DIVISION OF THEORETICAL BIOLOGY

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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 also useful in understanding pattern formation in development. Study of the mechanisms responsible for morphological differences between species is an important research focus of current developmental biology. Theoretical studies would be useful in identifying candidates of cell-cell interaction that are likely to be responsible for the systems in real organisms.

I. Transcriptional autoregulation in cyanobacterial circadian rhythms

Among the wide variety of organisms exhibiting circadian rhythms, cyanobacteria are the simplest organisms and may provide a model to understand the basic mechanism of the sustained rhythmicity physiologically. In the cyanobacterium *Synechococcus elongatus* PCC 7942, clock genes kaiA, kaiB and kaiC have been characterized as the indispensable clock regulators. The kai genes form a gene cluster and kaiB and kaiC are co-transcribed as kaiBC mRNA. 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.

The transcriptional regulation of kaiBC is not fully understood yet, though experimental evidences suggest the autoregulation by KaiC, which may work as a positive or negative regulator depending on the phosphorylation status. The gene of kaiB, which forms an operon with kaiC, also seems to affect this regulation process, inhibiting KaiA-enhanced phosphorylation of KaiC.

In this study, we investigated and predicted the possible mechanisms of the transcriptional regulation by KaiC and its phosphorylated state to realize circadian oscillation using a mathematical model. Considering the experimental results, phosphorylated and non-phosphorylated KaiC may play different roles in the transcriptional regulation. We developed a dynamical model including the concentrations of phosphorylated, non-phosphorylated KaiC, and kaiBC mRNA. We can choose different transcriptional regulation functions which may switch their values increasingly or decreasingly depending on both phosphorylated and non-phosphorylated KaiC. We examined all the possible regulation patterns without using biological assumption and determined the condition for oscillation by means of the linear stability analysis. We determined that there are only two possible patterns in the transcriptional regulation to realize circadian oscillation (Figures 1 & 2). One of the two patterns does not include the direct negative feedback regulation, but successfully exhibits the oscillatory behavior by positive feedback of the transcriptional regulation. Based on this result, we proposed that positive autofeedback may be a novel mechanism for circadian oscillation.

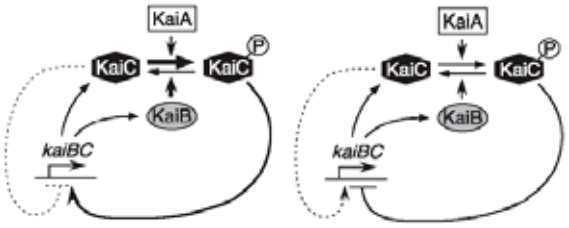

Figure 1. Functional schemes of two models. (Left) The Transcriptional Activation Model, where P-KaiC induces *kaiBC* transcription. NP-KaiC does not affect on transcription, or has a small effect of repressing transcription. (Right) The Transcriptional Repression Model, where P-KaiC represses *kaiBC* transcription.

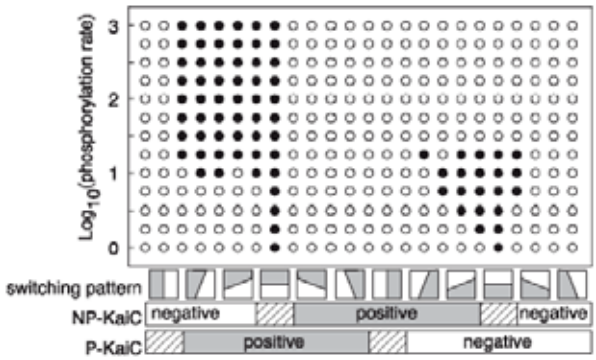

Figure 2. The result of linear stability analysis. The horizontal axis is θ, the angle of the threshold line of transcriptional switching, and the vertical axis is the phosphorylation rate. The θ specifies patterns of the transcriptional regulation, which are shown schematically below the horizontal axis. Along the horizontal axis, the regulation changes continuously; effects of NP-KaiC and P-KaiC are positive or negative. Open circles indicate conditions of the transcription and phosphorylation rates where oscillation never occurs, and filled circles indicate where oscillation can occur.

Secondly we verified if the determined conditions can explain the cyanobacterial circadian mechanism, comparing the behaviors in computer simulation with the experimentally observed phenotypes.

Experiments in circadian systems of *Neurospora*, *Drosophila*, and mammals as well as theoretical studies have long underlined the importance of the negative transcriptional regulation for the generation of oscillations.

The negative feedback regulation of clock genes has been considered responsible for generating oscillation. On the other hand, the contribution of positive feedback in circadian clock is much less well understood. The Transcriptional Activation Model we proposed in this report is distinct from circadian oscillator models that have been proposed previously.

II. Mathematical models for pattern formation of leaf vascular networks

The vascular system of a plant is a network of bundles that connects within major organs. The development or differentiation of vascular systems is one of the most important subjects in botany, and a lot of studies have focused on the problem. In dicot systems, veins in a leaf are usually diverse in their size and they can be classified distinctively by their appearance. The largest vein, located at the center of a leaf, is called the midvein (or primary vein), and is generated first. Veins differentiate progressively in an early developmental stage of the leaf. A vein, which is continuous with the stem vascular bundle, extends from the proximal edge to the distal end of the leaf, and then become a midvein. Secondary veins are generated from branches of the midvein and are thinner than the midvein. By repeated branching and extension, higher order veins are generated sequentially with the growth of the leaf and the reticulate network system is formed.

Although a lot of experimental studies have attempted to identify the mechanism of vascular patterning in leaves, it is still a problem to solve. It has been revealed that the auxin is important for vascular differentiation. Auxin is one of the major plant hormones. In the higher plant, indole acetic acid (IAA) is one of the most important auxin chemicals. Auxin is a diffusible molecule; however, it is believed that auxin is also transported from apical to basal in a stem, from distal to proximal in a leaf. This is called auxin polar transport. In a leaf, auxin is thought to be produced in the apical margins of leaves and transported toward the proximal regions. Many researchers believe that this kind of auxin flow plays an important role in the vascularization of plants.

Three hypotheses have been proposed so far to explain the leaf venation pattern formation. One is the auxin canalization hypothesis proposed by T. Sachs (1981) that is based on the assumption of the positive feedback regulation between auxin flow and flow capacity. Auxin is a diffusible plant hormone of small molecules and has important roles in various developmental events including vascular differentiation in plants. In this hypothesis, auxin pathways are thought to be generated from a uniform field and are extended in a sequential manner. The second is the activator-inhibitor type reaction-diffusion hypothesis proposed by H. Meinhardt (1982) that is based on two interactive factors of weakly diffusible activator and strongly diffusible inhibitor. We proposed the third hypothesis, the substrate-depletion type reaction-diffusion model, which is based on two interactive factors of strongly diffusible auxin resource and its consumer (Figure 3).

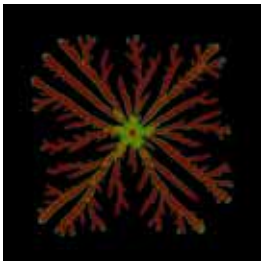

Figure 3. Branching pattern generated by computer simulation based on substrate-depletion type reaction-diffusion model. Veins grow toward the margin with repeating branching. In the final state, regular interval branching patterns are formed.

On the other hand, we tried to integrate the canalization hypothesis and the experimental knowledge. We introduce auxin transport by PIN1 efflux carrier into the auxin canalization hypothesis. We investigated a model based on the assumption of the positive feedback regulation between auxin flow and PIN1 localization (Figure 4).

The substrate-depletion type reaction-diffusion model can regenerate growth and branching patterns. On the other hand, the canalization model can generate closed circuits of leaf veins under a condition. Both mechanisms seem to be responsible for the leaf vein formation.

Figure 4. Leaf vein pattern generated by computer simulation based on canalization model. Veins grow toward the margin with repeating branching. The final patterns are similar to ones observed in maple leaves.

Publication List:

Original papers

Feugier, F.G., Mochizuki, A., and Iwasa, Y. (2005). Self-organization of the vascular system in plant leaves: Inter-dependent dynamics of auxin flux and carrier proteins. J. theor. Biol. *236*, 366-375.

Mochizuki, A. (2005). An analytical study of the number of steady states in gene regulatory networks. J. theor. Biol. *236*, 291-310.

Mochizuki, A., Yahara, K., Kobayashi, I., and Iwasa, Y. (2005). Genetic addiction: selfish gene's strategy for symbiosis in the genome. Genetics, in press.

Takigawa-Imamura, H., and Mochizuki, A. (2005).

Transcriptional autoregulation by phosphorylated and non-phosphorylated KaiC in cyanobacterial circadian rhythms. J. theor. Biol. in press.