"Biobje" as a new model system of gene expression

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Summary

The process of eukaryotic gene expression is a multi-step reaction system. Premature mRNAs produced at transcription sites are processed (capping, polyA addition and splicing), transport to ribosomes in the cytoplasm from the nucleus through nuclear pores, and translated to proteins. "Biobje" is a newly developed simulation model to analyze this complicated gene expression system in eukaryotic cells. In this paper Biobje is applied to circadian oscillation of Drosophila genes, and the model suggests that the stability of the mRNAs of *per, tim,* and *cyc* are crucial for the duration and robustness of the circadian rhythm.

Basic concept of "Biobje"

Living activities are bunch of thousands of chemical reactions. Various biological events in the living cells, metabolism, development, immunity, apoptosis, etc. are results of sequential chemical reactions, which respond to the environment. As the chemical reactions could be regarded as physical alternations of molecules in the cell, the nature of living activity may be described as the sum of property changes in thousands of molecules. Each molecule is movable, and when the molecule binds to the partner the chemical reaction starts. In the case of gene expression, mRNA is produced when RNA polymerase and transcription factors make an appropriate complex at the transcription site in the nucleus. The transcribed mRNA (premature mRNA) is processed (capping, polyA addition and splicing) by RNA-binding proteins and changed to the mature mRNA. The riboprotein complex is translocated to cytoplasm, bound to a ribosome, and translated to a protein. "Biobje" is a newly developed biological simulator to analyze the intracellular regulatory system of the gene expression.

What is "Biobje"?

In "Biobje" simulator (Figure 1), a multi-step reaction system is consisted of three simple steps. First step is "movement" of the molecular in the limited space (reaction point: transcription site, ribosome, etc). Second step is "association" with its reaction partner. The last step is "alteration" of its properties (transcription activity, degradation rate, phosphorylation state, etc). These reaction

steps were translated into simply programmed algorithms. An Object class was defined for representing character of a functional molecular. This class contains molecular identification code, molecular species identification code, lifetime and its location coordinate. These parameters are rewritten when the event like a movement or reaction occurred or when time passed. A functional molecular object produced form Object class moves around on the two-dimensional meshed space, which represented by two-dimensional array namely matrix object in the program. This movement uses random walk model. Although the direction using angle parameter is randomly changed and moving distance per unit of time is assumed as constant in this model, these factors could be changed to represent active transport of a specific molecule. As the result of the movement event, the location coordinate of the function molecular object was updated and the information is transferred to corresponding matrix object. In this step, each molecule has the lifetime and if the lifetime of the object becomes zero by its aging, this object data is deleted. The matrix object also has a compartment identification code and transcription site identification code. Biobje simulator uses this data to detect what types of reactions should occur.

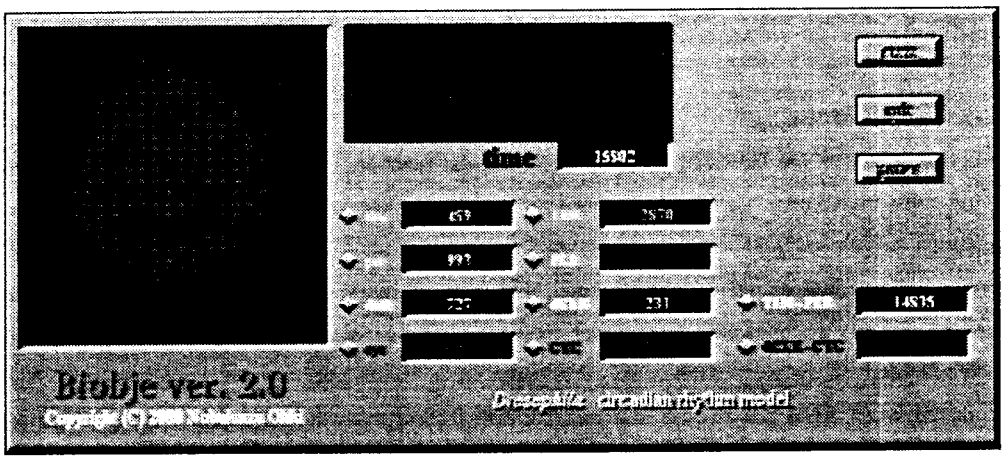

Figure 1. Graphical User Interface of Biobje

Biobje could display a location of the functional molecules in the cell (left on the panel). It also shows fluctuation of the number of the functional molecule (center on the panel).

Application of "Biobje" to Circadian Rhythm

Various organisms (microbes, plants, and mammals) have own circadian rhythm and an endogenous timekeeping. A period of this rhythm is almost same in any organisms and approximately 24 h. Organisms may use regulatory loops of gene expression to make daily rhythms of in physiology and behavior (reviewed by Dunlap, 1999). As essential components of the regulatory loops have been clarified in a fruit fly, *Drosophila melanogaster*, we here applied our "Biobje" to circadian regulation of clock genes in *Drosophila melanogaster*. Two genes, *period (per)*

and *timeless (tim)*, are negatively regulated by their own products, the PER and TIM proteins (reviewed by Reppert, 1998). PER and TIM repress the function of their own transcriptional activators, dCLOCK (dCLK), CYCLE (CYC) (Allada et al., 1998). Formation of a PER/TIM complex regulates oscillation of the mRNA and protein level (Sehgal et al., 1995). Heterodimerization of PER and TIM is necessary for their translocation to the nucleus (Saez and Young, 1996) and also required to protects PER from the activity of a kinase encoded by *double-time (dbt)*. DBT phosphate and promote turnover of monomeric PER proteins, which delays cytoplasmic accumulation of PER/TIM complex and produces endogenous oscillation (Price et. al., 1998). DBT has significant influences on the duration of part of the circadian rhythm by subordinating phosphorylation and turnover of nuclear PER proteins (Price et. al., 1998). There is another negative feedback loop, "*dClk* feedback loop", which connected to the "*per – tim* feedback loop". dClk mRNA level is decreased in per^{01} and tim^{0} mutants, imply that PER and TIM positively regulates expression of *dCLK* (Bae et. al., 1998; Glossop et al., 1999) On the other hand, per^{01};$dClk^{Jrk}$ and per^{01}; Cyc^{0} double mutant shows normal wild-type *dClk* mRNA level (Glossop et al., 1999). This recovery of *dClk* mRNA level suggests that dCLK and CYC prevent *dClk* transcription. Based on these experimental observations, we constructed a novel theoretical model (Ueda et al., submitted to J. Theor. Biol.). The proposed "two feedback model" is composed of two coupled negative feedback loops: a *per-tim* feedback loop, which is activated by dCLK-CYC and repressed by PER-TIM, and a *dClk* feedback loop, which is repressed by dCLK-CYC and derepressed by PER-TIM. Examination of this model revealed that two inter-locked feedback model has robust properties in oscillations. With a new simulation model of "Biobje", we tried to find the reason that *Drosophila melanogaster* can keep the circadian rhythm in any conditions and found that the degradation rates of the mRNAs of *per, tim,* and *cyc* are crucial for the duration and robustness of the circadian rhythm.

Discussion

Several theoretical models, which simulate the circadian rhythm, have been proposed. Leloup and Goldbeter constructed theoretical model for circadian oscillations of PER and TIM (Leloup and Goldbeter, 1999). They applied three kinds of formulation to construct the simulation model. The basic chemical reaction schemes has been used for represent the non-enzyme reactions. The standard Michaelis-Menten formulation is available to build enzyme reaction into the model. An equation of the Hill type is included to treat activation and suppression in a process of transcription. Result form this simulation, they show that for constant environmental conditions, this model is capable of generating autonomous chaotic oscillations. For other parameter values, the model can also display birhythmicity, i.e. the coexistence between two stable regimes of limit cycle oscillations. Novak's group proposed another mathematical model for *Drosophila* circadian rhythm (Tyson et al., 1999). Their model is similar in structure to that of Leloup-Goldbeter, but with a crucial difference. In the

Leloup-Goldbeter model, the role of PER phosphorylation is to introduce a time delay into the negative feedback loop. In Novak's group model, the role of PER phosphorylation is to introduce positive feedback in PER accumulation. As a result form their theoretical analyses, they suggested that a positive feedback loop, based on stabilization of PER by dimerization with TIM, might play an equally important role in generating oscillations. Our simulator predicts the degradation rates of mRNAs of these genes determine the duration and robustness of the circadian rhythm. "Biobje" should be a powerful tool to analyze the regulatory loops of gene expression.

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