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Theoretical Study for Circadian Rhythm in *Drosophila*: Condition for Generating A limit Cycle

by

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Abstract

It has been shown that PER and TIM play a crucial role in the gene network to generate the circadian rhythm of *Drosophila*. They make heterodimers and inhibit the transcriptions of their own in nucleus. Leloup(1998) proposed several mathematical models for this phenomenon, and analysed them by numerical simulations. He claimed that the model in which PER-TIM complex regulates the transcription of both of their genes is easier to generate a stable oscillation than the model in which only PER does feedback control, as the parameter region for oscillation is larger in the former model than in the latter. In this paper, we present 3 simple models and compare the condition for generating a limit cycle. We introduce a parameter "cooperativity" indicating the degree of non-linearity in the gene transcription term. We found that a larger cooperativity generally tends to lose the local stability of equilibrium point and generate a limit cycle in all of the three models. Compared with "three variables model" (*per* mRNA, PER in cytosol, and PER in nucleus), "four variables model" (PER is modified before entering the nucleus) can generate a stable oscillation with a smaller cooperativity. Compared with these, our "PER-TIM model" can also generate oscillation with a smaller cooperativity. They show that the modification and heterodimerizaion of proteins are important to generate a limit cycle.

Key words: circadian rhythm, limit cycle, PER, TIM

Introduction

Circadian rhythm is widely observed phenomena at various species and molecular mechanism of it is getting clear. It has been shown that these rhythms are autonomous and are generated by the circadian oscillations of the amount of the specific proteins in each cell. The *per* and *tim* genes of *Drosophila* commonly have elements of the transcriptions, CLK and CYC and they synthesise PER and TIM proteins. These two proteins make heterodimers and inhibit the transcriptions of their

own in nucleus, presumably inactivating the CLK-CYC heterodimers. This negative feedback is thought to generate the circadian rhythm of *Drosophila*.

Leloup proposed several mathematical models for circadian rhythm in *Drosophila* incorporating the formation of the PER-TIM complex and analysed them by numerical simulations (Leloup, 1998). He claimed that the model in which PER-TIM complex regulates the transcription of both genes is easier to generate a stable oscillation than the model in which only PER does feedback control, as the parameter region for oscillation is lager in the former model than that in the latter.

From the dynamical point of view, we have to consider the following two problems. First, is the modification of protein in cytosol necessary in order to generate a limit cycle? Second, why does genetic network of circadian rhythm of *Drosophila* consist of two genes, *per* and *tim* ? In this paper, in order to consider these two problems, we present three simple models, and analyse them in detail. The models have single non-zero equilibrium point. A limit cycle occurs when equilibrium point is locally unstable and system is globally stable. We studied whether it can occur or not in each model by analysing the local stability of equilibrium point. Although some theoretical analysis for simple model of circadian rhythms has been done (Griffith, 1968), our study is the first to compare the condition for generating a limit cycle between different models by theoretical method. We also studied whether stable oscillation can occur by numerical simulation where unstable equilibrium point can be found.

We studied three different models, "three variables model" (*per* mRNA, PER in cytosol, and PER in nucleus), "four variables model" (PER is modified before entering the nucleus), and "PER-TIM model" (PER and TIM protein make heterodimers before entering the nucleus). We use two types of reaction term in each model. First, transcriptional inhibition by protein binding is expressed by Hill type equation. Second, the other reaction terms are proportional to the amount of substrate.

We prove that equilibrium point is stable when n is very small. We could also show that equilibrium point can be unstable when n is larger than threshold. By numerical simulation, we show that equilibrium point is unstable and a limit cycle occurs when n is large.

Models and Results
"Three variables model"

First, we consider simplest model for gene regulation of *per*, which includes only three variables, the level of *per* mRNA, that of PER protein in cytosol and that of PER protein in nucleus. The amount of mRNA increase by the transcription and decrease by the enzymatic degradation. The amount of PER protein in cytosol increase by the translation and decrease by the transportation into nucleus and the degradation. The amount of PER protein in nucleus increase by this transportation and decrease by the reverse transportation into cytosol. In nucleus, this protein inhibits the transcription of its own. We call "n" in transcription term "cooperativity", which indicate the degree of non-linearity of the inhibition. The h is the threshold constant of that reaction.

$$\frac{dM}{dt} = \frac{1}{1+(P/h)^n} - aM \qquad (1.a)$$

$$\frac{dR}{dt} = M - (d+u)R + vP \qquad (1.b)$$

$$\frac{dP}{dt} = uR - vP \qquad (1.c)$$

, where M is concentration of mRNA and R and P are that of PER protein in cytosol and in nucleus, respectively. The a and d are rate of degradation and u is rate of transportation of R into the nucleus and v is that of P into the cytosol.

Small "n", generating stable equilibrium

By focusing on small perturbation about the equilibrium, we can linearize the dynamics and know the dynamical stability of the equilibrium points. The stability is shown by the sign of the real part of the eigenvalue of the matrix showing the linearized dynamics. When it is negative, the point is locally stable and cannot generate a limit cycle. However when it is positive, the point is not locally stable and the model can generate a limit cycle. We also studied by numerical simulation later whether the model actually generates a stable oscillation or not under this condition.

The eigenvalues of this model satisfy the equation

$$\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3 = 0 \qquad (2.a)$$

, where

$$a_1 = a + d + u + v \qquad (2.b)$$

$$a_2 = a(d+u) + v(a+d) \qquad (2.c)$$

$$a_3 = adv + u\frac{n(\hat{P}/h)^{n-1}}{h(1+(\hat{P}/h)^n)^2} \qquad (2.d)$$

The Routh-Hurwitz conditions give the necessary and sufficient conditions for $\mathrm{Re}\,\lambda < 0$ in general. For cubic equation, the conditions are

$$a_1, a_2, a_3 > 0 \qquad (3.a)$$

$$a_1 a_2 > a_3 \qquad (3.b)$$

We analysed this model by means of these two conditions.
The first three inequalities are always satisfied because all parameters are positive here.
Then let us consider $a_1a_2 - a_3$,

$$a_1a_2 - a_3 = (a+d+u+v)(a(d+u)+v(a+d)) - (adv + u\frac{n(\hat{P}/h)^{n-1}}{h(1+(\hat{P}/h)^n)^2}) \qquad (4)$$

After basic calculations, we proved that the above expression is always positive when $8 \geq n$. Then we can conclude that the equilibrium point is stable when $8 \geq n$ without depending on parameter values.

Large "n", generating unstable equilibrium

When $n \geq 9$, we can show that the equilibrium point can be unstable. For example, if we assume the following

$$a = d = v = x, u = kx \qquad (5)$$

, $a_1a_2 - a_3$ can be negative when $n \geq 9$. Then unstable equilibrium point can exist when $n \geq 9$. We carried out computer simulation by using above condition and confirmed that the trajectory became a limit cycle. In Fig. 1, we showed the value of mRNA with time. In conclusion, the equilibrium is stable and there is no limit cycle

for all the parameter value when $8 \geq n$, and there should be at least one set of parameter which make the equilibrium unstable when $n \geq 9$.

"Four variables model"

In this chapter, we consider a model in which PER protein is modified before entering nucleus. The variables are *per* mRNA, PER in cytosol, modified PER in cytosol, and PER in nucleus . We assumed here that PER has to be modified first before entering nucleus. In addition to " three variables model", we consider the amount of modified PER. The amount of modified PER protein in cytosol increase by the transition from unmodified PER into modified one and decrease by its reverse transition and the enzymatic degradation.

$$\frac{dM}{dt} = \frac{1}{1+(P/h)^n} - aM \qquad (6.a)$$

$$\frac{dR}{dt} = M - bR + cQ \qquad (6.b)$$

$$\frac{dQ}{dt} = bR - (c + d + u)Q + vP \qquad (6.c)$$

$$\frac{dP}{dt} = uQ - vP \qquad (6.d)$$

,where R is unmodified PER and Q is modified one. The b and c show the velocity of reversible reactions of transition from R into Q and that of Q into R, respectively.

We made stability analysis similarly as that of "three variables model". We prove that the equilibrium point is always stable when $3 \geq n$ without depending on parameter values. We could show that it could become unstable when $n \geq 5$. We carried out computer simulation by using above condition and confirmed that the trajectory became a limit cycle. The value of mRNA with time is similarly as shown in Fig. 1. In conclusion, the equilibrium is stable and there is no limit cycle for all the parameter value when $3 \geq n$, and there should be at least one set of parameter which make the equilibrium unstable when $n \geq 5$. The threshold n is smaller than the previous "three variables model." Then we can say that "four variables model" is more likely to generate a limit cycle than "three variables model".

"PER-TIM model"

In above two models, we just focused on *per* network. However *Drosophila* has also *tim* gene, which has the similar role as *per* gene acting as negative factor of transcription of both *per* and *tim* gene. In this chapter, we consider a model in which PER and TIM proteins make heterodimer. The dynamics of mRNA and monomers are same as previous models. The amount of heterodimer, complex in cytosol increase by the dimerization and decreases by the reaction of the separation of itself. The amount of complex in nucleus increase by the transportation of complex into nucleus and decrease by the transportation into cytosol. In nucleus this complex inhibits the transcription of both *per* and *tim* .

$$\frac{dM_1}{dt} = \frac{1}{1+(P/h)^n} - a_1 M_1 \qquad (7.a)$$

$$\frac{dM_2}{dt} = \frac{1}{1+(P/h)^n} - a_2 M_2 \qquad (7.b)$$

$$\frac{dR_1}{dt} = M_1 - d_1 R_1 - bR_1 R_2 + cQ \qquad (7.c)$$

$$\frac{dR_2}{dt} = M_2 - d_2 R_2 - bR_1 R_2 + cQ \qquad (7.d)$$

$$\frac{dQ}{dt} = bR_1 R_2 - (c+u)Q + vP \qquad (7.e)$$

$$\frac{dP}{dt} = uQ - vP \qquad (7.f)$$

, where M_1 and M_2 are concentrations of *per* mRNA and *tim* mRNA, R_1 and R_2 are those of PER protein and TIM protein, respectively, and Q is concentration of cytosolic form of complex, and P is that of nuclear one. The a_1, a_2, d_1 and d_2 are rate of degradation. The b and c are forward and reverse rate constants.

We made stability analysis similarly as that of "three variables model". We can't find any n that always makes the equilibrium point stable. However, we could show that the equilibrium could become unstable when $n \geq 3$ by giving a set of parameter value. We carried out computer simulation by using above condition and confirmed that the trajectory became a limit cycle. The value of mRNA against time is similarly as shown in Fig. 1. In conclusion, there should be at least one set of parameter which make the equilibrium unstable when $n \geq 3$. The threshold n is smaller than "three variables model" and "four variables model". Then we can say that "PER-TIM model" is more likely to generate a limit cycle than "three variables model" and "four variables model". Here we summarise the results of three models in Table, as follows.

Table

n	three variables model	four variables model	PER-TIM model
1	s	s	r
2	s	s	r
3	s	s	u,o
4	s	r	u
5	s	u,o	u
6	s	u	u
7	s	u	u
8	s	u	u
9	u,o	u	u

s: equilibrium point is always stable
u: equilibrium point can be unstable
o: stable oscillations are observed
r: we can't say whether equilibrium point is always stable or can be unstable.

Caption

Figure 1: Sustained oscillation of the amount of *per* mRNA generated by "three variables model" when n =9. Parameter values are $a = d = v = 1$, $u = 0.01$, $h = 0.00078$ which make expression (4) negative. Number of simulation steps is 10000 and at each step time unit is 0.001.

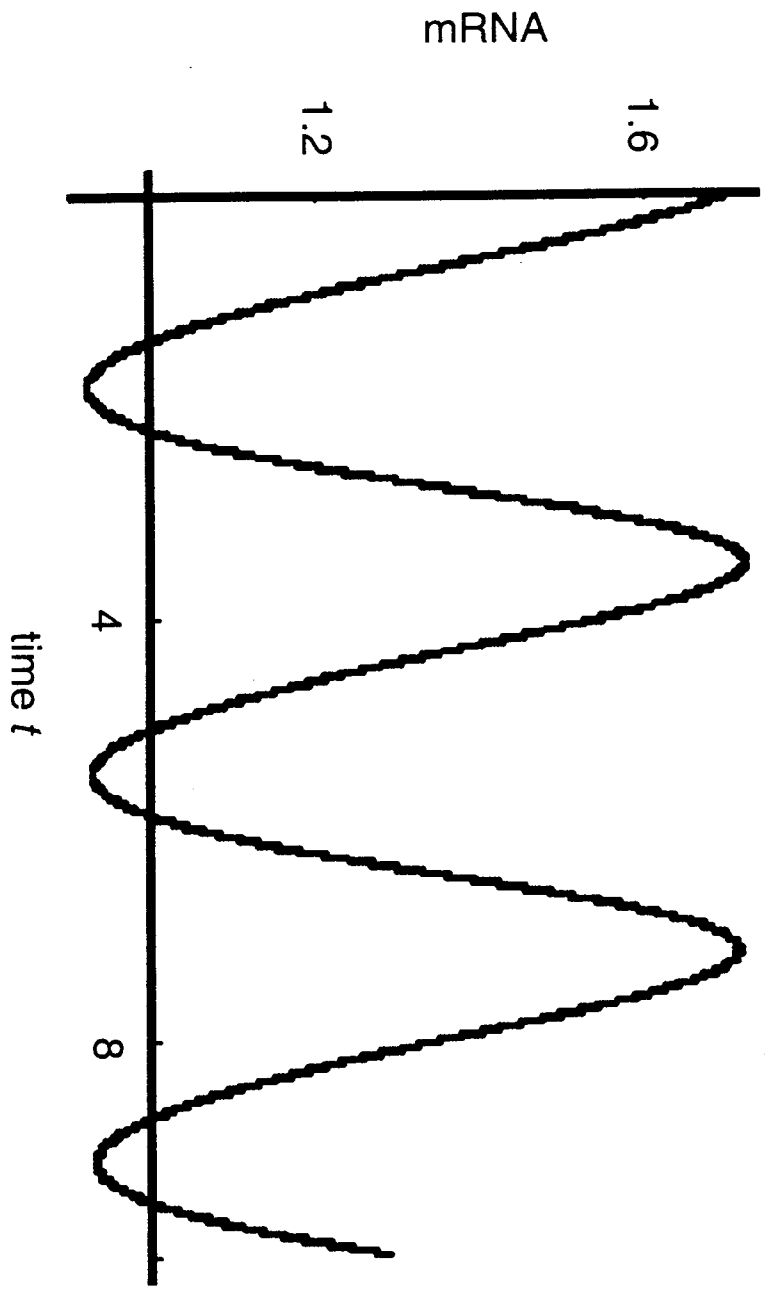

Fig. 1