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Modeling of Segmentation Clock Mechanism in Presomitic Mesoderm

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***Abstract*—Somite is sequentially generated in a head-to-tail order by segmentation of the mesenchymal tissue called presomitic mesoderm (PSM). The segmentation occurs periodically at the anterior end of the PSM, and this periodic segmentation has been suggested to be regulated by a molecular clock. In mouse PSM, the segmentation-related genes change their expression every 120 minutes, and this cyclic expression is essential for regular somite segmentation. In this study, a molecular mechanism of segmentation clock involving Wnt and Delta-Notch signaling pathways is modeled, and reality of the model structure is investigated through simulating biological findings. One dimensional array of the cellular clock models is constructed to simulate spatio-temporal dynamics of the gene-expressions in the PSM. The simulation result suggests that the Wnt gradient across the PSM is involved in the dynamics under concern.**

I. Introduction

SOMITE, the segmental units for the metametric structure such as skeletal muscles, vertebrate, and ribs is sequentially generated in a head-to-tail order by segmentation of the mesenchymal tissue called presomitic mesoderm (PSM). The segmentation occurs periodically at the anterior end of the PSM, and this periodic segmentation has been suggested to be regulated by a molecular clock [1,2]. The first evidence for the molecular clock for somite segmentation was provided by a report about the chick basic helix-loop-helix (bHLH) gene chairy1 [3]. chairy1 expression initiates as a broad band in the posterior PSM, moves anteriorly, and reaches the anterior end of the PSM as a narrow band, which corresponds to the posterior part of a newly formed somite [3]. Similar dynamic expression is also observed for other chairy1-related bHLH genes, such as zebrafish Her1 and mouse Hes1 and Hes7 [4,5,6,7]. Hes7 encodes a transcriptional repressor and its expression is controlled by Notch signaling. Hes7 expression was found to oscillate in 2-h cycles in the PSM synchronously with Lfng expression [8]. In mice mutant for Hes7, somites are not properly segmented and their anterior-posterior polarity is disrupted. Importantly, the oscillating expression of Lfng is disrupted in the mutant PSM. Lfng and Hes7 are the key regulators to convert the temporal component (oscillation) to the spatial component (segmentation) [8]. In addition to these molecules, Wnt signaling has recently been recognized to be involved in the somite-genesis, cell growth, and proliferation.

Modeling is an essential tool to integrate these diverse findings to explore mechanisms underlying the spatiotemporal organization of segmentation clock and a possible link between the oscillation and the formed segmentation. So far, the oscillating mechanisms of chairy1-related genes have been modeled as a delayed transcription-translation feedback loop [9,10] or inter-cellular interactions via Delta-Notch binding [11]. Among them, the former type of the model could not show how an inter-cellular interaction affects the oscillatory dynamics, because the Delta-Notch signaling pathway is not included. In the latter, the autonomous oscillatory mechanism of chairy1-related genes is not considered. In this paper, the Delta-Notch signaling pathway involving Hes and Lfng and the Wnt signaling pathway are modeled, which constructs a cellular model of segmentation clock mechanism. In addition, biological reality of the model structure is investigated. One dimensional array of the cellular models is composed to simulate spatio-temporal dynamics of gene expressions in the PSM. Through these investigations, a possible molecular mechanism underlying the somite genesis is clarified.

II. Cellular Model of Segmentation Clock

A. Molecular Interactions Involved in Segmentation Clock

A cellular mechanism of segmentation clock is considered to consist of two different molecular systems: Delta-Notch signaling system and Wnt signaling system.

In the Delta-Notch signaling system, when Delta (ligand) binds Notch (receptor) expressed on a neighboring cell

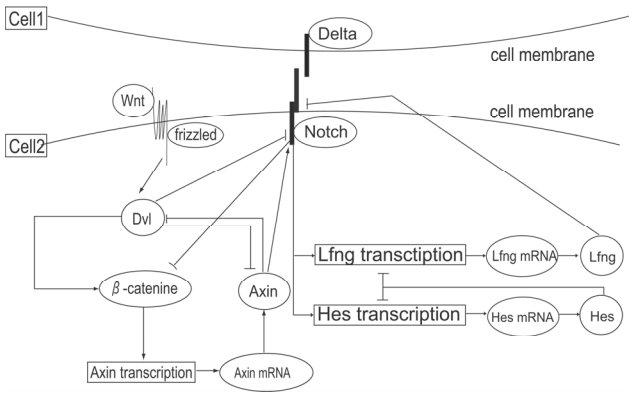

Fig. 1. Cellular model of segmentation clock which consists of the Wnt and the Delta-Notch signaling systems.

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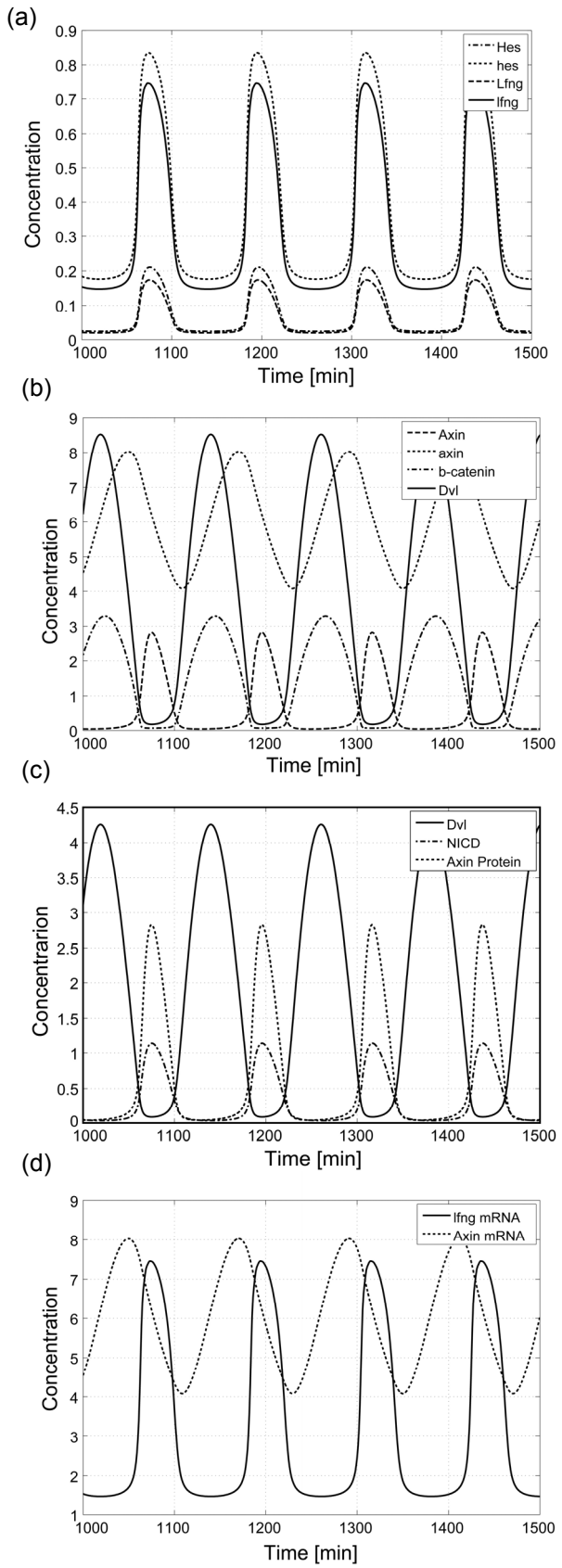

Fig. 2. Oscillation waveforms of model variables. (a) Hes protein, Hes mRNA, Lfng protein, and Lfng mRNA (b) Axin protein, Axin mRNA, β-catenin, and Dvl (c) Dvl, NICD, and Axin protein (d) Lfng mRNA and Axin mRNA

membrane, the intracellular domain of the Notch (NICD) is separated. Then, the NICD enters the nucleus to activate transcriptions of Hes and Lfng through binding transcription factors of the CSL family. In mouse PSM, expression levels of Delta and Notch are found to be kept constant in spite of oscillation of those of Hes and Lfng [9,13]. Hes transcription suffers a negative feedback via its protein, and the degradation of Hes protein is facilitated by ubiquitination [14]. On the other hand, NICD activates the transcription of Lfng through binding RBP-J site, and Hes protein suppresses it through binding E-box in the promoter region [13]. Taken all together, oscillation mechanisms of Hes and Lfng commonly involve activation by the Notch signaling and suppression by Hes protein.

Wnt is suggested to be essentially involved in the somite-genesis, cell growth, and proliferation [15]. Extracellular Wnt binds Fizzled (receptor), which stabilizes β-catenin and facilitates its accumulation in the cytoplasmic space. The accumulated β-catenin enters the nucleus and activates expression of the target genes such as Axin through making heterodimer with Tcf/Lef [16].

Both signaling systems are suggested to be coupled together to generate oscillatory expression of Lfng because in Wnt-knock-out mice the oscillation was found to be lost [12]. In the Wnt signaling system, Dvl inactivates Gsk-3β through binding Axin on its PDZ domain [17]. Dishevelled, a Drosophila homolog of Dvl, binds NICD on its PDZ domain to suppress the Notch signaling [18]. Taken all together, in mice Dvl is expected to suppress the Notch signaling by binding NICD on its PDZ domain. In addition, because over-expressed Notch was found to suppress production of β-catenin [19], this is taken into account as a possible mediator between both signaling systems.

Fig.1 shows the cellular model of segmentation clock which consists of the Wnt and Notch-Delta signaling systems interacting with each other. Here, in the Delta-Notch signaling system we assume Lfng's inactivating effect on Delta-Notch binding, and in the Wnt system Dvl is regarded as a Wnt signal by ignoring Gsk-3β. By this simplification, in the model the Wnt signal inactivates Axin through its direct activation of β-catenin.

B. Cellular Model of Segmentation Clock and Its Dynamics

The model equations are given as follows.

$$T\frac{dH}{dt} = S_1 h - D_1 \frac{H}{L_1 + H} - d_1 H$$

$$T\frac{dh}{dt} = S_{21}\frac{K_{21}}{K_{21} + H^2} + U_{22}\frac{k_f n}{K_{22} + k_f n} - D_2 \frac{h}{L_2 + h} - d_2 h$$

$$T\frac{dL}{dt} = S_3 l - D_3 \frac{L}{L_3 + L} - d_3 L$$

$$T\frac{dl}{dt} = S_{41}\frac{K_{41}}{K_{41} + H^2} + S_{42}\frac{k_f n}{K_{42} + k_f n} - D_4 \frac{l}{L_4 + l} - d_4 l$$

$$T\frac{dn}{dt} = k_i b - S_5 \frac{(Dv)n}{K_{51} + K_{52}(An) + n} - d_5 n$$

$$T\frac{db}{dt} = S_6 \frac{(De)N}{(K_6 + \alpha L + \beta < L >)} - k_i b - d_6 b$$

$$T\frac{d(An)}{dt} = S_{71}(an) - S_{72}\frac{(An)(Dv)}{K_{71} + K_{72}(An) + (An)(Dv)} - V_7(An)(Dv) - d_7(An)$$

$$T\frac{d(an)}{dt} = S_8 \frac{(Cn)}{L_8 + (Cn)} - d_8(an)$$

$$T\frac{d(Cn)}{dt} = Act - S_{91}\frac{(Cn)}{K_{91} + (1 + K_{92}(Dv))(Cn)} - S_{92}\frac{(Cn)n}{K_{93} + (Cn)} - d_9(Cn)$$

$$T\frac{d(Dv)}{dt} = W - V_{10}(An)(Dv) - D_{10}\frac{(Dv)}{K_{10} + (Dv)} - d_{10}(Dv)$$

where *H* and *h* denote Hess protein and mRNA, respectively, *L* and *l* for Lfng, *n* for NICD, *b* for Delta-Notch complex, *An* and *an* for Axin, *Cn* for β-catenin, *Dv* for Dvl, *N* for Notch, *De* for Delta, and *W* for Wnt. Parameter values are listed in the appendix. Typical model dynamics are shown in Fig.2, where the oscillation period is adjusted to 120min by tuning *T* after the actual one. One of paradoxical phenomena in actual PSM is an oscillation against the constant expression of Notch and Delta. In this model, instead of oscillating Delta and Notch expressions, an activation level of NICD oscillates by Lfng's oscillatory modulation on Notch-Delta binding. This dynamics of NICD reproduces the experimental finding [20]. Phase relationships between the Delta-Notch and the Wnt signaling systems have already been known: Axin protein and NICD oscillate in-phase, Dvl does in an anti-phase manner with them, and Lfng follows Axin with some delay [12]. The model is shown to reproduce these actual findings. Reality of the model dynamics is further verified by successful simulations (not shown here) of the perturbation experiments which showed that disturbing the Wnt signal prevented both of Lfng and Axin from oscillating, but disturbing the Delta-Notch signal stopped the Lfng oscillation while Axin continued oscillating [12].

III. Bifurcation Properties Of Wnt and Delta-Notch Signaling Systems

The reality of the cellular model of segmentation clock has been verified as shown in the preceding section. Here, we take a closer look into a mechanism generating oscillation. In order to achieve this, bifurcation properties of the Wnt and Delta-Notch signaling systems are explored separately. Bifurcation properties for the Wnt signaling system are differentiated by parameter value of S_8, where the concentration of NICD is set to be zero, i.e., an influence from the Delta-Notch system is excluded. For S_8=0.5, the Wnt system exhibits a subcritical bifurcation structure against the concentration of Wnt. In this case, stable and unstable limit cycles coexist outside of the range of Wnt concentration in which a stable limit cycle is only allowed. For S_8=2.5, a supercritical bifurcation is resulted against Wnt concentration, where a stable steady state is followed directly by a stable limit cycle. On the other hand, the Notch-Delta signaling system exhibits a focus under the conditions currently used. Taken all together, thus modeled dynamics suggest that the cellular segmentation clock is generated in a way that the Wnt signaling system exhibiting a limit cycle drives the stable Notch-Delta signaling system. This structure naturally underlies the finding that the abolition of Wnt signal exclusively stopped the oscillatory expressions both of Axin and Lfng [12].

IV. Modeling of Travelling Wave of Hes Expression

As shown before, the segmentation-related gene expressions are oscillating in the posterior region of the PSM (region I), but they are constantly expressed in the anterior side (region II). In region II, Lfng and Hes are separately and constantly expressed in the anterior and posterior halves of an expected segment area, respectively [21]. It has not yet been known what kind of mechanism underlies the regional separation of dynamics of gene expressions between region I and II, and the polarity formation in the segmentation area of region II. Here, a possible mechanism underlying the former phenomenon is discussed. It is known that there is a gradient in the concentration of Wnt protein across the PSM, i.e., higher in the posterior region of the PSM and lower in the anterior [12]. Our model has shown that a weakened Wnt signal can stop the segmentation clock totally. These findings naturally lead us to the idea that the Wnt signal below a certain threshold level stops the clock to cause the regional separation of gene expression dynamics in the PSM.

In the following simulations, provided that the gradient of the Wnt signal exists, it is examined whether the regional separation of gene expression dynamics and travelling of gene expression surge in the anterior part of PSM take place. In addition, how these phenomena depend on the bifurcation structure of the cellular segmentation clock is investigated as well. Fig.3 shows snap-shots of simulated spatial pattern of Hes expression in one-dimensional array of 100 PSM cells. Here, Lfng is assumed to diffuse to the nearest neighboring cells, where a geometric mean of Lfng concentrations of the neighboring cells are added with coupling coefficient:β of 0.2. For the cellular model showing the super-critical properties, the expression wave seems to travel from the posterior to the anterior. In contrast, for the model showing the sub-critical properties, the wave seems to travel to the opposite direction. As expected, regardless of the bifurcation structure the oscillation stops in the cells located more anterior beyond the vertical bar. Because actual traveling direction is from the posterior to the anterior, the simulation result suggests reality of the super-critical bifurcation structure. Further analysis showed that this traveling direction is due to a Wnt-dependency of oscillation frequency which is differentiated by the bifurcation properties (the results are not shown here). That is, the oscillation becomes faster as the Wnt increases for the super-critical case, and the opposite dependency is shown in the subcritical case. Although there

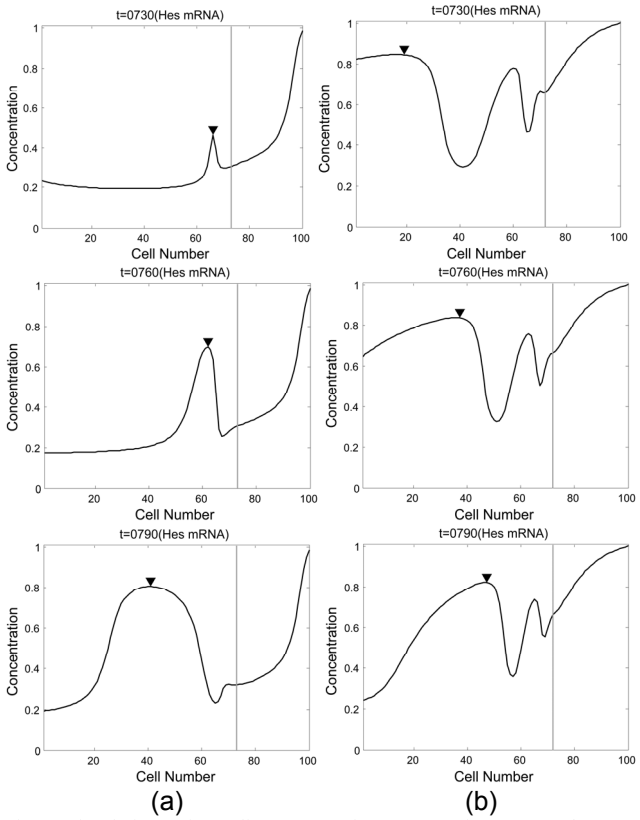

Fig. 3. Simulations of traveling waves of Hes expressions across the PSM. (a) sub-critical Wnt signaling system (b) super-critical Wnt signaling system. Arrows indicate peaks of the corresponding waves.

are other possible mechanisms underlying the traveling gene expression, e.g., Zhu and Dhar [22], none of them has been established. In this sense, the Wnt-gradient is worth investigating further as a possible factor involved in the traveling gene expression in the PSM.

V. Conclusions

In this study, the cellular segmentation clock mechanism was modeled by the oscillating Wnt signaling system and the stable Notch-Delta signaling system. This organization was shown to be essential for simulating the experimental results concerning the segmentation clock. Biological reality of super-critical bifurcation structure of the Wnt signaling system was suggested through simulations of the traveling of segmentation-related gene expressions by using the one-dimensional array of the cellular clock model. On the other hand, a mechanism of segmentation itself still remains to be modeled. How dynamically changing gene expressions in the PSM contribute toward forming static segmentation will be studied as a future subject.

Appendix

Parameter values: S_1=0.5, S_{21}=4, S_3=0.5, S_{41}=4, S_{42}=5, S_5=20,S_6=1, S_{71}=0.136, S_{72}=1.153, S_8=0.5, S_{91}=1.343, S_{92}=0.5, D_1=0.8, D_2=5.5, D_3=0.8, D_4=5.5, D_{10}=0.5, d_1=0.031, d_2=0.028, d_3=0.001, d_4=0.001, d_5=0.1, d_6=0.01, d_7=0.1, d_8=0.001, d_9=0.001, d_{10}=0.01, d_{11}=0.1, K_1=0.1, K_2=2.5, K_3=0.1, K_4=2.5, K_{51}=15, K_{52}=2, K_6=0.11, K_{71}=1, K_{72}=5, K_{91}=0.1, K_{92}=1, K_{93}=1, K_{10}=1, L_1=0.2, L_2=0.06, L_3=0.2, L_4=0.05, L_8=10, U_{22}=5, V_7=0.5, V_{10}=1, Act=0.5, α=0.9, β=0.2, k_i=0.25.

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