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THEORETICAL INVESTIGATION ON MODELS OF CIRCADIAN RHYTHMS BASED ON DIMERIZATION AND PROTEOLYSIS OF PER AND TIM

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Abstract. Circadian rhythms of physiology and behavior are widespread mechanisms in many organisms. The internal biological rhythms are driven by molecular clocks, which oscillate with a period nearly but not exactly 24 hours. Many classic models of circadian rhythms are based on a time-delayed negative feedback, suggested by the protein products inhibiting transcription of their own genes. In 1999, based on stabilization of PER upon dimerization, Tyson et al. [J. J. Tyson, C. I. Hong, C. D. Thron, B. Novak, Biophys. J. 77 (1999) 2411–2417] proposed a crucial positive feedback to the circadian oscillator. This idea was mathematically expressed in a three-dimensional model. By imposing assumptions that the dimerization reactions were fast and dimeric proteins were in rapid equilibrium, they reduced the model to a pair of nonlinear ordinary differential equations of mRNA and total protein concentrations. Then they used phase plane analysis tools to investigate circadian rhythms. In this paper, the original three-dimensional model is studied. We explore the existence of oscillations and their periods. Much attention is paid to investigate how the periods depend on model parameters. The numerical simulations are in good agreement with their reduced work.

1. **Introduction.** Wide-type fruit flies, *Drosophila melanogaster*, might be the most extensively studied organism in circadian rhythm research. The researches of endogenous activity rhythm on *Drosophila* generally involve two different kinds of *clock genes*, called *period* (*per*, for short) [14, 10] and *timeless* (*tim*, for short) [20, 27]. Their encoded proteins, PER and TIM, bind to each other [5, 20, 27, 29].

PER protein and *per* mRNA cycle in a 24-hour period [7]. When PER protein is at a high level, *per* mRNA expression is repressed, suggesting that PER is an inhibitor of *per* mRNA accumulation [7]. The expression of *per* and *tim* genes is

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regulated by dCLOCK and CYC, and PER inhibits the transcription of *per* and *tim* by inactivating dCLOCK and CYC [1, 3, 18]. This negative feedback, introduced by PER inhibiting its own mRNA transcription, is the basis of many classic theoretical models of circadian rhythms [6, 17, 15, 19].

An alternative way to study circadian rhythms is based on a positive feedback, introduced by PER phosphorylation being an activator to PER [26]. Phosphorylation of PER is operated by a *double-time* gene encoded kinase, DOUBLE-TIME (DBT, for short) [13, 16]. As suggested by the *dbt* mutants phenotypes, PER phosphorylation might be precluded to its degradation. PER and TIM stimulate transcription of *per* and *tim* genes by activating dClOCK [2]. Experimental results suggest that *per* mRNA is stabilized by PER/TIM dimers [24], and PER is stabilized by dimerization with TIM [13, 16].

The idea that PER phosphorylation introduces a positive feedback in PER accumulation can be expressed in a model of three-dimensional ordinary differential equations [26] (see (1) below). In [26], by imposing assumptions that the dimerization reactions were fast and dimeric proteins were in rapid equilibrium, they reduced the three-dimensional model to a pair of nonlinear ordinary differential equations of mRNA and total protein concentrations (see (2) below). Then they used the powerful phase plane portraiture to study the simplified two-dimensional model. In this paper, we explore the original three-dimensional model directly. It is shown that the circadian rhythms occur if the model possesses a unique equilibrium which is unstable. Furthermore, we deeply investigate how circadian rhythms are affected by several model parameters, including mRNA translation, mRNA degradation, monomer phosphorylation, protein proteolysis, association of PER/TIM protein and equilibrium constant for dimerization. The results help to explain some former-observed phenomena of circadian rhythms. In particular, our numerical results extremely agree with those given in [26], indicating that their reduction work is greatly reasonable.

2. **Mechanism and mathematical model.** In this section, we restate the model proposed by Tyson et al. [26]. The molecular mechanism for the circadian rhythm in *Drosophila* is summarized in Figure 1. Here the total PER (monomer + dimer) degradation rate does not increase proportionally with the total PER concentration's increasing.

The mechanism in Figure 1 could be translated into a set of six differential equations, for *per* and *tim* mRNAs, PER and TIM monomers, and PER/TIM dimers in the cytoplasm and nucleus. Such a complicated set of equations could not efficiently illustrate the importance of positive feedback in the reaction mechanism. So by noticing that PER and TIM messages and proteins followed roughly similar time courses in vivo, Tyson et al. [26] lumped them into a single pool of clock proteins. In addition, they assumed that the cytoplasmic and nuclear pools of dimeric protein were in rapid equilibrium. Then they established the following differential equations for [mRNA]=M, [monomer]=P_1, and [dimer]=P_2:

$$\begin{cases} \dfrac{\mathrm{d}M}{\mathrm{d}t} &= \dfrac{v_m}{1+(P_2/P_{crit})^2} - k_m M, \\ \dfrac{\mathrm{d}P_1}{\mathrm{d}t} &= v_p M - \dfrac{k_{p_1}^{'} P_1}{J_P + P_1 + rP_2} - k_{p_3} P_1 - 2k_a P_1^2 + 2k_d P_2, \\ \dfrac{\mathrm{d}P_2}{\mathrm{d}t} &= k_a P_1^2 - k_d P_2 - \dfrac{k_{p_2} P_2}{J_P + P_1 + rP_2} - k_{p_3} P_2. \end{cases} \tag{1}$$

Here monomer was assumed to be phosphorylated more quickly than dimer, i.e., $k_{p_1}^{'} \gg k_{p_2}$. The parameter r determined the inhibition of dimer to monomer

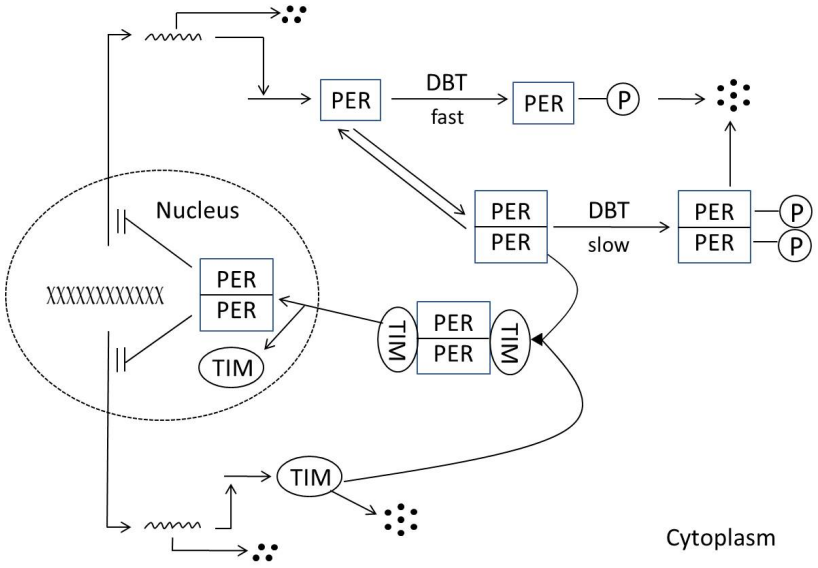

FIGURE 1. A simple molecular mechanism for the circadian clock in Drosophila. Redrawn from [26]. PER and TIM proteins are synthesized in the cytoplasm, where they may be destroyed by proteolysis or they may combine to form relatively stable heterodimers. Heteromeric complexes are transported into the nucleus, where they inhibit transcription of *per* and *tim* mRNA. Here it is assumed that PER monomers are rapidly phosphorylated by DBT and then degraded. Dimers are assumed to be poorer substrates for DBT.

phosphorylation. For convenience, in this paper we follow them by taking $r = 2$.

In their work, it was further assumed that the dimerization reactions were fast (k_a and k_d are large) such that monomers and dimers were always in equilibrium with each other. Then, by equilibrium conditions: $P_2 = K_{eq}P_1^2$, $K_{eq} = k_a/k_d$, they obtained the reduced two-dimensional system:

$$\begin{cases} \dfrac{\mathrm{d}M}{\mathrm{d}t} &= \dfrac{v_m}{1+(P_t(1-q)/(2P_{crit}))^2} - k_m M, \\ \dfrac{\mathrm{d}P_t}{\mathrm{d}t} &= v_p M - \dfrac{k_{p_1}P_t q + k_{p_2}P_t}{J_P + P_t} - k_{p_3}P_t, \end{cases} \tag{2}$$

where $P_t = P_1 + 2P_2 = [\,\text{total protein}\,]$, $k_{p_1} = k_{p_1}^{'} - k_{p_2} \approx k_{p_1}^{'}$, and

$$q = q(P_t) = \frac{2}{1+\sqrt{1+8K_{eq}P_t}}.$$

Two widely concerned points of circadian rhythms are whether the endogenous rhythms exist and how long the periods are. Since the mechanism has already been translated into mathematical models, attentions are drawn to examine the existence of periodic orbits and calculate the periods. In their work, system (2) has been thoroughly analyzed. In this paper, we try to study system (1). A typical oscillating solution of system (1) is illustrated in Figure 2, where the corresponding parameter values are chosen from Table 1.

TABLE 1. Parameter values suitable for circadian rhythm of wild-type fruit flies

Name	Value	Units	E_a/RT	Description
v_m	1	$\frac{\mathrm{C_m}}{\mathrm{h}}$	6	Maximum rate of synthesis of mRNA
k_m	0.1	h^{-1}	4	First-order rate constant for mRNA degradation
v_p	0.5	$\frac{\mathrm{C_p}}{\mathrm{C_m h}}$	6	Rate constant for translation of mRNA
k_{p_1}	10	$\frac{\mathrm{C_p}}{\mathrm{h}}$	6	V_{max} for monomer phosphorylation
k_{p_2}	0.03	$\frac{\mathrm{C_p}}{\mathrm{h}}$	6	V_{max} for dimer phosphorylation
k_{p_3}	0.1	h^{-1}	6	First-order rate constant for proteolysis
K_{eq}	200	$\mathrm{C_p}^{-1}$	-12	Equilibrium constant for dimerization
P_{crit}	0.1	$\mathrm{C_p}$	6	Dimer concen at the half-maximum transcription rate
J_P	0.05	C_p	-16	Michaelis constant for protein kinase (DBT)

This table is adapted from Tyson et al. [26]. Parameters $\mathrm{C_m}$ and $\mathrm{C_p}$ represent characteristic concentrations for mRNA and protein, respectively. E_a is the activation energy of each rate constant (necessarily positive) or the standard enthalpy change for each equilibrium binding constant (may be positive or negative). The parameter values are chosen to ensure temperature compensation of the wild-type oscillator.

3. **Method and result.** It is well-known that for higher dimensional ordinary differential equations, there is no so-called Poincaré-Bendixson theory: any limit set is a limit cycle if it contains no steady state. So, in order to use the powerful phase plane analysis tools, Tyson et al. [26] reduced (1) into (2) by imposing some assumptions. Fortunately, we observe that (1) is a three-dimensional competitive system in some sense [8, 9, 22, 23]. For n-dimensional competitive ordinary differential equations, the dynamics is co-dimensional one. Every limit set lies on a Lipschitz manifold with one dimension lower, and this manifold is homeomorphic to an $(n-1)$-dimensional Euclidean space [28]. As for a three-dimensional competitive system, though there is no phase plane, one has a two-dimensional Lipschitz manifold (qualitatively exists but is unknown), where recurrent motions of system lie in. As a result, any limit set for such a system consists of either limit cycle, or steady state, or steady states connected with homoclinic or heteroclinic orbits. Suppose that all forward orbits for a three-dimensional competitive system are bounded and the system has a unique steady state. Then by the Perron-Frobenius theory, the linearized matrix at the steady state has a negative eigenvalue. The system has a one-dimensional stable manifold which is a strictly monotone curve [21], so it also rules out the third choice for limit set. For more details, please see [23] or the Appendix. We summarize the above discussion into the following theorem which can be found in [30]:

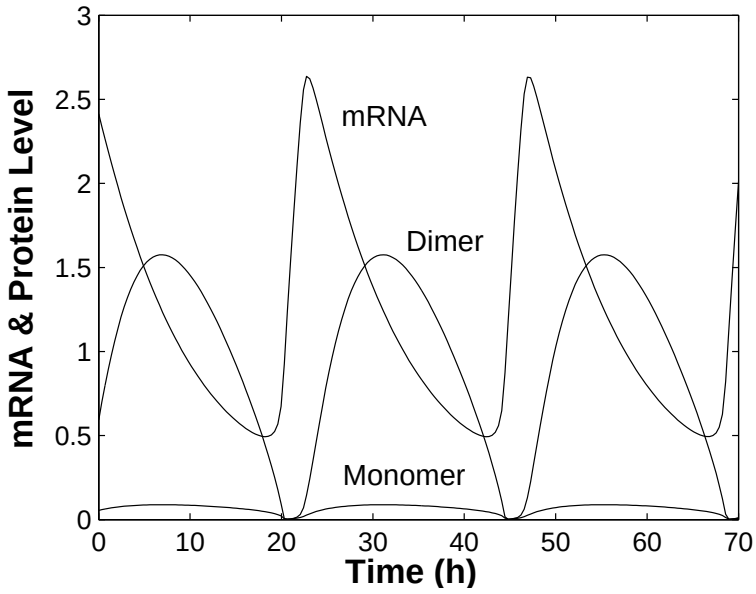

FIGURE 2. Numerical solution of (1). Parameter values are chosen as in Table 1. We take $k_a = 10^6$ and $k_d = k_a/K_{eq}$.

Theorem 3.1. *Suppose* (1) *has a unique steady state* E. *If the linearized matrix of* (1) *at* E *has one negative eigenvalue and two positive real part eigenvalues, then* (1) *has at least one stable limit cycle.*

Numerical calculation suggests that (1) has a unique equilibrium in a large region of parameter values. However, limit cycles do not exist all the time. According to Theorem 3.1, when either K_{eq} or k_a is small, (1) has no limit cycles but a unique equilibrium (see Table 2), and the equilibrium appears to be a global stable steady state.

Comparing with the two-dimensional system (2), there are two more parameters k_a and k_d in system (1). Considering the equilibrium condition, $K_{eq} = k_a/k_d$, one only needs to detect how periods of (1) are influenced by k_a. As shown in Table 3 and Figure 3E, if we take $K_{eq} = 200$, periodic orbits occur when k_a is larger than a critical value $k_a^* = 0.9$. In that region, as k_a goes up, the period starts with a rapid decline, and then becomes quite insensitive. At first, we guess that the period is decreasing when k_a is sufficiently large, but numerical calculations tell that it is not the case. In fact, the period even has a tendency to increase when k_a is larger than 2.9×10^6 (see Table 3). The similar situations are observed with $K_{eq} = 15$ (see Table 4).

Based on Tables 3 and 4, one can choose a suitable value of k_a to calculate the rhythms for wild-type and mutant flies. The numerical results are presented in Table 5, where temperature compensation is found in wild-type flies but not in per^L mutant flies.

Table 5 is due to the original three-dimensional system (1). As a comparison, we state Table 6, which is cited from [26] and based on the reduced two-dimensional system (2). Clearly, one can see that Table 5 and Table 6 are almost the same, which indicates that the reduction in [26] is greatly reasonable from this perspective.

TABLE 2. Equilibrium of (1) and corresponding eigenvalues of its Jacobian matrix vary with K_{eq} and k_a.

K_{eq}	k_a	Equilibrium[1]	Eigenvalues
200	10^{-6}	$(10.00, 0.05, 0)$	$\{-50.20, -0.40, -0.1\}$
	10^{-3}	$(10.00, 0.05, 6 \times 10^{-6})$	$\{-50.19, -0.40, -0.1\}$
	1	$(8.62, 0.10, 0.04)$	$\{-25.96, 0.01 \pm 0.11\mathrm{i}\}$
	10^3	$(1.38, 0.04, 0.24)$	$\{-164.97, 0.11 \pm 0.41\mathrm{i}\}$
	10^6	$(1.36, 0.04, 0.25)$	$\{-1.47 \times 10^5, 0.12 \pm 0.42\mathrm{i}\}$
15	10^{-6}	$(10.00, 0.05, 0)$	$\{-50.20, -0.40, -0.1\}$
	10^{-3}	$(10.00, 0.05, 6 \times 10^{-6})$	$\{-50.19, -0.40, -0.1\}$
	1	$(9.60, 0.08, 0.10)$	$\{-30.77, -0.03 \pm 0.08\mathrm{i}\}$
	10^2	$(5.09, 0.08, 0.10)$	$\{-63.98, 0.66 \pm 0.28\mathrm{i}\}$
	10^3	$(5.03, 0.08, 0.10)$	$\{-417.94, 1.43, 0.54\}$
	10^6	$(5.02, 0.08, 0.10)$	$\{-3.9 \times 10^5, 1.57, 0.52\}$
1	10^{-6}	$(10.00, 0.05, 0)$	$\{-50.20, -0.40, -0.1\}$
	10^{-3}	$(10.00, 0.05, 6 \times 10^{-6})$	$\{-50.19, -0.40, -0.1\}$
	1	$(10.00, 0.05, 2 \times 10^{-3})$	$\{-46.81, -1.13, -0.10\}$
	10^3	$(10.00, 0.05, 3 \times 10^{-3})$	$\{-1240, -28.12, -0.10\}$
	10^6	$(10.00, 0.05, 3 \times 10^{-3})$	$\{-1.2 \times 10^6, -28.49, -0.10\}$

[1] Those zeros in equilibrium terms are actually very small positive numbers. Other parameter values are as given in Table 1.

TABLE 3. Period of endogenous rhythms of wild-type flies varies as k_a ($K_{eq} = 200$) varies.

k_a	0.001	0.1	0.8	0.9	1	10	100
Period	none	none	none	72.44	63.10	50.89	32.51
k_a	500	1000	5000	10^4	5×10^4	10^5	5×10^5
Period	28.61	26.90	24.86	24.54	24.27	24.24	24.21
k_a	10^6	2×10^6	2.5×10^6	2.9×10^6	3×10^6		
Period	24.21	24.21	24.21	24.30	24.44		

Periodic oscillations happen when k_a is larger than the bifurcation value $k_a^* = 0.9$. Other parameter values are as given in Table 1.

In the next section, we will see more about the relation between circadian rhythms and parameters of (1).

4. **Discussion.** In the actual experiment, parameters of the circadian rhythms models are hard to be measured, or even unmeasurable. Parameter values in Table

TABLE 4. Period of endogenous rhythms of per^L mutant varies as k_a ($K_{eq} = 15$) varies.

k_a	0.001	0.1	1.1	1.2	2	10	100	500
Period	none	none	none	57.19	55.67	41.34	30.98	29.21
k_a	1000	2000	5000	10^4	10^5	7×10^5	7×10^5	7×10^5
Period	28.94	28.80	28.71	28.67	28.65	28.65	29.20	30.37

Periodic oscillations occur when k_a is beyond the bifurcation value $k_a^* = 1.2$. Other parameter values are as in Table 1.

TABLE 5. Period of the endogenous rhythms of wild-type and mutant flies based on (1).

Genotype	K_{eq}	Temp	Period	Genotype	k_{p_1}	k_{p_2}	Period
Wild type	245	20	24.2	$dbt^+(1\times)$	10	0.03	24.2
	200	25	24.2	$dbt^+(2\times)$	15	0.06	24.3
	164	30	24.2	$dbt^+(3\times)$	20	0.09	25.7
per^L	18.4	20	26.5	dbt^S	10	0.3	17.6
	15.0	25	28.7	dbt^+	10	0.03	24.2
	12.3	30	30.4	dbt^L	10	0.003	25.1

To simplify the integration, we take $k_a = 10^6$ for wild-type flies and $k_a = 5000$ for mutant flies. Other conditions are as in Table 6.

1 have been chosen to yield a period close to 24-hours and ensure temperature compensation of the wild-type oscillator. The parameter values are arbitrary. Other combinations of parameter values may also yield circadian oscillations with possibly different periods.

It is significant to study how parameters of (1) affect its periodic oscillations. The numerical results are given in Figure 3, where the following parameters are considered: mRNA translation, mRNA degradation, monomer phosphorylation, protein proteolysis, association of PER/TIM protein and equilibrium constant for dimerization.

As shown in Figure 3A, periodic oscillation disappears when the protein synthesis rate v_p is below a critical value. That coincides with the truth that the inhibiting effect of protein synthesis may eventually suppress the circadian rhythmicity [11, 4, 25]. Moreover, the period decreases when the protein synthesis rate is greater than

TABLE 6. Period of the endogenous rhythms of wild-type and mutant flies based on (2).

Genotype	K_{eq}	Temp	Period	Genotype	k_{p_1}	k_{p_2}	Period
Wild type	245	20	24.2	$dbt^{+}(1\times)$	10	0.03	24.2
	200	25	24.2	$dbt^{+}(2\times)$	15	0.06	24.4
	164	30	24.2	$dbt^{+}(3\times)$	20	0.09	25.7
per^{L}	18.4	20	26.5	dbt^{S}	10	0.3	17.6
	15.0	25	28.7	dbt^{+}	10	0.03	24.2
	12.3	30	30.5	dbt^{L}	10	0.003	25.2

This table is copied out of Tyson et al. [26]. It is assumed that each parameter k varies with temperature according to $k(T) = k(298)\exp\{\varepsilon_a(1 - 298/T)\}$, with values for $k(298)$ and $\varepsilon_a = E_a/(0.592\text{kcalmol}^{-1})$ given in Table 1. The $dbt^{+}(n\times)$ means n copies of the wild-type allele.

a certain value. That matches the observations of anisomycin in the mollusk *Bulla* [12].

The PER/TIM complex formation plays a key role in the model. Circadian rhythm is markedly affected by the dimerization reaction, precisely in the model, by the association rate constant k_a and dissociation rate constant k_d. In Figure 3E the period decreases as k_a increases, which coincides with the suggestion that the heterodimeric dimerization is attenuated in the long-period per^{L} mutant [5]. Here the attenuation is probably due to the competition of PER homodimeric complexes [10]. Note again that it always has $k_d = k_a/K_{eq}$ in this paper. The results of Figure 3F imply that circadian rhythm occurs only when k_d is in a bounded range, and the period can be recognized as an increasing function of the dimer disassociation rate.

In Figure 3B we show how the oscillation is affected by mRNA synthesis. Periodic rhythm requires the mRNA synthesis rate k_m to be bounded, implying that the oscillation may be destroyed if mRNA synthesizes either too slow or too fast. Moreover, the period of oscillation becomes shorter as the mRNA synthesis rate goes up. The qualitatively similar results (Figure 3D) are detected when we consider the proteins proteolysis rate k_{p_3}, except that periodic oscillation happens even if there is no proteins proteolysis.

According to Theorem 3.1, in Figure 4 we inspect the dependence of oscillations on parameters K_{eq} and k_{p_1}. A U-shape region is found, whose boundary is almost the same as the locus of Hopf bifurcation in [26] (see Figure 4 in [26]). Within that region the system exhibits periodic oscillations, and in the outside area the system exhibits no limit cycle but a stable steady state. Figure 3C and 3F help to investigate the variation of period in this U-shape region. In Figure 3C, periodic oscillation

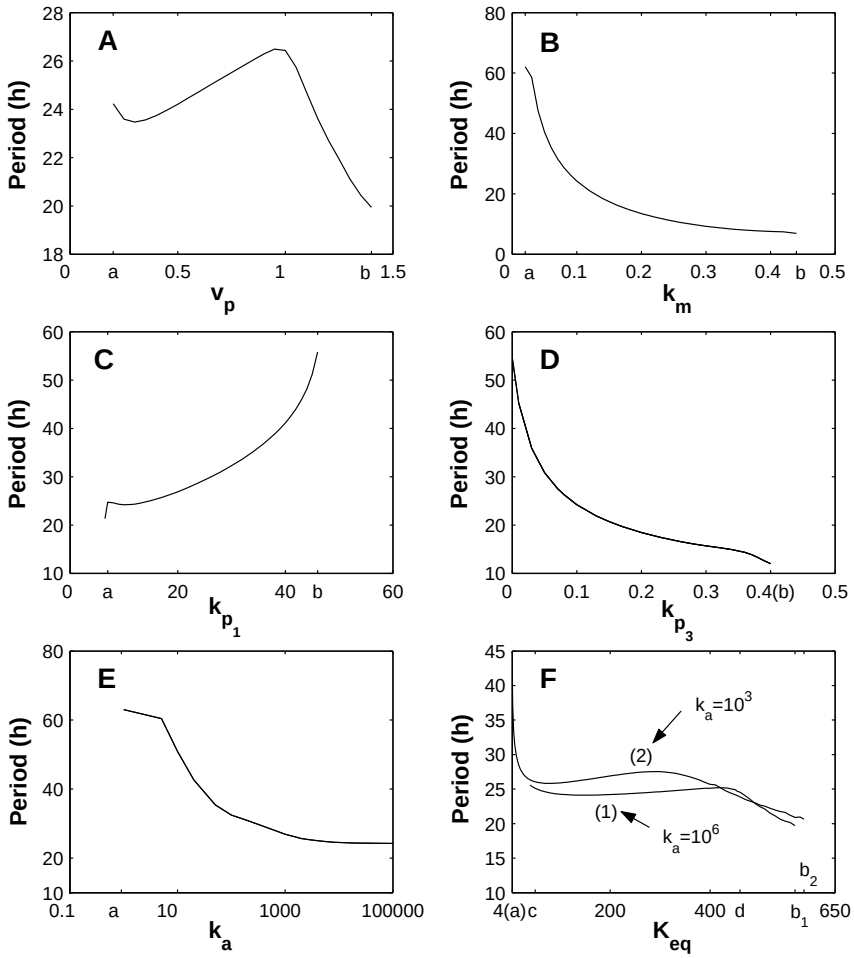

FIGURE 3. Relation between the oscillator period of (1) and some parameter values. In each diagram, other parameter values are chosen as in Table 1 and $k_a = 10^6$, and periodic oscillations occur only when the correlate parameter is in the interval $[a, b]$. In case A, $a = 0.2$ and $b = 1.4$; in case B, $a = 0.02$ and $b = 0.44$; in case C, $a = 7$ and $b = 46$; in case D, $a = 0$ and $b = 0.4$; in case E, $a = 0.9$ and $b = \infty$; in case F, $a_1 = a_2 = 4$, $b_1 = 570$ and $b_2 = 588$. For the convenience of numerical integration, curve (1) is shown only with $K_{eq} \geq 40$ in case F. As for $4 \leq K_{eq} \leq 40$, a decreasing period is suggested by curve (2) with increasing K_{eq}. Particularly, on curve (1) the period maintains 24.2–25.2 when the parameter K_{eq} varies in the interval $[c, d] = [50, 460]$.

requests that k_{p_1} is neither too small nor too large, which means that protein monomers are sufficient but not too unstable. In Figure 3F, periodic oscillation vanishes when K_{eq} is smaller than a critical value, which implies that the proteins tend to dimerize. Meanwhile, when K_{eq} varies within a quite large region beyond a certain value, the period of (1) remains virtually unchanged, suggesting that the

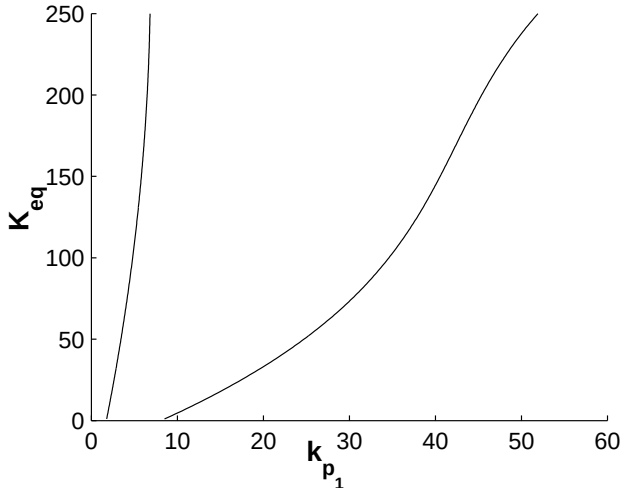

FIGURE 4. Two-parameter (K_{eq} and k_{p_1}) bifurcation diagram for system (1). Here K_{eq} and k_{p_1} are allowed to vary, and other parameter values are fixed as in Table 1. We take $k_a = 10^6$. Periodic oscillations happen only within the U-shape region bounded by the two curves. Outside this region the system evolves toward a stable steady state. We note that for any K_{eq} one can find a k_{p_1} such that oscillations happen, which differs from the boundedness requirement of K_{eq} as in Figure 3F.

wild-type oscillation has temperature compensation (see also Table 5). Furthermore, when K_{eq} decreases in a large region the period increases, which agrees with the consensus that per^L mutant introduces a longer period for the per^L-encoded protein to reduce its tendency to form dimers [5, 10]. By Table 5, one can also tell that per^L mutant loses temperature compensation.

Appendix. The concentrations of mRNA, monomers and dimers are naturally nonnegative. We therefore focus on the first orthant $\mathrm{R}^3_+ = \{(M, P_1, P_2) : M \geq 0, P_1 \geq 0, P_2 \geq 0\}$. It is easy to see that R^3_+ is a positively invariant set of system (1), i.e., any solution $((M(t), P_1(t), P_2(t))$ of system (1) through a point in the first orthant lies in it when $t \geq 0$.

Let $a > v_m/k_m$, $b > v_p\, a/k_{p_3}$, $c = K_{eq} b^2$ and $B(a, b, c) = \{(M, P_1, P_2) : 0 \leq M \leq a,\ 0 \leq P_1 \leq b,\ 0 \leq P_2 \leq c\}$. Denote (f_1, f_2, f_3) the vector field of (1). By estimating the sign of the vector field at vertexes on the boundary of $B(a, b, c)$, one has

$$\begin{cases} f_1(a, P_1, P_2) &= \dfrac{v_m}{1 + (P_2/P_{crit})^2} - k_m a < v_m - k_m a < 0, \\ f_2(M, b, P_2) &= v_p M - \dfrac{k_{p_1} b}{J_P + b + r P_2} - k_{p_3} b - 2k_a b^2 + 2k_d P_2 \\ &< v_p a - k_{p_3} b < 0, \\ f_3(M, P_1, c) &= k_a P_1^2 - k_d c - \dfrac{k_{p_2} c}{J_P + P_1 + rc} - k_{p_3} c \\ &< -\dfrac{k_{p_2} c}{J_P + P_1 + rc} - k_{p_3} c < 0. \end{cases}$$

The vector field for (1) on the boundary of $B(a,b,c)$ is shown in Figure 5, which

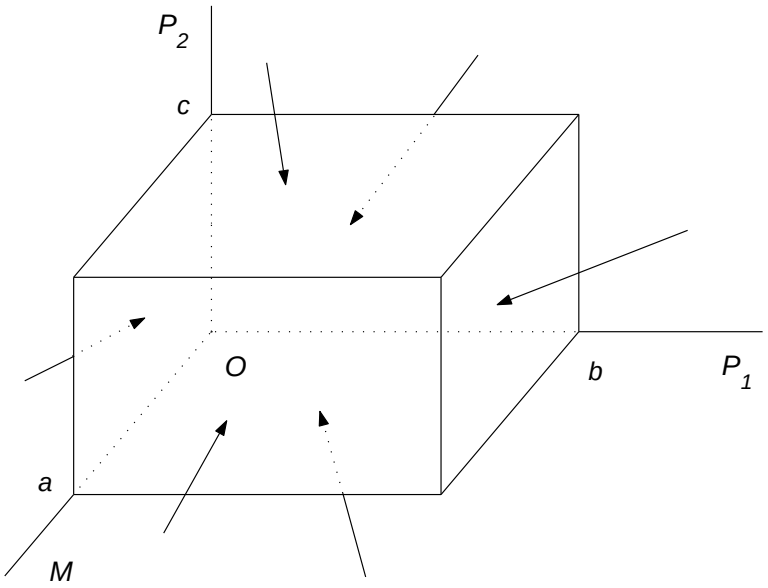

FIGURE 5. The vector field for (1) on the boundary of $B(a,b,c)$.

implies that $B(a,b,c)$ is positively invariant. Note that for any point in R^3_+, one can find such (a,b,c) satisfying that box $B(a,b,c)$ contains the point. It follows immediately that any forward solution of system (1) is bounded. We summarize the above discussion into the following proposition:

Proposition 1. *For any $a > v_m/k_m$, $b > v_p\, a/k_{p_3}$ and $c = K_{eq}b^2$, $B(a,b,c)$ is positively invariant for (1), that is, all forward solutions for (1) are bounded.*

From Proposition 1, there are at least one steady state in $B(a,b,c)$. Suppose that the steady state E is unique and there is no zero real part eigenvalue for the linearized matrix at E. Then the equilibrium E is either locally asymptotically stable, or has a two-dimensional unstable manifold. The following arguments show that the latter case provides the existence of limit cycles.

By computing the Jacobian matrix of (1), one has

$$Df = \begin{pmatrix} - & 0 & - \\ + & - & + \\ 0 & + & - \end{pmatrix},$$

where "$-$" represents that the entry is strictly negative and "$+$" means strict positivity. According to [23], the system is competitive with respect to the cone $K = \{(M,P_1,P_2) \in \mathrm{R}^3 : M \geq 0, P_1 \leq 0, P_2 \geq 0\}$. By applying the theory on competitive systems in [23], we have

Theorem A. *Suppose (1) has a unique steady state E. If the linearized matrix of (1) at E has one negative eigenvalue and two positive real part eigenvalues, then (1) has at least one stable limit cycle.*

Therefore, in order to study the oscillations for (1), one only needs to discuss its steady state and the local stability of the steady state.

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