CIRCADIAN RHYTHM AS A PHASE-LOCKED LOOP

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Abstract: The circadian rhythm is a sort of biological clock that works in the cell level synchronized with a day rhythm of light condition. It is ubiquitous in living organisms. This paper tries to establish an analogy of circadian rhythm with the Phase Locked Loop (PLL) which is widely used in various fields of engineering. Though the analogy is not complete, the PLL framework is shown to give a nice new paradigm to discuss the intrinsic structure of the circadian rhythm.

Keywords: Biocontrol, Biocybernetics, Phase-locked loop, Oscillation, Synchronization

1. INTRODUCTION

Most living organisms, from bacteria to insects, plants and mammals, have developed the capability of generating endogenous oscillations with a period close to 24h to adjust their life rhythms to light/dark cycle of the earth. These oscillations are called *circadian rhythms*.

The endogenous oscillations which occur in the constant environment (e.g. complete darkness) can synchronize with the daily change of environment and oscillate with the period of precisely 24 hours.

Experimental studies during the last decade have disclosed the molecular mechanisms of circadian rhythms, initially in *Drosophila* (Goldbeter A, 1995; Hardin P.E. et al., 1990; Leloup J.C. and Goldbeter A., 1998) and *Neurospora* (Liu Y. et al., 1987), and then to plants and mammals (Dunlap J.C., 1999; Shearman L.P. et al., 2000). In these studies, the intrinsic mechanism of the oscillations is ascribed to the autoregulation through negative feedback exerted by a protein on the expression of its gene. This is a simple feedback loop embodied by a protein life cycle. It contains a significant time delay before produced protein can give influence on its own expression rate. It is a well-known fact that a negative feedback loop generates oscillations if the delay in the feedback path is significant. From control theoretical point of view, this sort of oscillations is popular, but it does not explain the entrainment to the outer signals, since the period of oscillation is solely determined by the delay time which does not depend on the environmental change.

A well-known synchronization mechanism which is extensively used in engineering to generate oscillations with precise frequency is the *phase locked loop* (PLL). The PLL has some similar characteristic features to circadian rhythm. It contains a mechanism of generating autonomous oscillations just like circadian rhythm's endogenous oscillation with a period close to 24h. The PLL can be entrained by the periodic inputs which is the most

salient characteristic feature of the circadian rhythm. It is natural to exploit some analogies between PLL and circadian rhythm in the hope that the circadian rhythm can be translated to the framework of PLL.
This paper exploits an analogy between circadian rhythm and PLL in the hope that it may give a new insight to the intrinsic mechanisms of circadian rhythm.

2. A SIMPLE MODEL OF THE CIRCADIAN RHYTHM OF *DROSOPHILA*

Based on the accumulation of experimental data concerning the circadian rhythm of *Drosophila*, Goldbetter proposed a quantitative model of its molecular mechanism (Goldbeter A., 1995). According to his model, the circadian rhythm is represented by a protein *PER* which forms a negative feedback loop. The produced *PER* after forming dimmers through phosphorylation enters the nucleus and inhibits its own transcription, as is shown in the diagram of Figure 1.

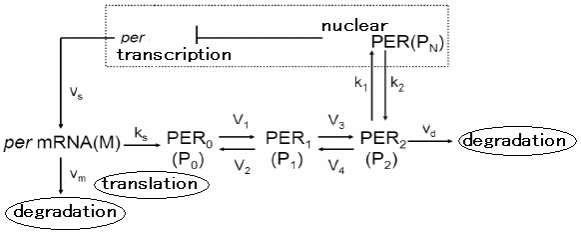

Fig.1 Molecular Mechanism of Drosophila Circadian

In Figure 1, the phosphorylation is considered to occur twice (PER_0 to PER_1 to PER_2) for simplicity, but actually it occurs more than twice.
The mathematical model of the chemical process of Figure 1 is derived based on the enzyme kinetics of Michaelis-Menten-Hill. It is described by the four states dynamics as follows:

$$\frac{dM}{dt} = \upsilon_s \frac{K_I^n}{K_I^n + P_N^n} - \upsilon_m \frac{M}{K_m + M} \tag{1a}$$

$$\frac{dP_0}{dt} = k_s M - V_1 \frac{P_0}{K_1 + P_0} + V_2 \frac{P_1}{K_2 + P_1} \tag{1b}$$

$$\frac{dP_1}{dt} = V_1 \frac{P_0}{K_1 + P_0} - V_2 \frac{P_1}{K_2 + P_1} - V_3 \frac{P_1}{K_3 + P_1} + V_4 \frac{P_2}{K_4 + P_2} \tag{1c}$$

$$\frac{dP_2}{dt} = V_3 \frac{P_1}{K_3 + P_1} - V_4 \frac{P_2}{K_4 + P_2} - k_1 P_2 + k_2 P_N - \upsilon_d \frac{P_2}{K_d + P_2} \tag{1d}$$

$$\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N \tag{1e}$$

The glossary of the symbols is given in Table 1.

M	Concentration of messenger RNA for *per*
P_0	Concentration of expressed PER
P_1	Concentration of singly phosphorylated PER
P_2	Concentration of doubly phosphorylated PER
P_N	Concentration of phosphorylated PER in the nucleus
U_m	Rate of transcription of PER
U_s	Rate of inhibition
K_s	Rate of translation of PER
$V_1 \sim V_4$	maximum rates of phosphorylations
$K_1 \sim K_4$	Michaelis constants
n	Hill coefficient

Table 1. Glossary of Symbols

The model (1) involves only one protein, *PER*. After the model (1) was proposed, several proteins have been identified to be responsible for the circadian rhythm of *Drosophila*. The model has been evolved gradually as a new protein participating is discovered. The second protein which is responsible for the *Drosophila* circadian rhythm is *TIM*. It was experimentally shown by Hardin et. al. (Hardin P.E. et al., 1990) that *PER* cannot enter the nucleus, unless it forms a complex with *TIM*. It was also shown that *TIM* is very sensitive to light. Leloup et. al. (Leloup J.C. and Goldbeter A., 1998) proposed a model of *Drosophila* circadian rhythm involving both *PER* and *TIM* (Fig.2). In their model, *PER* and *TIM* mutually inhibit their transcriptions inside the nucleus.

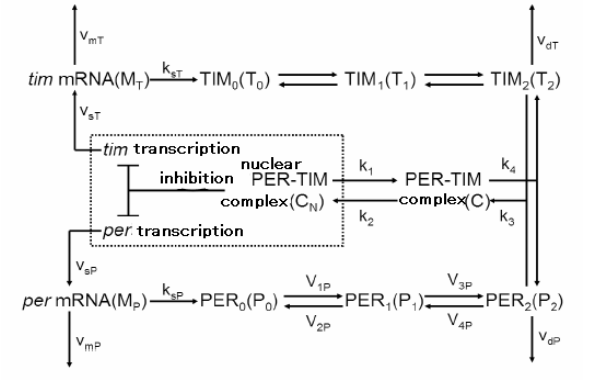

Fig.2 PER/TIM Model of Drosophila Circadian Rhythm

The mathematical description of the *PER-TIM* model is rather complex and is given as follows:

$$\frac{dM_P}{dt} = \upsilon_{sP}\frac{K_{IP}^n}{K_{IP}^n + C_N^n} - \upsilon_{mP}\frac{M_P}{K_{mP}+M_P} - k_d M_P \tag{2a}$$

$$\frac{dP_0}{dt} = k_{sP}M_P - V_{1P}\frac{P_0}{K_{1P}+P_0} + V_{2P}\frac{P_1}{K_{2P}+P_1} - k_d P_0 \tag{2b}$$

$$\frac{dP_1}{dt} = V_{1P}\frac{P_0}{K_{1P}+P_0} - V_{2P}\frac{P_1}{K_{2P}+P_1} - V_{3P}\frac{P_1}{K_{3P}+P_1} + V_{4P}\frac{P_2}{K_{4P}+P_2} - k_d P_1 \tag{2c}$$

$$\frac{dP_2}{dt} = V_{3P}\frac{P_1}{K_{3P}+P_1} - V_{4P}\frac{P_2}{K_{4P}+P_2} - k_3 P_2 T_2 + k_4 C - \upsilon_{dP}\frac{P_2}{K_{dP}+P_2} - k_d P_2 \tag{2d}$$

$$\frac{dM_T}{dt} = \upsilon_{sT}\frac{K_{IT}^n}{K_{IT}^n + C_N^n} - \upsilon_{mT}\frac{M_T}{K_{mT}+M_T} - k_d M_T \tag{2e}$$

$$\frac{dT_0}{dt} = k_{sT}M_T - V_{1T}\frac{T_0}{K_{1T}+T_0} + V_{2T}\frac{T_1}{K_{2T}+T_1} - k_d T_0 \tag{2f}$$

$$\frac{dT_1}{dt} = V_{1T}\frac{T_0}{K_{1T}+T_0} - V_{2T}\frac{T_1}{K_{2T}+T_1} - V_{3T}\frac{T_1}{K_{3T}+T_1} + V_{4T}\frac{T_2}{K_{4T}+T_2} - k_d T_1 \tag{2g}$$

$$\frac{dT_2}{dt} = V_{3T}\frac{T_1}{K_{3T}+T_1} - V_{4T}\frac{T_2}{K_{4T}+T_2} - k_3 P_2 T_2 + k_4 C - \upsilon_{dT}\frac{T_2}{K_{dT}+T_2} - k_d T_2 \tag{2h}$$

$$\frac{dC}{dt} = k_3 T_2 T_2 - k_4 C - k_1 C + k_2 C_N - k_{dc} C \tag{2i}$$

$$\frac{dC_N}{dt} = k_1 C - k_2 C_N - k_{dN} C_N \tag{2j}$$

In the above model, *PER* and *TIM* follow a similar form as described in model (1) except that the effect of degradation is introduced. The *PER/TIM* complex is now introduced. C denotes the concentration of the complex outside the nucleus, while C_N denotes the concentration of the complex inside the nucleus. The two proteins interact each other through the formation of the complex. It should be noted that the concentrations of *TIM* directly influence dynamics of *PER* through the third term in (2d).

3. PRINCIPLE OF PLL

The PLL is a feedback control systems that tracks the phase of input signal. The basic principle of the PLL utilizes the fact that the phase is the integral of the angular velocity. Thus, the PLL is essentially an integral action which guarantees the robustness of the tracking performance.

The usual PLL is composed of the three main parts:

(1) Phase Detector,

(2) Low-Pass Filter,

(3) Voltage Controlled Oscillator (VCO).

The basic architecture of the PLL is shown in Figure 3.

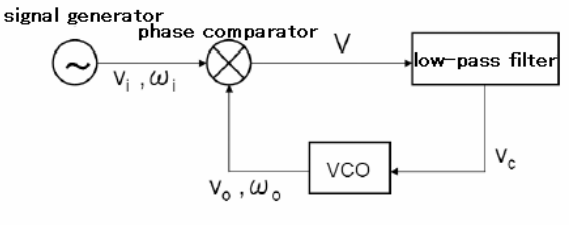

Fig.3 Basic Architecture of PLL

PLL generates a spontaneous oscillation of a fixed frequency even if the input is zero or constant. This corresponds to the *free-run* oscillation in the circadian rhythm. In PLL, all the oscillations are regarded as an entrainment of this basic oscillation to the input signal.

Let us briefly describe a mechanism of PLL. Denote by ω_0 the frequency of the basic oscillation. Assume that the input signal $\upsilon_i(t)$ and the output signal $\upsilon_0(t)$ are given as follows:

$$\upsilon_i(t) = \sqrt{2}\sin(\omega_0 t + \theta_i(t)), \tag{3a}$$

$$\upsilon_0(t) = \sqrt{2}\cos(\omega_0 t + \theta_0(t)), \tag{3b}$$

where $\theta_i(t)$ and $\theta_0(t)$ denotes the deviated phase functions of the input and output from the basic phase $\omega_0 t$, respectively. The amplitudes of the signals are of no importance here, and chosen to be $\sqrt{2}$ for notational convenience. The output of the phase comparator which is actually the product of $\upsilon_i(t)$ and $\upsilon_0(t)$, is calculated to be

$$\upsilon_i(t)\cdot\upsilon_0(t)=\sin(\theta_i(t)-\theta_0(t))+\sin(2\omega_0 t+\theta_i(t)+\theta_0(t)). \quad (4)$$

The second term is eliminated through low-pass filter and only the first term is the input to the VCO, where the output frequency of the VCO is proportional to the input voltage. Thus, the behaviors of the VCO is denoted by

$$\frac{d\theta_0(t)}{dt}=K\sin(\theta_i(t)-\theta_0(t)), \quad (5)$$

where K denotes the gain of the VCO. If the input frequency is $\omega_0+\omega_i$, then the above equation becomes equal to

$$\frac{de(t)}{dt}=-K\sin e(t)+\omega_i \quad (6)$$

where $e(t)$ denotes the phase difference $\theta_i(t)-\theta_0(t)$. If $\omega_i \le K$, there exists a stable equilibrium point of (6), where $de(t)/dt$ tends to 0. This implies that the output phase $d\theta_0(t)/dt$ approaches to ω_i.

4. CIRCADIAN RHYTHM AS A PLL

Now, we consider the circadian rhythm in the framework of PLL. For that purpose, we take the *PER/TIM* model where *TIM* is regarded as an independent outer signal representing light/dark alterations. For simplicity, we omit all phosphorylations assuming that *PER* and *TIM* can form a complex without phosphorylation. The diagram of this model is given in Figure 4.

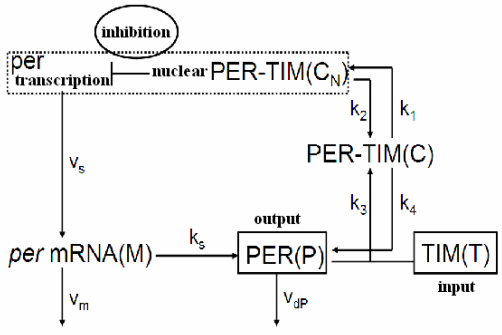

Fig.4 Model of Circadian Rhythm for Analogy with PLL

Mathematical description of the model is given as follows:

$$\frac{dM}{dt}=\upsilon_s\frac{K_I^n}{K_I^n+C_N^n}-\upsilon_m\frac{M}{K_m+M}-k_dM \quad (7a)$$

$$\frac{dP}{dt}=k_sM-k_3P\cdot TIM+k_4C-\upsilon_d\frac{P}{K_d+P}-k_dP \quad (7b)$$

$$\frac{dC}{dt}=k_3P\cdot TIM-k_4C+k_2C_N-k_{dC}C \quad (7c)$$

$$\frac{dC_N}{dt}=k_1C-k_2C_N-k_{dN}C_N \quad (7d)$$

Here, M denotes the concentration of the m-RNA for *PER* transcription, P the concentration of *PER*, C and C_N denotes the concentration of *PER/TIM* complexes outside and inside the nucleus, respectively. *TIM*, the concentration of *TIM*, is written explicitly to represent it as an independent signal. Note that *TIM* appears twice in this model but always as the product with P. We divide the system (7) into the two subsystems.

The system I is composed of the equations (7a)(7c)(7d) with the input P・TIM, and $F=k_sM-k_3P\cdot TIM+k_4C$ as an output, i.e.,

$$\frac{dM}{dt}=\upsilon_s\frac{K_I^n}{K_I^n+C_N^n}-\upsilon_m\frac{M}{K_m+M}-k_dM \quad (8a)$$

$$\frac{dC}{dt}=k_3P\cdot TIM-k_4C+k_2C_N-k_{dC}C \quad (8b)$$

$$\frac{dC_N}{dt}=k_1C-k_2C_N-k_{dN}C_N \quad (8c)$$

while the system II represents the dynamics of P, i.e.,

$$\frac{dP}{dt}=F-\upsilon_d\frac{P}{K_d+P}-k_dP \quad (9)$$

The analogy with Figure 2 is established, if we identify system I as a low pass filter and system II VCO.

Since the system I is nonlinear, it cannot be regarded as a usual filter because sinusoidal input may not generate pure sinusoidal output. However, we can pick up the amplitude of the main lobe of the output and approximately evaluate its filter characteristics. The model (8) (9) is represented in the block diagram of Figure 5. The parameter values are taken from (Gonze D. et al., 2003) as

$$\upsilon_s = 1.0nMh^{-1}, \upsilon_m = 0.7nMh^{-1},$$
$$k_d = 0.01nMh^{-1}, k_s = 0.9h^{-1},$$
$$k_3 = 1.2nM^{-1}h^{-1}, k_4 = 0.6h^{-1},$$
$$\upsilon_d = 2.0nMh^{-1}, k_2 = 0.2h^{-1},$$
$$k_{dC} = 0.01nM^{-1}, k_{dN} = 0.01nMh^{-1},$$

It is confirmed through simulation that the model (5) has satisfactory characteristics as a model of circadian rhythm, i.e., free-run oscillation of approximately 24h, and capability of entrainment with various waveforms of *TIM* with appropriate selections of kinetic parameters, as is shown in Figures 6 and 7.

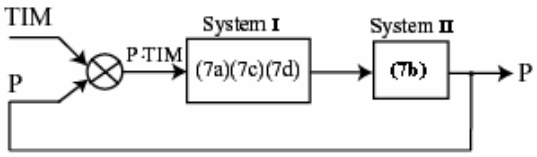

Fig. 5 Block Diagram of Model (7)

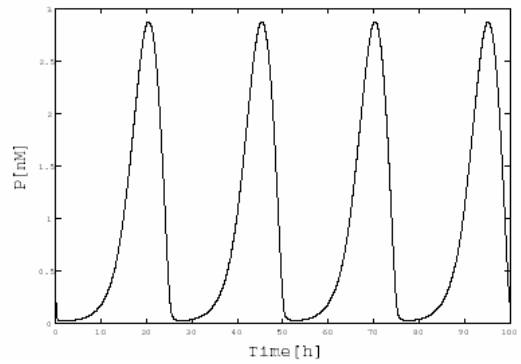

Fig. 6 Free-run Oscillation of PER

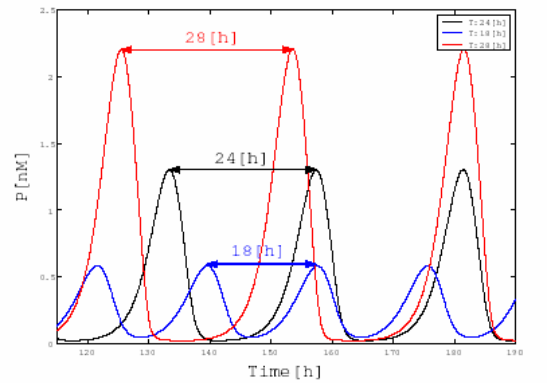

Fig. 7 Entrainments of Model (7) for Signals with Various Periods

Figure 8 shows the "frequency response" of the system I. Very sharp low pass characteristics is observed. As a comparison, we show the frequency response of the linear part of system I (labeled C_N), where C and C_N are chosen as states. It is remarkable that addition of nonlinear part M enhances the low pass characteristics greatly.

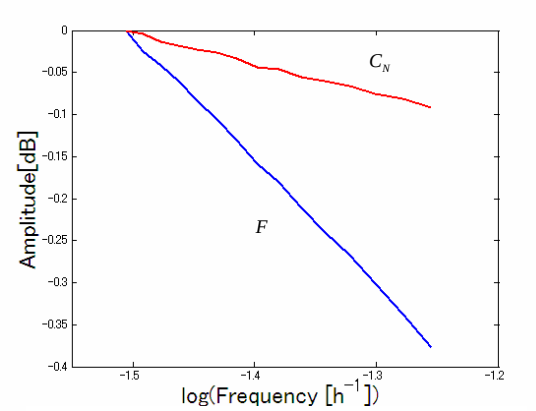

Fig. 8 Low Pass Characteristic of System

As for the system II, we neglect the second and the third terms and regard it as a simple integrator. If the input to the integrator is $Ae^{j\theta(t)}$, then the output frequency $\dot{\theta}(t)$ is proportional to A. This can be regarded as a VCO whose output frequency is proportional to the input voltage, not proportional to the instantaneous strength but proportional to the amplitude of the input. The analogy of VCO with integrator is justified if we consider the average effect of input to the output frequency. With this interpretation, the analogy between the circadian rhythm and the PLL has now been established satisfactory.

The model (7) does not involve phosphorylation which is known to play an essential role in circadian rhythm. We can establish similar analogy in more complicated models involving several steps of phosphorylation, by taking phosphorylation as a part of low pass filter. The speed of entrainment is almost the same in these more complicated models.

It is not clear for the moment that whether our analogy gives a new *biological* insight into the basic mechanism of circadian rhythm. We cannot say any clear anticipation whether PLL analogy can be extended to more complicated circadian rhythm models like an interlocked model of two network (Ueda H.R. et al., 2001). However, our analogy gives a more comprehensive and correct view of the mechanism of the circadian rhythm than delayed feedback oscillation which has been the main paradigm of the circadian rhythm.

5. CONCLUSION

The circadian rhythm of the biological organisms has many features in common with the PLL (Phase-Lock Loop) which is widely used in engineering for generating oscillations with precise frequency. The paper tries to establish some analogies between the circadian rhythm and PLL for the purpose of understanding some intrinsic mechanism of circadian rhythms.

It has been shown that, corresponding to each part of the PLL, namely, the phase comparator, the low pass filter and the voltage controlled oscillator, there exists a counterpart in the circadian rhythm. The framework of PLL is shown to give a good paradigm to discuss intrinsic mechanism of the circadian rhythm.

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