Simulation of Biological Systems by Hybrid Petri Net with an Enhancement

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Abstsuct **– The following two matters should be resolved in order for biosimulation tools to be accepted by users in biology/medicine; (1) Remove issues which are irrelevant to biological importance, and (2) Allow users to represent biopathways intuitively and understand/manage easily the details of representation and simulation mechanism. From these criteria, we firstly define a novel notion of Petri net called *hybrid functional Petri net* (HFPN). Then, we introduce a software tool, *Genomic Object Net*, for representing and simulating biopathways, which we have developed by employing the architecture of HFPN.**

In order to show the effectiveness of *Genomic Object Net* for representing and simulating biopathways, we show an HFPN representation of gene regulation mechanism of *Drosophila melanogaster* (fruit fly) circadian rhythm. The simulation results of this mechanism are also presented. The software is available to academic users from *http://www.GenomicObject.Net* **.**

I. INTRODUCTION

Considerable attentions have been paid to the biopathway representation and simulation in the literature. The most traditional approach is to employ ordinary differential equations (ODEs) such as Michaelis-Menten equations and to represent biochemical reactions as a systems of ODEs. This approach provides mathematically well-founded and fine interpretations of biopathways, especially for enzyme reactions. Gepasi [1] is a software package based on this approach for modeling biochemical systems and it aims at assisting users in translating reaction processes to matrices and ODEs. E-Cell [2] develops a system for representation and simulation with GUI and, with this tool, a model of a hypothetical cell with only 127 genes sufficient for transcription, transration, energy production and phospholipid synthesis is constructed.

As is stressed in [3] and [4], in order for software tools to be accepted by users in biology/medicine for biopathway modeling, we consider the following two matters should be resolved, at least: (1) Remove issues which are irrelevant to biological importance; Otherwise, users might be trained to understand some special notions in mathematics, physics and computer science which are irrelevant to biology/medicine. (2) Allow users to represent biopathways intuitively and understand/manage easily the details of representation and simulation mechanism; Otherwise, users could not have a confidence that the understanding and knowledge in their minds coincides with the object represented with the software tools.

From these criteria, in this paper we firstly define a novel notion of Petri net called *hybrid functional Petri net* (HFPN) by extending the notions of hybrid Petri net [5] and functional Petri net [6] so that the notion will be suited for modeling biopathways.

Then, we introduce a software tool, *Genomic Object Net*, for representing and simulating biopathways, which we have developed by employing the architecture of HFPN. *Genomic Object Net* has an editor and a simulator of HFPN with GUI which shall resolve the matters (1) and (2). In order to demonstrate the effectiveness of *Genomic Object Net* for representing and simulating biopathways, we show a circadian rhythm of *Drosophila* which is one of the typical models of gene regulatory mechanism.

II. HYBRID FUNCTIONAL PETRI NET

The hybrid Petri net (HPN) [5] has been introduced as an extension of the discrete Petri net model so that it can handle real numbers in the continuous way and it allows us to express explicitly the relationship between continuous values and discrete values while keeping the good characteristics of discrete Petri net soundly.

From the definition of HPN, the firing speed of a continuous place must be the same as the consuming speed through each arc from its its source place and the contents of all source places are consumed with the same speed. This speed is also the same as the production speed through each arc from the transition. This is the unfavorable feature of HPN for biopathway simulation.

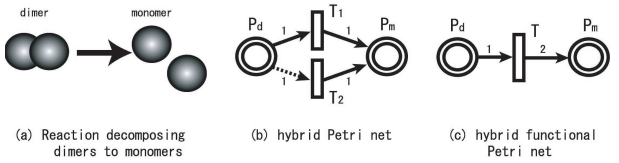

Fig. 1: (a) is a reaction model decomposing dimers to monomers. (b) and (c) are the HPN and HFPN representations of (a), respectively.

For example, consider a reaction in which a dimer is cleaved to two monomers (Fig. 1 (a)). This reaction in the HPN model could be represented as shown in Fig. 1 (b) by using a test arc and a transition for amplification (note that the amounts consumed and produced in places by continuous transition firing is the same by definition while the amount of monomers is twice as large as that of dimers). But it is neither intuitive nor natural at all.

Definition 1 A *hybrid functional Petri net* (HFPN) is defined by extending the notion of transition of HPN [5] in the following way: HFPN has five kinds of arcs; *discrete input arc*, *continuous input arc*, *test input arc*, *discrete output arc*, and *continuous output arc*. A discrete input arc (continuous input arc) is directed to a discrete transition (continuous transition) from a discrete/continuous[1] place (continuous place) from which it consumes the content of the source place by firing. A test input arc is directed from a place of any kind to a transition of any kind. It does not consume the content of the source place. These three arcs are called *input arcs*. A discrete output arc is directed from a discrete transition to a place of any kind. A continuous output arc is directed from a continuous transition to a continuous place. These two arcs are called *output arcs*.

(1) Continuous transition: A *continuous transition* T of HFPN consists of continuous/test input arcs $a_1, \ldots, a_p$ from places $P_1, \ldots, P_p$ to T and continuous output arcs $b_1, \ldots, b_q$ from T to continuous places $Q_1, \ldots, Q_q$. Let $m_1(t), \ldots, m_p(t)$ and $n_1(t), \ldots, n_q(t)$ be the contents of $P_1, \ldots, P_p$ and $Q_1, \ldots, Q_q$ at time t, respectively. The continuous transition T specifies the following:

   (a) The *firing condition* given by a predicate $c(m_1(t), \ldots, m_p(t))$. As long as this condition is true, T fires continuously.

   (b) For each input arc a_i,T specifies a function $f_i(m_1(t), \ldots, m_p(t)) \geq 0$ which defines the speed of consumption from P_i when it is firing. If a_i is a test input arc, then we assume $f_i \equiv 0$ and no amount is removed from P_i. Namely, $d[a_i](t)/dt = f_i(m_1(t), \ldots, m_p(t))$, where $[a_i](t)$ denotes the amount removed from P_i at time t through the continuous input arc a_i during the period of firing.

   (c) For each output arc b_j, T specifies a function $g_j(m_1(t), \ldots, m_p(t)) \geq 0$ which defines the speed of amount added to Q_j at time t through the continuous output arc b_j when it is firing. Namely, $d[b_j](t)/dt = g_j(m_1(t), \ldots, m_p(t))$, where $[b_j](t)$ denotes the amount of the contents added to Q_j at time t through the continuous output arc b_j during the period of firing.

(2) Discrete transition: A *discrete transition* T of HFPN consists of discrete/test input arcs $a_1, \ldots, a_p$ from places $P_1, \ldots, P_p$ to T and discrete output arcs $b_1, \ldots, b_q$ from T to places $Q_1, \ldots, Q_q$. Let $m_1(t), \ldots, m_p(t)$ and $n_1(t), \ldots, n_q(t)$ be the contents of $P_1, \ldots, P_p$ and $Q_1, \ldots, Q_q$ at time t, respectively. The discrete transition T specifies the following:

   (a) The *firing condition* given by a predicate $c(m_1(t), \ldots, m_p(t))$. If this is true, T gets ready to fire.

   (b) The *delay function* given by a nonnegative integer valued function $d(m_1(t), \ldots, m_p(t))$. If the firing condition gets satisfied at time t, T fires in delay $d(m_1(t), \ldots, m_p(t))$. However, if the firing condition is changed during this delay time, the transition T looses the chance of firing and the firing condition will be reset.

   (c) For each input arc a_i, T specifies a nonnegative integer valued function $f_i(m_1(t), \ldots, m_p(t)) \geq 0$ which defines the the number of tokens (integer) removed from P_i through arc a_i by firing. If a_i is a test input arc, then we assume $f_i \equiv 0$ and no token is removed.

   (d) For each output arc b_j, T specifies a nonnegative integer valued function $g_j(m_1(t), \ldots, m_p(t)) \geq 0$ which defines the number of tokens (integer) are added to Q_j through arc b_j by firing.

From the above definition, it may be obvious that in the HFPN model, the dimer-to-monomers reaction can be intuitively represented as Fig. 1 (c). Not only this simple example but also more complex interactions can be easily and intuitively described with HFPN. The software *Genomic Object Net* is developed and implemented based on this HFPN architecture.

III. GENOMIC OBJECT NET

The current version of *Genomic Object Net* consists of two tools, *Genomic Object Net Assembler* (GON Assembler) (the Largest window in Fig. 2) and *Genomic Object Net Visualizer* (GON Visualizer) (the window at the lower left in Fig. 2). Both tools work on Windows 98/NT/2000.

Fig. 2 shows the the circadian rhythms of *Drosophila melanogaster* (a fruit fly) modeled and visualized with *Genomic Object Net*. The mechanism in biopathway

[1] A/B means A or B.

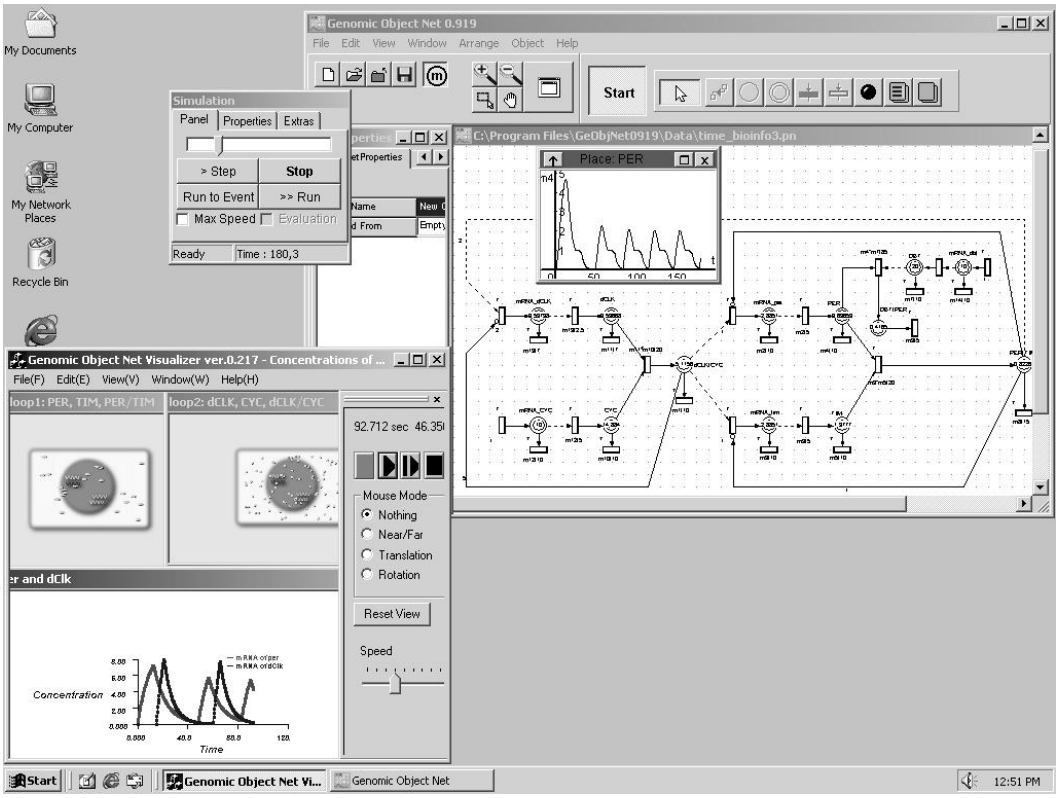

Fig. 2: *Genomic Object Net*

is described as HFPN with GON Assembler and the system behavior can be observed in the animated way with GON Visualizer.

GON Assembler has an editor and a simulator of HFPN with GUI. GON Visualizer is developed on the basis of XML technology. Users in biology/medicine can realize visualizations of simulation results of the aimed biological phenomenon by creating XML document in which CSV files produced by GON Assembler are included as basic data for simulations.

The concept of XML and DTD structure is quite successful in representing biopathways, since this structure enable us to separate complicated mathematical descriptions to the DTD file from biologically essential descriptions. This concept contributes to reduce efforts of users in biology/medicine to learn programming languages which are basically computer technology, but to put it the other way around, computer scientists should make efforts to create DTD files based on the discussion with the users in biology/medicine.

IV. BIOPATHWAY MODELING EXAMPLE BY GON

In order to demonstrate how the tool "Genomic Object Net" is useful in representing and simulating biological phenomenon, we show a biopathway modeling of gene regulation about circadian rhythms in *Drosophila*. We have also modeled some other biopathways such as the Fas ligand induced apoptosis signaling pathway and *lac* operon gene regulatory mechanism with glycolytic metabolic pathway [7].

The control mechanism of autoregulatory feedback loops of *Drosophila* circadian rhythms has been intensively studied [8, 9, 10, 11, 12, 15] and some fine modelings by ODEs with detailed coefficients have also been reported [13, 14].

Fig. 3 shows the scheme of the regulatory mechanism of five genes contributing to the *Drosophila* circadian rhythms; *period* (*per*), *timeless* (*tim*), *Drosophila Clock* (*dClk*), *cycle* (*cyc*) and *double-time* (*dbt*). It is known that the *Drosophila* circadian feedback system is composed of two interlocked negative feedback loops [9]. Roughly speaking, PER and TIM proteins collaborate in the regulation of their own expression in *Drosophila*, assembling in PER-TIM complexes that permit nuclear translocation, inactivation of *per* and *tim* transcription in a cycling negative feedback loop, and activation of *dClk* transcription which participates in the dCLK-CYC negative feedback loop. The *dCLK* and *CYC* form heterodimers that activate *per* and *tim* transcriptions and inhibit *dClk* transcription. Among these five genes, three genes, *per*, *tim*, and *dClk*, are rhythmically expressed: *per* and *tim* mRNA levels begin to rise in the subjective day and to peak early in the subjective evening, and *dClk* mRNA level peaks late at night to early in the morning. Although *per* and *tim* mRNAs reach peak levels in the evening, PER and TIM levels do not peak until late evening. It is considered that this delay results from the initial destabilization of PER by DBT-dependent phosphorylation followed by the stabilization of PER by dimerization with TIM [11, 12]. The details of the mechanism are surveyed in

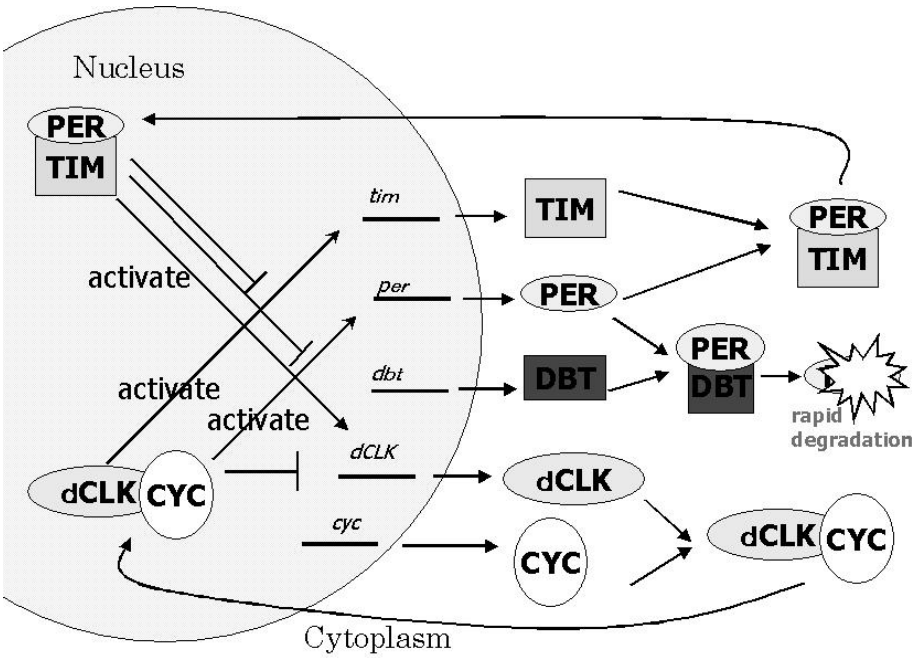

Fig. 3: The gene regulation in the *Drosophila* circadian oscillator is schematized.

[8, 10, 15].

Ueda et al. [14] have modeled the two interlocked negative feedback loop system [9] with ODEs and made extensive simulation and mathematical analysis. We have translated it into an HFPN as in Fig. 4 and further computational experiments based on this model are possible on *Genomic Object Net* with this HFPN file.

A series of ten ODEs, e.g. $\frac{dPer_m}{dt} = C_1 + S_1 \frac{\left(\frac{CC_n}{A_1}\right)^a + B_1}{1+\left(\frac{PT_n}{R_1}\right)^r + \left(\frac{CC_n}{A_1}\right)^a + B_1} - D_1 \frac{Per_m}{L_1 + Per_m} - D_0 Per_m$ are realized in this network, where Per_m (CC_n, PT_n) represents the concentration of *per* mRNA (dCLK-CYC complex in the nucleus, PER-TIM complex in the nucleus) and $C_1 = 0$nM/h, $S_1 = 1.4$nM/h, $A_1 = 0.45$nM/h, $B_1 = 0$, $L_1 = 0.3$nM/h, $D_0 = 0.012$nM/h, $D_1 = 0.94$nM/h, $R_1 = 1.02$nM/h.

By using *Genomic Object Net*, we designed a HFPN from scratch by interpreting the facts and observations in [8, 9, 10, 11, 12, 15]. Fig. 5 is a naïve representation of the gene regulatory mechanism of *Drosophila* circadian oscillator, where the functions for continuous transitions are defined and tuned so that the simulation results will coincide with the facts and observations.

In Fig. 5, smaller value of tokens $m2$ or $m4$ is taken as the complex forming rate of the proteins dCLK ($m2$) and CYC ($m4$) at the transition T_1. *Genomic Object Net* can assign a perl script to any transition for realizing such a sophisticated function. The perl script of this case describes the program which takes smaller ones of tokens $m2$ or $m4$. Complex forming rates of PER/TIM and PER/DBT are similarly realized at the transitions T_2 and T_3, respectively, by using this function. Transitions T_4, T_5, and T_6 represent the degradation rates of complexes of the corresponding proteins. Fig. 6 is the simulation result of the HFPN in Fig. 5. It indicates that this HFPN model representing two negative feedback loops, the PER-TIM feedback and the dCLK-CYC feedback, successfully produce periodic oscillations of *per* mRNA ($m6$), *tim* mRNA ($m8$), and *dClk* mRNA ($m1$), while the concentration of *cyc* mRNA ($m3$) keeps constant expression.

V. CONCLUSION

Genomic Object Net based on HFPN architecture shall provide a useful platform with high potential abilities for designing and simulating biopathways. *Genomic Object Net* is intended to be a naïve platform where we can create hypotheses and evaluate them by simulation. This feature is especially important when only rough modeling is enough or enough information is not available for fine modeling.

VI. REFERENCES

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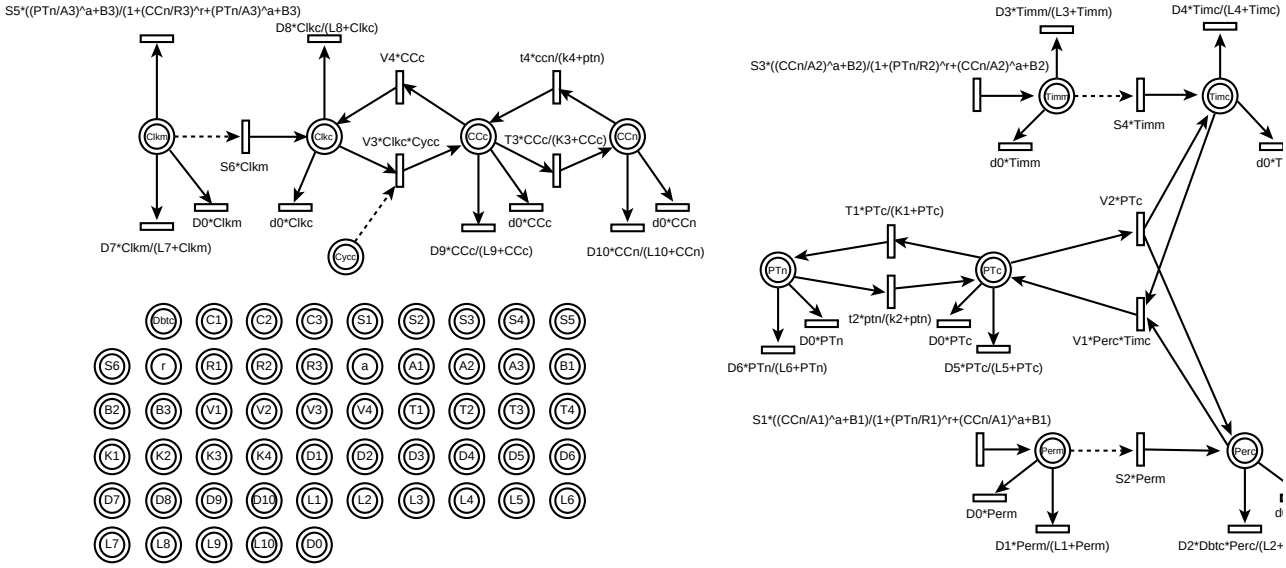

Fig. 4: An HFPN realization of the circadian rhythm model due to Ueda et al. [14].

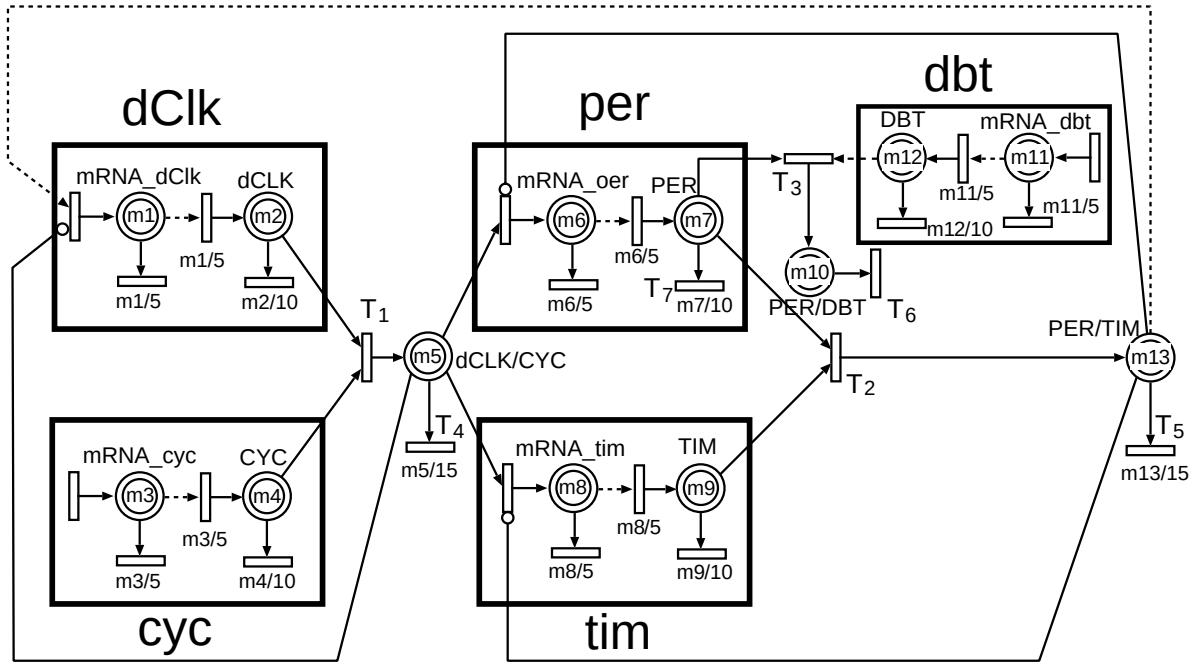

Fig. 5: A naïve HFPN representation of *Drosophila* circadian mechanism

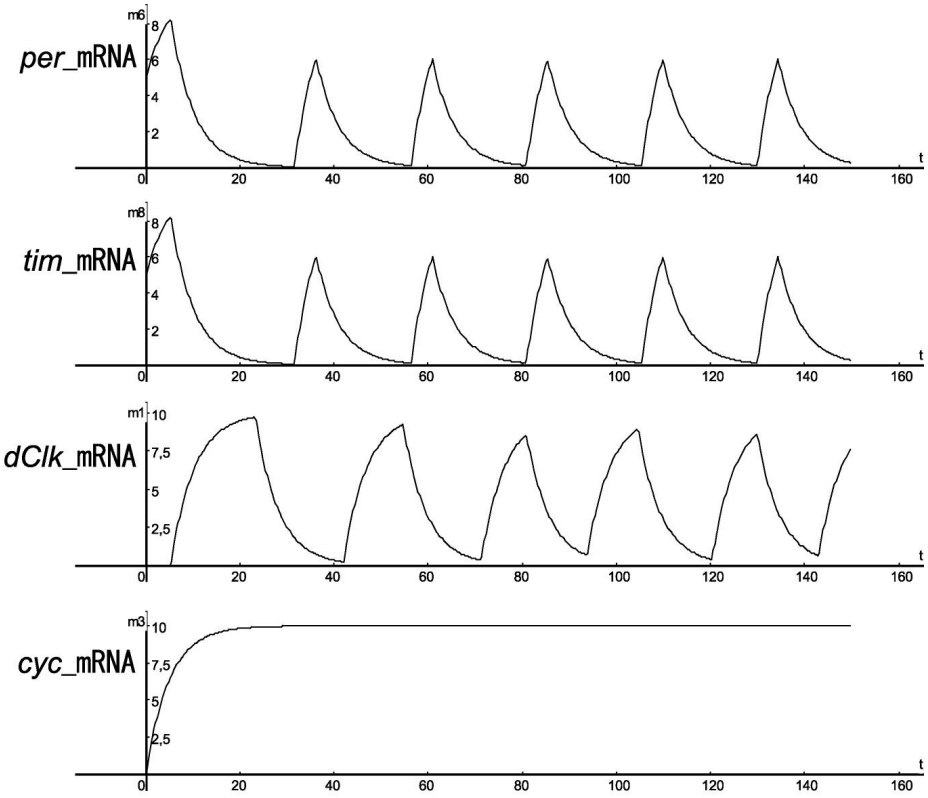

Fig. 6: Behaviors of concentrations of four mRNAs simulated on *Genomic Object Net.*

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