}$ is superior to the low stable steady state for constant signal. When BM88 switch to a high level, the oscillatory signal can remain $\bar{B}$ in a relatively low value than sustained signal.

For oscillatory signal, we find that $\bar{B}$ is obviously increased at a certain threshold level of $\bar{H}_N$. To explain the phenomenon, we roughly choose two points (blue dots marked as points A, B in Fig. 3), which can represent the sharp increase in BM88, to analysis BM88 dynamics associated with Hes1 oscillations. We scrutinize the changes in BM88 as Hes1 oscillates with the average values equaling to $\bar{H}_N$ at the two points of A and B, respectively. Two limit cycles are obtained in the phase portrait where Hes1 protein is plotted against BM88, shown as blue curves in Fig. 4(a) and Fig. 4(c). We find that the behavior of suddenly rising in the expression of BM88 can be understood by saddle-node bifurcation from sustained signal. Once the limit cycle exceeds the saddle-node, a transition of states is likely to happen. We also analyze the time-series of Hes1 and BM88 at the two points of A and B, shown in Fig. 4(b) and Fig. 4(d), respectively. It is visible that there is significant difference between the two waves of BM88 in the shape. For a smaller Hes1, BM88 performs a small amplitude but high average level, shown as green curve in Fig. 4(b); While for a larger Hes1, the trough value of BM88 is obviously down-regulated, shown as green curve in Fig. 4(d).

C. The effect of inhibitory strength on BM88

K_8 is the inhibitory constant for repression of BM88 by Hes1. To explore whether the inhibitory strength of BM88 performed by Hes1 has an essential impact on the cell states, we examine the BM88 expression by changing the strength of inhibition from Hes1, shown in Fig. 5. In response to an oscillatory signal, larger inhibitory strength

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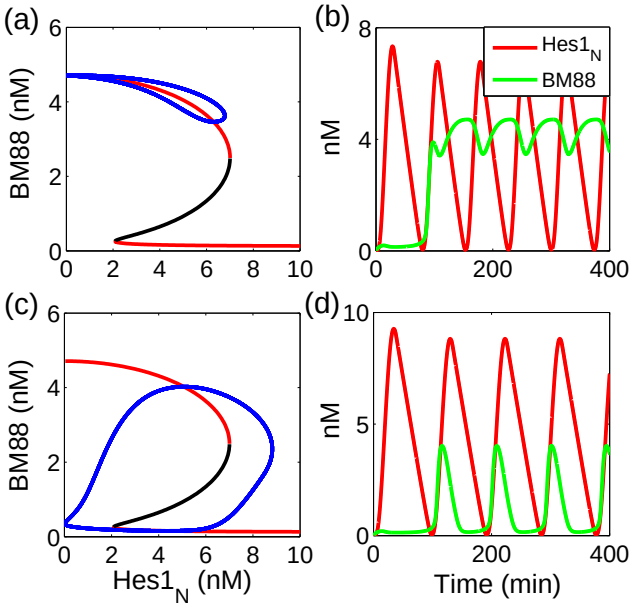

Fig. 4. Decoding the sharp changes in BM88 for oscillatory signal. (a) For point A in Fig. 3, the relation between oscillatory signal and sustained signal is shown. A stable limit cycle is shown as blue curve. The saddle-node bifurcation is represented by red and black curves. (b) Time-series of Hes1 and BM88 at point A are shown. (c) The relation between limit cycle for oscillatory signal and saddle-node bifurcation for sustained signal at point B in Fig. 3. A stable limit cycle is shown as blue curve. The steady stable state and unstable state of the saddle-node bifurcation are represented by red and black curves, respectively. (d) Time-series of Hes1 and BM88 at point B.

can get lower $\bar{B}$ for the same $\bar{H}_N$ (Fig. 5(a)). Meanwhile, for amplified inhibiting signals, BM88 expression is more likely to prevail at low level as $\bar{H}_N$ decreases. In addition, it has the same tendency for constant signals, shown in Fig. 5(b). We also compare the response of BM88 to Hes1 generating from oscillatory signal and constant signal. We find that the expression of BM88 for oscillatory input exhibits better robustness in the rising stage. The dose-dependent area is larger than constant signal. However, BM88 jumps from the branch of low steady state to high steady state as Hes1 is continuously decreased for constant Hes1 input, indicating a diminished dose-dependent response. It demonstrates that Hes1 oscillations contribute to BM88 robustness. Meanwhile, as Hes1 decreases, cell is more 'excited' for oscillatory signal when the repression strength from Hes1 is weak. It is more sensitive to initiate the switch of BM88 to a high state. So, for those cells presenting oscillatory patterns, Hes1 concentration need to be less decreased to promote differentiation.

These results strongly suggest that the oscillatory pattern of Hes1 contribute to the robustness of BM88 in proliferating state in order to maintain cells in progenitor states. Once NPCs get ready, cells are more easily to promote the differentiating procedure. As the repression strength of BM88 from Hes1 get larger, the cells become less excited. There are not so much differences between sustained and oscillatory signals. They all prefer staying in a low state of BM88 unless Hes1 is low enough.

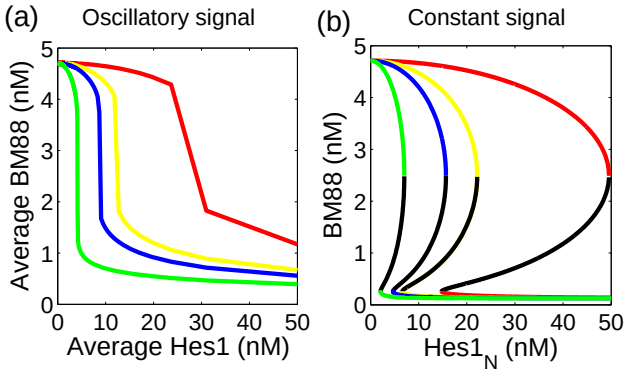

Fig. 5. Effects of different inhibitory strength on the expression of BM88. (a) Different values of inhibitory constant K_8 are used to test the impacts of Hes1 as oscillatory inputs. The different colored curves denote average levels of BM88 as Hes1 varies for different values of K_8. K8=0.001, 0.005, 0.01, 0.05 are represented by red, yellow, blue and green curves, respectively. (b) We also test the impact of different K_8 for constant signal.

IV. DISCUSSION

It is well known that Hes1 expression oscillates in NPCs, but the reason for existence is less clear. In this paper, we present a simple model involving Hes1 and BM88 to dissect the impact of Hes1 oscillatory mode on BM88 in neural progenitors. The model reveals the critical roles of Hes1 oscillations in neural fate decisions and therefore provides an essential resource for theoretically analysis of the mechanisms underlying fate choice process.

To unravel Hes1 oscillations critical for progenitor maintenance and differentiation, we compare the roles of oscillatory versus sustained expression of Hes1 in regulating the dynamics of our Hes1-BM88 system. Our investigation reveals that sustained Hes1 expression can form bistable steady state of BM88. Similarly, Hes1 oscillations can also lead to high and low states of BM88 but in a switch-like manner. It is coordinate with the experimental findings that in those NPCs whose daughter cells underwent differentiation to neuron during asymmetric cell division, Hes1 expression is repressed before cell division. At the same time, Ascl1 is up-regulated. Transient down-regulation of Hes1 together with up-regulation of Ascl1 before cell division direct NPCs toward neuronal fate decision.

In addition, transmitting information via oscillatory signal has an advantage. It can keep BM88 in a suitable state, neither too high to differentiation nor too low to affect normal proliferation.

Moreover, in the process of BM88 transition to high state, it is more robust for oscillatory signal regardless of the initial value of BM88. However, the BM88 levels are affected by initial values for sustained signal due to the saddle-node bifurcation. These results indicate that sustained Hes1 expression make the NPC state easily to be broken by

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intrinsic and extrinsic fluctuating, while oscillatory expression of Hes1 enables a metastable state to noise.

Furthermore, though both of sustained and oscillatory Hes1 expression patterns can lead to two states of BM88, in the form of saddle-node bifurcation versus switch-like tendency, the response concentration of Hes1 for BM88 to switch from low to high state is not the same. Comparing with sustained input, the required concentration of Hes1 in oscillatory manner to promote differentiation is higher. The cells seem to be more excited when Hes1 is in the oscillatory pattern. For sustained expression of Hes1, cells are more likely to stay in a low state of BM88 unless Hes1 level is low enough to cross the saddle-node. It is known that unlike embryonic NPCs, adult NPCs are quiescent and only occasionally divide into neurons, although both of them express Hes1. From our results, we speculate that the different expression patterns of Hes1 can lead to this phenomenon, but this possibility remains to be addressed.

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