DIVISION OF THEORETICAL BIOLOGY

Associate Professor: *MOCHIZUKI, Atsushi*
NIBB Research Fellow: *FUJITA, Hironori*
Postdoctoral Fellow: *ISHIHARA, Shuji*
Graduate Student: *IMAMURA, Hisako*
Secretary: *UMEBAYASHI, Hiromi*

We are studying biological phenomena using mathematical models. This method gives us an integrative understanding of the behavior of complex systems in biology including gene regulatory networks.

Mathematical models are especially useful in understanding pattern formation in development. The study of the mechanisms responsible for morphological differences between species is an important research focus of current developmental biology.

I. Predicting regulation of the phosphorylation cycle of KaiC clock protein using mathematical analysis

Cyanobacteria are the simplest organisms exhibiting circadian rhythms. In the bacterium, clock genes kaiA, kaiB and kaiC have been characterized as the indispensable clock regulators. KaiC plays a central role and exhibits rhythms in transcription, translation and phosphorylation status under continuous illumination conditions. The other clock proteins KaiA and KaiB modulate KaiC autophosphorylation: KaiA enhances autophosphorylation of KaiC, and KaiB inhibits this action of KaiA. It was recently revealed that periodic oscillation of the phosphorylation level of KaiC persists even under continuous dark conditions, where transcription and translation have almost ceased. The KaiC phosphorylation cycle was reconstituted even *in vitro*, thus confirming that the interaction between Kai proteins generates the cycle, although the specific mechanism that drives the clock remains unclear.

Using mathematical models, we investigated the mechanism for the transcription-less KaiC phosphorylation cycle. We developed a simple model based on the possible KaiC behavior, which was suggested by previous experimental studies. In the model the KaiC-KaiA complex formation followed by a decrease in free KaiA molecules may attenuate the KaiC phosphorylation rate, and it acts as negative feedback in the system. However, our mathematical analysis proved that simple dynamics based on the experimentally suggested model never show the KaiC phosphorylation cycle.

We then developed the generalized formulae of models and determined the necessary condition to generate the KaiC phosphorylation cycle. Linear stability analysis revealed that oscillations can occur when there is sufficient distance of feedback between the recipient reaction and the effector. Furthermore, we found that the negative feedback regulations in closed systems can be classified into two types: *destabilizing inhibition* and *stabilizing inhibition*.

Figure 1. Schematic representation of closed circuit model and the condition for the possible oscillation by inhibition of the transition from state V_1 to V_2. Red-colored state (V_1, V4-V_7) indicate that inhibition from the states can destabilize the system and possibly cause oscillation. Inhibition from the blue-colored state never induces oscillation.

# of states	inhibitor V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	
3	○	×	×	-	-	-	-	-	× : always stable
4	○	×	×	○	-	-	-	-	○ : oscillation possible
5	○	×	×	○	○	-	-	-	
6	○	×	×	○	○	○	-	-	
7	○	×	×	○	○	○	○	-	
8	○	×	×	○	○	○	○	○	

Table 1. Summary of the results of the general state transition model with conservation of molecules. The system could oscillate when the inhibiting state is more than two steps ahead of the inhibited reaction (from V_1 to V_2). If the inhibiting state is less than three steps ahead of the reaction, the system is always stable. The necessary distance between the inhibiting state and reactant state does not depend on the system size.

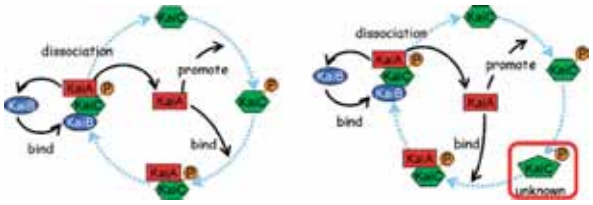

Figure 2. Schematic representations of "Basic model" (left) and "Multiple-phosphorelation-state model" (right). The basic model is determined from experimental results. It was proven that the model never shows oscillation. The multiple-phosphorelation-state model was developed based on the mathematical analysis. The model shows clear periodic oscillations. There are at least two different phosphorelated states. The time-delay caused by the transition between the states is essential for generating oscillation.

Based on this result, we predicted that, in addition to the identified states of KaiC, another unknown state must be present between KaiC phosphorylation and the complex formation. By incorporating the unknown state into the previous model, we realized the periodic pattern reminiscent of the KaiC phosphorylation cycle in computer simulation. This result implies that the KaiC-KaiA complex formation requires more than one step of posttranslational modification including phosphorylation or conformational change of KaiC.

II. Mathematical models for pattern formation of dendrites of neurons

Dendrite is a neuronal process which is specialized for receiving and processing synaptic or sensory input. A remarkable feature of dendrite is its morphological diversity. The shapes of dendritic trees are characteristic of individual neuronal types and they are highly variable from one neuronal type to another. This diversity contributes to differential processing of information in each type of neuron. Therefore, patterning neuronal class-specific dendrites is a process to produce forms that realize physiological functions of neurons. However, a comprehensive logic of dendrite development has not been formulated yet.

Previously proposed mathematical models to explain the pattern formation of dendrites assumed that dendrite development is a consequence of stochastic sprouting and subsequent growth arrest. Different forms of branching functions were postulated and modified so that simulated dendrograms fit dendritic arbors of real neurons. One of the problems of the previous models is that those dendrograms represent limited features of dendritic patterns such as order of branches and degree (the number of branches at each order) and do not reproduce the full range of the morphological features of the original dendrites. Other problems include the fact that many of the models cannot specify experimentally confirmed mechanisms to account for their assumptions. To overcome these problems, we developed a new class of dendrite growth model, which represents all extension, orientation of growth and branching of dendrites in a single scheme. In addition, this model has explicitly incorporated an underlying biological mechanism, that is, competitive interactions between neighboring dendrites.

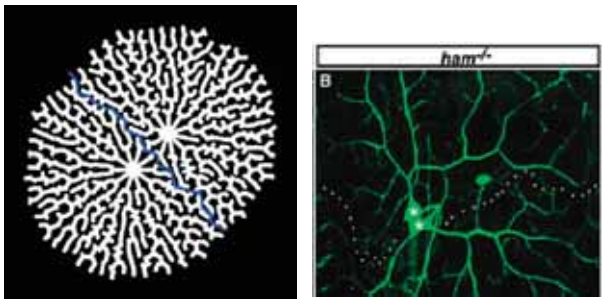

Figure 3. Examples of obtained dendrite patterns by computer simulation of the model (left) and the correspondence observed in an experiment (Grueber et al., 2003; Moore et al., 2002). Dendrites from the cells spread and cover the space. However, they never interfere with each other.

A key point in our modeling is to couple chemical dynamics to dendrite growth. In our model, we distinguish two spatial compartments: inside and outside regions of neurons. The cell compartment dynamically grows under the regulation of a chemical reactant activator. Thus we call our model a ìc ell compartment modelî. The activator reacts with another reactant suppressor in the way of the reaction-diffusion (RD) model of the so-called "Activator-Inhibitor type" (Turing, 1952; Gierer and Meinhardt, 1972). We set a restriction in the 2D space so that the activator only diffuses inside of the cells. These settings endow the system with feedback loop regulations at two different levels: one between two chemicals, and another between the dynamics of the chemicals and the expansion of the cell compartment. Using this formula, we study the dynamics of dendritic branch formation. Computer simulation showed that the cell compartment model developed dendritic branching autonomously and numerical analysis determined the conditions that allow it. This work is collabolation with Dr. T. Uemura and Dr. K. Sugimura in Kyoto University.

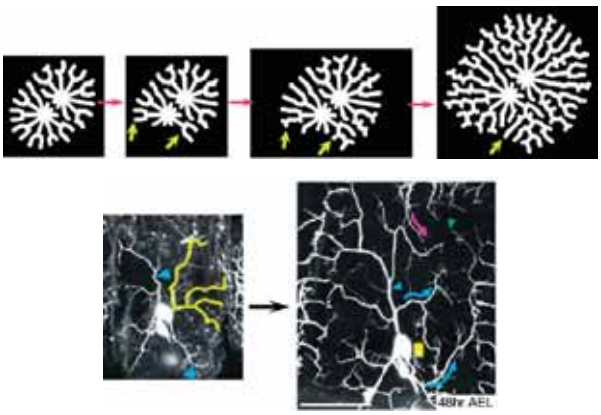

Figure 4. Regeneration after artificial severing. Patterns obtained by computer simulation of the model (up) and the corresponding pattern obtained in an experiment (down).

Publication List:

Original papers

Nakamura, T., Mine, N., Nakaguchi, E., Mochizuki, A., Yamamoto, M., Yashiro, K., Meno, C., and Hamada, H. (2006). Generation of robust left-right asymmetry in the mouse embryo requires a self-enhancement and lateral-inhibition system. Dev. Cell *11*, 495-504.

Takigawa-Imamura, H., and Mochizuki, A. (2006). Predicting regulation of the phosphorylation cycle of KaiC clock protein using mathematical analysis. J. Biol. Rhythms *21*, 405-416.

Takigawa-Imamura, H., and Mochizuki, A. (2006). Transcriptional autoregulation by phosphorylated and non-phosphorylated KaiC in cyanobacterial circadian rhythms. J. theor. Biol. *241*, 178-192.

Mochizuki, A., Yahara, K., Kobayashi, I., and Iwasa, Y. (2006). Genetic addiction: selfish gene's strategy for symbiosis in the genome. Genetics *172*, 1309-1323.

Fujita, H., and Mochizuki, A. (2006). The origin of the diversity of leaf venation pattern. Dev. Dyn. *235*, 2710-2721.

Fujita, H., and Mochizuki, A. (2006). Pattern formation by the positive feedback regulation between flow of diffusible signal molecule and localization of its carrier. J. theor. Biol. *241*, 541-551.