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Analysis of Biological Smooth Oscillators Inspired by the Relay Control Tuning Method ⋆

Yan Zhang, Jian Chen, Guang Li

Department of Control Science and Engineering, Zhejiang University, Hangzhou 310027, China (e-mail: jchen@zju.edu.cn)

Abstract: Biological oscillation is generated by different genetic circuit structures, such as the smooth oscillator and the relaxation oscillator. Studies on the complex interactions among genes and proteins are essential for understanding the generating mechanism underlying the circadian clock or other biological oscillators. In this paper, the biological oscillation is investigated from a new perspective. It is studied here, through principles in the nonlinear system and control theory, the important factors for the existence of oscillation in the smooth oscillation system. Conclusions have been drawn that both the degradation rates of genes and proteins and the switch-like behavior of the translation process are essential for generating oscillation. The prediction in the Goodwin oscillators model, that the degradations of the clock mRNA and clock protein play important roles in regulating the oscillation period, is discussed here through classic control theory, i.e., the relay control tuning method.

Keywords: Smooth oscillators, circadian rhythms, dynamical systems, relay control, biodegradation rate

1. INTRODUCTION

Circadian clock is known to be essential for regulating life activities. Its malfunction can lead to disruption of biological rhythms and cause severe damage to the organism. Researches on mechanism of the biological oscillations have matured rapidly during the past decades. Many genetic circuits, exhibiting sustained oscillation, have been constructed both experimentally and theoretically(Judd (2000),Chang (2013), and Relogio (2011)). in Biological oscillators have been classified into several types(Alon (2010)). The most common structures are known as the relaxation oscillation and the repressilator.

It has been commonly recognized that the negative feedback mechanism plays a crucial role for the system to generate robust oscillation. Further study(Becker-Weimann (2004)) denotes that the function of positive feedback, existing in the circadian clock is to regulate output process, such as the peak concentration of proteins.

Modeling the system of the mammalian circadian oscillator is a useful tool to understand its basic mechanism. In a previous study(Buhr (2010)), by using different sets of modeling parameter values, researchers compared the effect of the various parameters and showed that both the occurrence and the period of the oscillations are generally most sensitive to parameters related to the synthesis or the degradation of clock mRNA and clock protein. Sustained oscillations might arise from the sole negative autoregulation of gene expression.

Researchers(Zhou (2008), Reznik (2013)) denote that perhaps the most challenging task is to understand the kinetic details of oscillation models. They used generalized modeling to study the dynamical behavior and illustrated that widely separated time scales could promote stability in the genetic circuit, exhibiting oscillation. Hence, design principles have been concluded recently(Novak (2008)), declaring that negative feedback, time delay, sufficient nonlinearity of the reaction kinetics and proper balancing of the timescales opposing chemical reactions are the four general requirements for biochemical oscillations.

Among many types of biological oscillator, the smooth oscillator is the most basic structure for the limit-cycle oscillation. The Goodwin oscillators and Repressilators all belong to this class(Hasegawa (2013)). A feature of the Goodwin oscillators is that the degradations of the clock mRNA and clock protein play important roles in controlling the length of the oscillation period(Ruoff (1999)).

Many efforts(Sturk (2010), Menolascina (2011)) have been devoted to controlling the biological systems. Classic control theories have been applied to understanding complex biological systems or networks. When the biological systems have been modeled, the next task is to derive a control algorithm that forces the system to meet the required specification. Scientists are often faced with different challenges, such as non-observable or non-controllable biological events(Cury (2013)).

The primary focus of this paper is to analyze the biological smooth oscillator based on the relay control tuning

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method(Astrom (1984), Schei (1992)). A special circuit is developed in the relay control tuning method, the core feature of this circuit satisfies the four general requirements for biochemical oscillations mentioned above. After all, the relay control tuning method is to analyze the characteristics of the oscillation, which is generated by the system with a relay controller.

This paper is organized in the following order. In Section 2 and Section 3, the mathematical model for the genetic circuit of smooth oscillators is constructed. In Section 4, a mathematical tool, i.e., the Poincare-Bendixson theorem(Smith (1979)) is used to prove the existence of oscillation in the system. The determinant factors for the existence of oscillation is found. In Section 5, simulation results are provided. Certain roles that model parameters play in generating the robust oscillation are analyzed in Section 5. Determinant factors for the oscillation period are also investigated, the conclusion of which is in good accordance with the predictions made in the Goodwin oscillator model. Further analysis in Section 5 leads to the conclusion that the self-regulating mechanism, i.e., the degradation of biological components is essential for the oscillation. Conclusions are given in Section 6.

2. MODEL DESCRIPTION

In control theory, a commonly used method(Astrom (1984), Schei (1992)) for tuning PID regulators is to substitute the PID controller with a relay controller, then to analyze the dynamics of the relay control system. The detailed structure is shown in Fig.1((Astrom (1984)). The method is based on the observation that a system with a phase lag of at least π at high frequencies may oscillate in a certain period. Obviously, the relay controller added to the system can provide the phase lag. This nonlinearity part plays a fundamental role in generating oscillation. The critical period of oscillator is mainly determined by characteristics of the *Process* part. Also, the relay control system is robust against disturbance and stochastic noise.

In Fig.2, the genetic circuit of the smooth oscillator(Hasegawa (2013)) is presented. Note that this circuit can be seen as simplified genetic oscillator networks or the motif, because it shows the fundamental mechanism of biological oscillator(Alon (2010)).

This genetic circuit consists of four major components: the clock gene, the clock mRNA, the unphosphorylated clock proteins, and the phosphorylated activated clock proteins. Once the transcription factors combine with the clock gene, the activated clock proteins will be synthesized eventually. Thus, the concentration of activated clock proteins, which serves as the output of the system, will oscillate. Also, there are three main biological processes in the genetic circuit: the transcription process of the clock genes, which is inhibited by the activated proteins, the mRNAtranslation process, and the clock proteins phosphorylation process. And the translation of clock mRNA is a fast process. These processes together form a negative feedback loop of the genetic circuit.

Comparing the circuit structure in Fig.1 and Fig.2, it can be noticed that in a relay control circuit, the time delay is realized by combining the nonlinear part, i.e., the relay

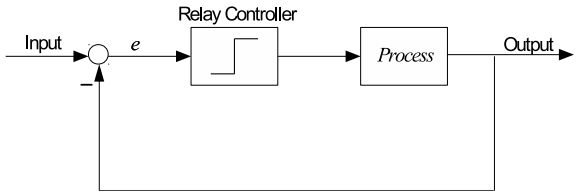

Fig. 1. Circuit Structure in the relay control tuning method

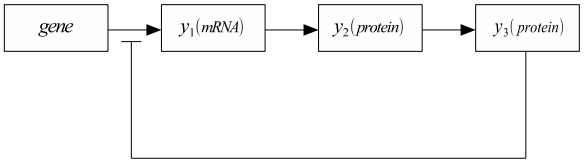

Fig. 2. Structure for genetic circuit of Smooth oscillators

controller, with a linear *Process* part. Similarly, in a genetic circuit, combining a fast translation process exhibiting switch-like behaviors and a relatively slow phosphorylation process is necessary for smooth oscillators. Hence, the time delay and the sufficient nonlinearity of the reaction kinetics are satisfied the smooth oscillators. The other two requirements in biological oscillators, i.e., a negative feedback and the proper balancing of the timescales opposing chemical reactions are also satisfied in in this model.

The translation process with nonlinear characteristics can be seen as the relay controller part in Fig.1, the inhibition of the transcription process by activated proteins forms a negative feedback in the circuit, and the phosphorylation process is similar to the *Process* part in Fig.1.

In the relay control tuning method, the amplitude of the oscillator is determined by the critical gain of the relay controller. The oscillation period is determined by the dynamic characteristics of the *Process* part. Similarly, in the genetic circuit, the existence of sustained oscillation is determined by a switch-like behavior of the translation process. The oscillation period is determined by characteristics of the phosphorylation process. The investigation about the oscillation period is in good accordance with the prediction in the Goodwin oscillator, because the degradation rate of proteins is the characteristic of the phosphorylation process. Detailed conclusions will be shown in the following section. Because the preciseness and robustness of the oscillation period are more important, the amplitude of the oscillation is not discussed here.

Note that even though the switch-like behavior of the translation process is not quite similar to a nonlinear relay controller, a high Hill coefficient can still lead to a phase lag of at least π in the smooth oscillator circuit.

3. MATHEMATICAL MODEL

In model (1) (2) (3), which is derived from previous works(Gonze (2013), Hasegawa (2013))$y_1(t)$, $y_2(t)$ and $y_3(t)$

represent the concentration of the clock mRNA, the unphosphorylated clock proteins, and the phosphorylated activated clock proteins, respectively. The dynamics of $y_1(t)$, $y_2(t)$ and $y_3(t)$ are described by the following differential equations:

$$\frac{dy_1}{dt} = f_1(y_1, y_3) = \frac{r_1}{1 + (y_3/k_1)^m} - k_{1d}y_1 \quad (1)$$

$$\frac{dy_2}{dt} = f_2(y_1, y_2) = \frac{r_2 y_1^n}{k_2^n + y_1^n} - k_{2d}y_2 \quad (2)$$

$$\frac{dy_3}{dt} = f_3(y_2, y_3) = r_3 y_2 - k_{3d}y_3. \quad (3)$$

It is clear that $y_1(t) \geq 0$,$y_1(t) \geq 0$,$y_1(t) \geq 0$. Also, meanings of each model parameter are shown in Table.1. The unit nM and h represent nano-mole and hour, respectively. All values of model parameters, which are within a biologically plausible range, are derived from previous works(Ruoff (1999), Becker-Weimann (2004)).

Table 1. Model Parameters

Parameter	Value	Meaning
r_1	1 $nM{\cdot}h^{-1}$	Maximal rate of gene transcription
k_1	1 nM	Inhibition constant of gene transcription
k_{1d}	0.3 h^{-1}	Degradation rate constant of mRNA
m	3	Hill coefficient of inhibition of gene transcription
r_2	1 $nM{\cdot}h^{-1}$	Maximal rate of mRNA translation
k_2	1 nM	Activation constant of mRNA translation
k_{2d}	0.3 h^{-1}	Degradation rate constant of inactivated protein
n	8	Hill coefficient of activation of mRNA translation
r_3	1 h^{-1}	Phosphorylation rate constant of protein
k_{3d}	0.5 h^{-1}	Degradation rate constant of activated protein

In the model, the Hill functions are used in (1) and (2) to describe transcription and translation. With a relatively high Hill coefficient, the translation process in (2), showing the switch-like behavior of the translational effectors and the saturation of translational activity, has the similar feature of a relay controller.

The Michaelis-Menten kinetics are used to describe the phosphorylation process in (3). In time scale, comparing with the switch-like behavior of the mRNA translation process in (2), the clock protein phosphorylation process in (3) is a relatively slow one.

All processes are regulated by the concentration of its own products, which means all components in the genetic circuit will degrade eventually. This self-regulating mechanism is essential for exhibiting oscillatory behaviors. It will be further analyzed in the following section.

Previous work(Becker-Weimann (2004)) shows that in a negative feedback loop, high Hill coefficients, an explicit delay and Michaelis-Menten kinetics can reduce the number or reaction steps that are needed to obtain oscillation. For this purpose, the mathematical model is constructed based under relative high Hill coefficients, as these keep the number of the model parameters low.

4. PROOF FOR THE EXISTENCE OF THE OSCILLATORY BEHAVIOR

4.1 Poincare-Bendixson Criterion

The Poincare-Bendixson theorem is a useful tool to prove the existence of periodic orbits of the second-order system. Researchers(Hartley (1989)) found that this theorem could be applied to a higher order system. A detailed corollary of the theorem is presented here. The corollary(Hassan (2002)), called the Poincare-Bendixson Criterion, summarizes how the theorem is applied to prove the existence of sustained oscillatory behaviors in the system.

The corollary of Poincare-Bendixson Criterion :
Consider a dynamic system:

$$\frac{dy}{dt} = f(y). \quad (4)$$

Let M be a closed bounded subset of the space such that:

1) M contains no equilibrium points, or only one equilibrium point, such that the Jacobian matrix $[\partial f/\partial x]$ at this point has eigenvalues with positive real parts. (Hence, the equilibrium point is an unstable focus or node.)

2) Every trajectory starting in M stays in M for all future time. Then, M contains a periodic orbit of (4).

Note that since all trajectories are trapped in the subset M, they must either go to the equilibrium point of the system, or go to a limit cycle. If the subset M can be chosen so that it does not contain any equilibrium point, but all trajectories stay in it for all time, then the trajectories must go to a limit cycle. Likewise, if M contains only one equilibrium point of the system and satisfies the condition stated above, then in the vicinity of the equilibrium point all trajectories will move away from it. Thus, there must be a periodic orbit inside the subset M.

4.2 Proof for the Poincare-Bendixson Criterion

It can be obtained that

$$\frac{dy_1}{dt} = \frac{r_1}{1 + (y_3/k_1)^m} - k_{1d}y_1 < r_1 - k_{1d}y_1.$$

If $y_1 > r_1/k_{1d}$, then $dy_1/dt < 0$. Likewise, choose $y_2 > r_2/k_{2d}$, it can be obtained that $dy_2/dt < 0$.

Hence, define region $\Omega_1 = \{0 \leq y_1 < r_1/k_{1d}\}$ and $\Omega_2 = \{0 \leq y_2 < r_2/k_{2d}\}$. When $y_1 \in \Omega_1$ and $y_2 \in \Omega_2$, the following conclusion can be obtained:

If

$$y_3 > \frac{r_3 y_2}{k_{3d}} > \frac{r_2 v_3}{k_{2d}k_{3d}},$$

then

$$\frac{dy_3}{dt} = r_3 y_2 - k_{3d}y_3 < 0.$$

Define region as $\Omega_3 = \{0 \leq y_3 < \frac{r_2 r_3}{k_{2d}k_{3d}}\}$. Hence, define the subset M_1 as

$$M_1 = \{(y_1, y_2) | y_1 \in \Omega_1, y_2 \in \Omega_2\}.$$

Outside M_1, all trajectories have the feature that

$$\frac{dy_1}{dt} < 0, \frac{dy_2}{dt} < 0.$$

When $y_2 \in \Omega_2$ and $y_3 \notin \Omega_3$, all trajectories have the feature that

$$\frac{dy_3}{dt} < 0.$$

Hence, by choosing $M_2 \subset M_1$, a subset M_2 can be further defined as

$$M_2 = \{(y_1, y_2, y_3) | y_1 \in \Omega_1, y_2 \in \Omega_2, y_3 \in \Omega_3\}.$$

By choosing the subset M_2, every trajectory will be trapped inside M_2 for all future time, which satisfies the second condition of the Poincare-Bendixson Criterion.

The next step is to find the conditions for existence of unstable equilibrium points. To calculate the equilibrium point of the system, let $dy_1/dt = dy_2/dt = dy_3/dt = 0$. Simplify these into two equations:

$$y_1 = g_1(y_2) = \frac{r_1}{k_{1d}} \cdot \frac{1}{1 + (\frac{r_3}{k_1 k_{3d}} y_2)^m}$$

$$y_2 = g_2(y_1) = \frac{r_2}{k_{2d}} \cdot \frac{1}{1 + (k_2/y_2)^n}.$$

It is clear that $y_1 = g_1(y_2)$ and $y_2 = g_2(y_1)$ are monotone decreasing function and monotone increasing function, respectively. The intersection point of these two functions is the equilibrium point of the system. Obviously, inside the subset M_2, there is only one equilibrium point. Consider the unique equilibrium point as (y_1^*, y_2^*, y_3^*). Calculate the Taylor series expansion near the equilibrium point. Hence, the Jacobian matrix, $J = \frac{\partial f}{\partial y}|_{y=y^*}$, at the equilibrium point shows as follow:

$$\begin{bmatrix} \frac{\partial f_1}{\partial y_1} & \frac{\partial f_1}{\partial y_2} & \frac{\partial f_1}{\partial y_3} \\ \frac{\partial f_2}{\partial y_1} & \frac{\partial f_2}{\partial y_2} & \frac{\partial f_2}{\partial y_3} \\ \frac{\partial f_3}{\partial y_1} & \frac{\partial f_3}{\partial y_2} & \frac{\partial f_3}{\partial y_3} \end{bmatrix} = \begin{bmatrix} -k_{1d} & 0 & a \\ b & -k_{2d} & 0 \\ 0 & c & -k_{3d} \end{bmatrix}$$

where

$$\begin{cases} a = -\frac{r_1 m}{k_1} \cdot \frac{(y_3^*/k_1)^{m-1}}{[1 + (y_3^*/k_1)^m]^2} \\ b = \frac{r_2 n}{y_1^*} \cdot \frac{(k_2/y_1^*)^n}{[1 + (k_2/y_1^*)^n]^2} \\ c = r_3. \end{cases}$$

The characteristic equation of the system shows as follow:

$$\lambda^3 + (k_{1d} + k_{2d} + k_{3d})\lambda^2 + (k_{1d}k_{2d} + k_{2d}k_{3d} + k_{1d}k_{3d})\lambda + k_{1d}k_{2d}k_{3d} - abc = 0.$$

Here the Routh stability criterion is applied. If the eigenvalues have positive real part, then

$$k + abc < 0. \quad (5)$$

where

$k = (k_{1d}+k_{2d}+k_{3d})(k_{1d}k_{2d}+k_{2d}k_{3d}+k_{1d}k_{3d}) - k_{1d}k_{2d}k_{3d}$.

When the parameter k, which is related to the degradation rate constants in the model, satisfies inequation (5), the eigenvalues of the system have positive real part, and the equilibrium point is unstable. Hence, when $k + abc < 0$, the first condition of the Poincare-Bendixson Criterion is satisfied, which means the sustained oscillation exists.

Upon (5), it is clear that the degradation rate constants in all three processes play decisive roles in generating oscillation in the model. In the following section, the quantitative relation between the degradation rate constants and the existence of oscillatory behaviors will be investigated.

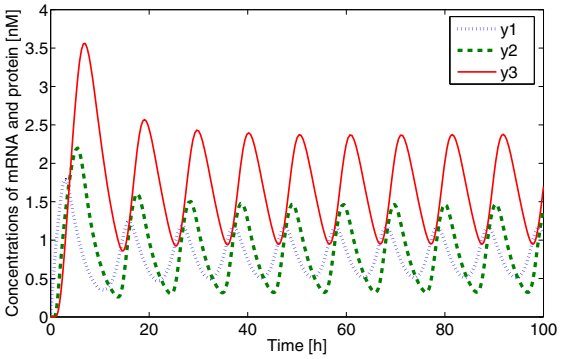

Fig. 3. The oscillatory behaviors in the system

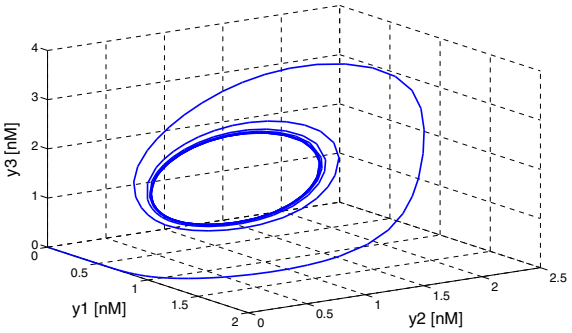

Fig. 4. The limit cycle of the system

5. SIMULATION RESULTS

Fig.3 shows the oscillatory behaviors in the model. Fig.4 shows the limit cycle of the system. Values of the model parameters are listed in Table.1. For simplicity, all parameters are given without units in the following discussions.

5.1 Importance of the degradation rates

The degradation rates of components in the system are essential for the sustained oscillatory behaviors. When (5) is satisfied, the concentration of the phosphorylated activated clock proteins can oscillate. Therefore, it is further investigated under what specific degradation rate range can the system generate sustained oscillation. Since the degradation of the activated clock proteins, y_3, is the most controllable process under experimental condition, degradation rate constant k_{3d} is chosen to be our simulation subject. Results in Fig.5 show how the value of the degradation rate constant of the phosphorylated proteins, i.e., k_{3d}, is related to oscillatory behaviors in quantity, while values of the degradation rate constants of mRNA in (1) and the inactivated proteins in (2) remain unchanged.

In Fig.5, when $k + abc < 0$, there is the sustained oscillation in the system. It is shown that $0.0410 < k_{3d} < 0.9170$ is the feasible value range of the degradation rate constant of phosphorylated proteins, which means the value of k_{3d} can neither be too small nor too large for the system exhibiting oscillatory behaviors. Simulation results in Fig.5 also indicate that the value of the degradation rate constant of phosphorylated proteins, i.e., k_{3d}, can vary in a wide range, while the system can still generate the

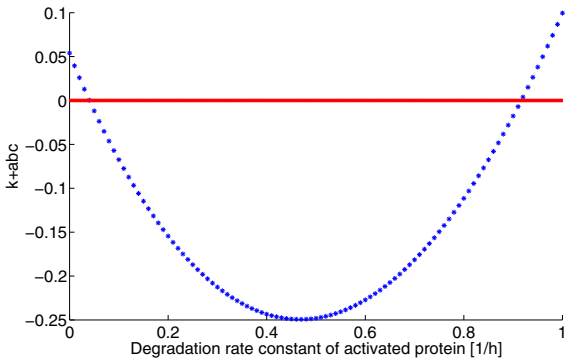

Fig. 5. The value range of the activated proteins degradation rate constant

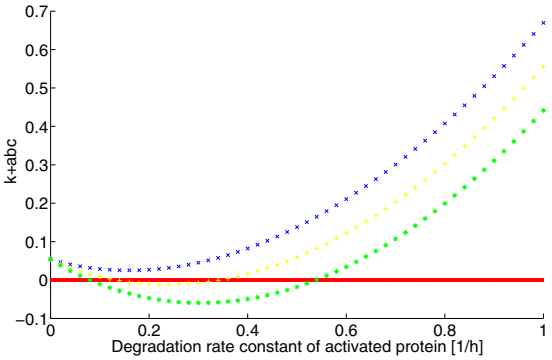

Fig. 6. The feasible value range of the degradation rate constant with different Hill coefficient n

sustained oscillation. This unique feature leads us to the conclusion that there is strong robustness in the system. The oscillatory behaviors tend to be stable against the disturbance of the degradation rate change.

5.2 Importance of the switch-like behavior in translation

The degradation rate constant is not the only factor that influences the existence of oscillatory behaviors. It is also determined by the rule that rates of gene expression in (1) and mRNA translation in (2) are substantially faster than the protein phosphorylation and activation process in (3). This rule indicates the necessity of the switch-like behavior of the transcriptional/translational effectors and the saturation of transcriptional/translational activity.

It can be inferred that high similarity similarity between the real behavior of translation process and the ideal switch-like behavior, is an important factor for the existence of oscillation. According to the feature of Hill function, the higher value of Hill coefficient n, indicates the higher similarity between the real translation process and ideal process with switch-like behaviors.

In Fig.6, the green, yellow and blue curve represent different conditions when $n = 3, 4, 5$, respectively. When Hill coefficient n in (2) decreases, the value range of the degradation rate constant, under which sustained oscillation exists, narrows down. Upon Poincare-Bendixson Criterion, with a low value of parameter n, the equilibrium point of the system only have positive real part, which means

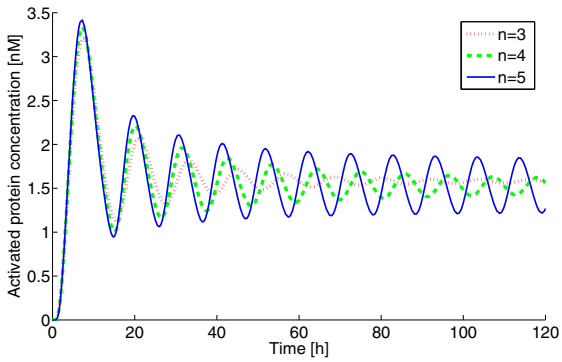

Fig. 7. The oscillation behavior with different Hill coefficient n

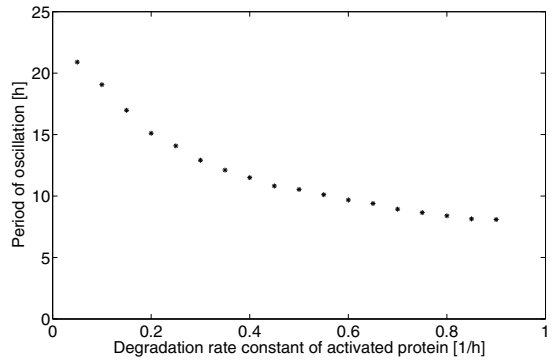

Fig. 8. The oscillation period change over the value of the degradation rate constant of activated proteins

the equilibrium point of the system is stable. Hence, the system output tends to be stable, not oscillatory.

Upon the results in Fig.7, it can be concluded that only when the Hill coefficient n is large enough, can the sustained oscillation exists in the model. Hence, with a relatively low value of the Hill coefficient n in the mRNA translation process, the genetic circuit tends to be stable without oscillatory behaviors.

5.3 Factors for the change of the oscillation period

As mentioned above, in the relay control circuit, the characteristics of the *Process* part play a decisive role in determining the oscillation period. The degradation rate constant of activated proteins, i.e., k_{3d}, which is the characteristic of the phosphorylation process in (3), can determine the oscillation period. In order to observe the oscillation period length of activated protein concentration through simulation, the value of k_{3d} is changed steadily.

Upon the results in Fig.8, when the degradation rate constant of the phosphorylated proteins, i.e., k_{3d}, increases from 0.05 to 0.90, the oscillation period gradually decreases from 20.90 h to 8.09 h. This result is in good accordance with previous theoretical analysis. Simulation results related to the other two degradation rates are not given here, because they share the same feature with the results in Fig.8. Moreover, with three degradation rate constants regulating the oscillation period, the oscillatory behaviors in the system are strongly robust in terms of both oscillation existence and oscillation period. It is

further concluded that the oscillation period of the system is determined mainly by the degradation rate constants, not other parameters in the processes of the model. This conclusion indicates that the self-regulating mechanism of all components plays significant role in the genetic circuit.

6. CONCLUSION

An analyze method is used to investigate the generating mechanism of oscillation in the smooth oscillators. The Poincare-Bendixson theorem is used to prove the existence of oscillation in the mathematical model of smooth oscillators. To the best of our knowledge, the analyze method in this paper, i.e., comparing the structure and mechanism of the biological smooth oscillators with the circuit in the relay control tuning method, is used to investigate smooth oscillators for the first time.

The conclusion is drawn that the self-regulating mechanism is the determinant factor for the existence of oscillation. When the value of the degradation rate constants are under certain range, the system can generate oscillation. Simulation results indicate that the length of the oscillation period is closely related to the value of the degradation rate constants. It is also shown that the oscillation period is robust against parameter value change.

Another conclusion is that combining fast gene transcription process and mRNA translation process with a relatively slow protein phosphorylation process is the core mechanism in the model. In the translation process, high value of the Hill coefficient n, can lead to robust oscillation, while the system with a small-value parameter n tends to be stable without oscillatory behaviors.

The specific structure of the model, taking into account mainly the essential processes, should make it a valuable tool for further experimental studies and better understanding of the mammal circadian clock. It is also shown that control theories can provide efficient ways to investigate biological oscillators.

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