Cell Simulation for Circadian Rhythm Based on Michaelis-Menten Model

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Abstract. We report here the development of a cell biological simulation system based on ordinary differential equations and the results on the simulation of the heat pulses' effects on the circadian rhythm in *Drosophila*. The simulator implements intra-cellular processes: transcription, translation, transport, modification (association, disassociation), degradation. It simulates the temporal behavior of concentrations of proteins and mRNA involved in various biological phenomena. Moreover, the system is able to determine the exact type of reaction for a given regulatory pathway. In order to prove the usefulness of the simulator we construct a model of the circadian rhythm in *Drosophila* and we simulate the effect of the heat pulses applied in early afternoon on the circadian clock proteins PER and TIM. Our simulation results show the robustness of the genetic network as well as the important role played by dClk mRNA in the mechanism of phase-shift responses.

Key words: Circadian rhythm, fourth order Runge-Kutta method, regulatory pathway

1. Introduction

Genome sequencing projects and further functional analysis of complete gene sets provide a huge mass of molecular information for a wide range of model organisms. The large number of interdependent chemical reactions and structural components that combine to regulate a typical cell biological process forces one to start building biological models within the computer.

We were motivated to develop a software SIBIL (SIMulator for BIO-Logical systems) for building models based on known regulatory pathways and running simulations to conduct experiments *in silico*. SIBIL allows to the user to indicate the interacting factors (DNA, mRNA, proteins, etc.), the involved pathways and the parameters which characterize them. Then the software is able to decide what kind of reaction occur (transcription, translation, modification, transport, degradation, etc.) and to generate automatically the corresponding ordinary differential equations based on the Michaelis-Menten model. Next, it simulates the cell behavior

Figure 1. Simulator architecture.

by integrating numerically the system of ordinary differential equations previously generated. The user can observe on the display the behavior of concentrations of different factors on the pathway.

Since 1940, there exists several attempts to develop simulators in Japan and abroad (GEPASI, E-CELL, V-CELL, etc.). Compared with these the main features of SIBIL are: the user can be a biologist and does not need any knowledge of computer science; the system is thought as a server-client system, so it can be used on the PC, the calculation time is fairly short (a few seconds for the circadian rhythm in *Drosophila*) and the error is within some acceptable limits.

We applied our system to simulate the effect of heat pulses on the *per-tim* feedback loop [5, 7] in *Drosophila*. We observed the behavior of the clock proteins PER and TIM. Based on the assumption that the action of heat inhibits the translation of the *tim* and *per* mRNA, respectively, we simulated the delay in PER and TIM biochemical cycles caused by exposure to a 37 °C heat pulse of 30 minutes beginning at 15:00. Our results correspond with the experimental data [5]. Moreover, the simulations conducted by us show the importance of the dClk mRNA to the phase shift of the circadian clock proteins PER and TIM.

2. Simulator's Architecture

The present simulation system consists in a solver (a simulation kernel) and a graphic user interface (GUI). The user specifies the involved pathways, the factors (mRNA, proteins, etc.) through the converter. The system generates automatically the deterministic model (system of ordinary differential equations) and solves it by

Figure 2. Per-Tim feedback loop in *Drosophila*.

numerical integration on a server machine. Finally, the viewer allows to observe the temporal behavior of the concentration of the chemical of interest (Figure 1).

In order to simulate a cellular process the user has to input first the substances and the factors.

The system will recognize the nature of the reaction and will generate automatically the corresponding system of differential equations based on the Michaelis-Menten model. The converter allows the user to input the factors, the reactions and the parameters that characterize them. This part of the system was written in Java language with the image of a draw soft in mind. The solver is the engine that solves numerically (fourth order Runge-Kutta method) the system of ordinary differential equations generated by the converter. The various parameters as sampling steps and error margin can be specified through an interactive interface. The viewer is the part of the simulation system that allows the user to observe the temporal behavior of the factors on the pathways indicated to the converter.

3. The Model

In the present simulation system, the mRNAs, proteins and their compounds are defined as factors. Their interaction is described by ordinary differential equations based on Michaelis-Menten model ([6]). We describe here on short the main chemical reactions understood by the simulator. In this model there are several ways to describe the chemical reactions: translation, transcription, modification, transport, degradation. The regulatory pathway that induces the circadian rhythm in *Drosophila* can be described using SIBIL as in Figure 2.

Its corresponding system of differential equations generated by SIBIL coincides with the one discussed in [7].

Figure 3. The temporal behavior of the clock protein TIM in normal conditions (solid line) compared with the behavior when the fly was heat pulsed starting from 15:00 (dotted line).

4. Simulation of the Heat Effects on the *Drosophila*'s circadian Clock Proteins

It is a known fact that various organisms (microbes, plants, mammals) have their own circadian rhythm and an endogenous timekeeping. A period of this rhythm is almost the same in any organism, namely 24h. From the biological point of view, it is assumed that organisms are using regulatory loops of gene expression to maintain their rhythmical physiology.

In a wide variety of organisms they can be phase-shifted (i.e. delayed or advanced) by brief exposure to light and changes in temperature. In the following we are going to simulate the last one. The reason of our choice is that the way how the changes in temperature reset circadian timekeeping mechanisms is not known. To study this phenomena we simulated the action of the heat pulses on *Drosophila* seeking to obtain the same temporal behavior as the one obtained experimentally [5]. Heat pulses at all times in a daily cycle elicit dramatic and rapid decreases in the levels of PER and TIM proteins. We observe in our simulation mainly the protein TIM. This suggests the fact that the resetting mechanism of the circadian rhythm is induced by delays in the *per-tim* transcriptional-translational feedback loop when heat pulses are administrated in early afternoon.

By Sidote *et al.* [5], the phenomena that heat-induced phase delays in the behavioral rhythms are accompanied by long-term delays in the TIM biochemical oscillations. Incubating the flies at 15:00 with 37 °C heat pulse lasting 30 minutes elicit long-time delays in the TIM cycles in abundance and phosphorylation. The experimental data show that the TIM protein in pulsed flies reaches the peak concentration at 20:00, compared to the control case (constant temperature 25 °C) of 15:00.

To simulate this experiment *in silico* we have first reseted the translation of the *tim* and *per* mRNAs, respectively. However, our results were different from the experimental data. This shows two facts. First, the *per-tim* feedback loop is very robust to the variation of transcription and translation parameters of *tim* and *per* mRNAs, respectively. Second, the transcriptional-translational mechanism of

tim and *per* mRNAs cannot explain the experimental data. Therefore, it should be involved an other mechanism in the rapid decrease of TIM protein caused by the exposure to heat pulses. Indeed, if we reset the translation of the dClk mRNA also, we would obtain the same behavior as in the experiment (Figure 3). The conclusion is that the per-tim feedback loop is not the only translational mechanism affected by the heat pulses, but also the dClk translational mechanism is severely affected. We mention here that there is no other translations in the whole cycle (Figure 2).

5. Conclusion

In the present report we presented a simulation system that should satisfy the needs of pharmaceutical companies concerning drug discovery. The system models various chemical reaction in a single cell, recognizes by default the corresponding chemical reaction and generates the system of ordinary differential equations. By the simulation process the temporal behavior of the substrates of interest (drug targets) can be observed on the viewer and the simulation can be repeated for various values of the parameters simulating the action of the drug on some specific target inside the cell.

Our simulations have shown that the experimental data from Sidote *et al.* [5], i.e. the delay with several hours of the peak of the protein is caused not only by the inhibition of the translation of the *per* and *tim* mRNAs, but also by the inhibition of the translation of the dClk mRNA. It seems that this fact is not specified in Sidote *et al.* [5]. In other words, the heat pulses inhibit at the same time all the translations in the pathway.

References

1. Arkin, A., *et al.*: Stochastic kinetic analysis of developmental pathway bifurcation in phage lambda-infected *Escherichia coli* cells, *Genetics* **149**(4) (19984), 1633–1649.
2. Barkai, N. and Leibler, S.: Robustness in simple biochemical networks, *Nature* **387**, June 1997.
3. Leloup, C. and Goldbeter, A.: Chaos and birhythmicity in a model for circadian oscillations of the PER and TIM proteins in *Drosophila*, *J. Theor. Biol.* **198** (1999), 445–459.
4. Murray, J.D.: *Mathematical Biology*, Springer-Verlag, Biomathematics Texts 19, Berlin, Heidelberg, New York, Tokyo, 1993.
5. Sidote, D., *et al.*: Differential effects of light and heat on the *Drosophila* circadian clock proteins PER and TIM, *Mol. Cell. Biol.*, April 1998, pp. 2004–2013.
6. Tomita, M., *et al.*: E-CELL: software environment for whole-cell simulation, *Bioinformatics* **15**(1) (1999), 72–84.
7. Ueda, H., Hagiwara, M. and Kitano, H.: Robust Oscillations within the Interlocked Feedback Model of *Drosophila* Circadian Rhythm, *J. Theor. Biol.* (to appear), 2001.
8. Ueda, H., Hirose, K. and Iino, M.: Coupling Mechanism for Synchronized and Noise-resistant Circadian Oscillators, *Pacific Symposium on Biocomputing (PSB)*, Hawaii, 2001.