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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 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. Structure of regulatory networks and diversity of gene expression patterns

The complexity of gene regulatory networks is considered responsible for the diversity of cells. Different types of cells, characterized by the expression patterns of genes, are produced in early development through the dynamics of gene activities based on the regulatory network. However, very little is known about the relationship between the structure of regulatory networks and the dynamics of gene activities.

In this study I introduce the new idea of "steady state compatibility," by which the diversity of possible gene activities can be determined from the topological structure of gene regulatory networks. The basic premise is very simple: the activity of a gene should be a function of the controlling genes. Thus a gene should always show unique expression activity if the activities of the controlling genes are unique. Based on this, the maximum possible diversity of steady states is determined using only information regarding regulatory linkages and without knowing the regulatory functions of genes.

Using the concept of "steady state compatibility," three general properties of the relationship between the topology of regulatory networks and the maximum number of steady states can be derived (Figure 2). (A) Cascade structures in regulatory networks do not increase the number of possible steady states (Figure 2a). (B) Loop structures in networks are necessary to generate multiple steady states. The number of separated loops increases the maximum diversity of steady states (Figure 2b). (C) Multiple loops that are connected by sharing the same genes do not increase the maximum diversity of steady states (Figure 2c).

The method was applied to a gene regulatory network responsible for early development in a sea urchin species. A set of important genes responsible for generating diversities of gene activities was derived based on the concept of compatibility of steady states.

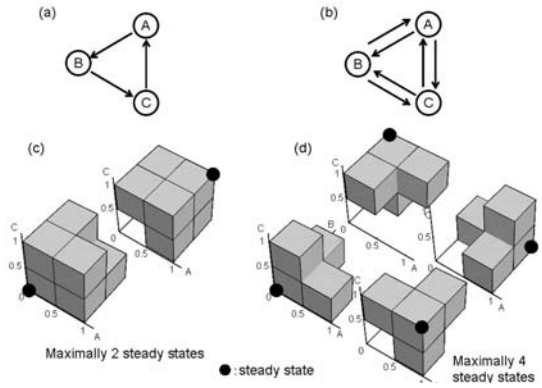

Figure 1. An intuitive explanation of "steady state compatibility". (a) An example of the regulatory links of a mono-directional loop with three genes. (b) Another example of the regulatory links of a bi-directional loop with three genes. (c) The shaded domains show the region where other steady states should not appear except for the original point (0,0,0) and (1,1,1)based on the network in (a). The network (a) has two steady states at maximum. (d) The network (b) determines the different shapes of the domains of no-steady-state except for the points (0,0,0), (0,1,1), (1,0,1) and (1,1,0). This network allows four steady states at maximum.

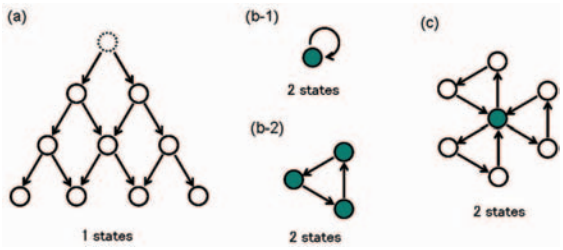

Figure 2. General properties showing the relationships between the structure of regulatory networks and the maximum diversity of steady states.

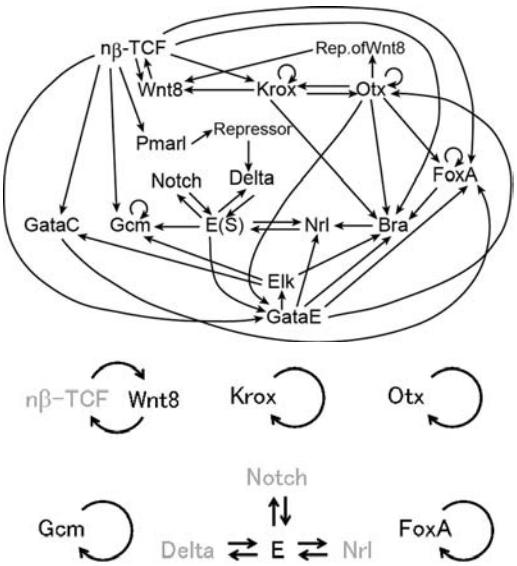

Figure 3. Analysis of an actual gene network responsible for the early development of a sea urchin species. (a) The network is simplified from the one of Fig. 3 in Davidson *et al*. (2002). The maximum diversity generated from this network is determined by the analysis as 64. (b) All of the "reduced observation point" ROP genes are derived. At least one of the ROPs should change its activities in the alternative steady states.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

Ⅱ. 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 possible KaiC behavior 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 may 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*.

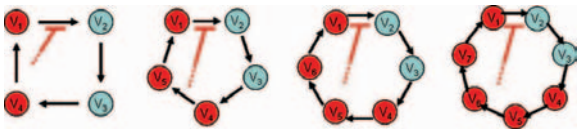

Figure 4. Schematic representation of closed circuit model and the condition for the possible oscillation by inhibition of the transition from state V_1 to V_2. Red-colored state (V_1, V_4-V_7) indicate that inhibition from the states can destabilize the system and possibly cause oscillation. Inhibition 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	O	×	×	-	-	-	-	-
4	O	×	×	O	-	-	-	-
5	O	×	×	O	O	-	-	-
6	O	×	×	O	O	O	-	-
7	O	×	×	O	O	O	O	-
8	O	×	×	O	O	O	O	O

×: always stable
O: oscillation possible

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.

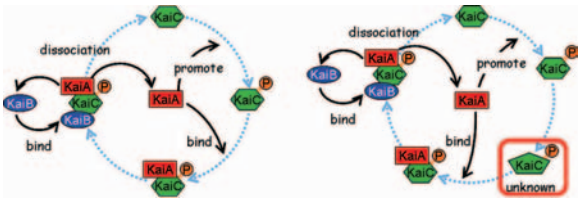

Figure 5. Schematic representations of "Basic model" (left) and "Multiple-phosphorelation-state model" (right). The basic model is determined from experimental results. It was proven that the model never shows oscillation. The multiple-phosphorelation-state model was developed based on a mathematical 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. This prediction has recently been confirmed by experimental methods.

Publication List

〔Original paper〕

- Mochizuki, A. (2008). Structure of regulatory networks and diversity of gene expression patterns. J. theor. Biol. *250*, 307-321.