Comparative Study of Circadian Oscillatory Network Models of *Drosophila*

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Abstract The circadian clock of *Drosophila* is a model pathway for research in biological clock mechanisms, both with traditional experimental approaches and with emerging systems biology approaches utilizing mathematical modeling and in silico computer simulation. Dynamic diurnal oscillations are achieved by the complex interaction of components as a system, and mathematical reconstruction has proven to be an invaluable means of understanding such systematic behavior. In this study, we implemented eight published models of the *Drosophila* circadian clock in Systems Biology Markup Language (SBML) for comparative systems biology studies using E-Cell Simulation Environment version 3, to examine the system-level requirements for the clock mechanism to be robust, by calculating the period and amplitude sensitivity coefficients with simulation experiments. While all models were generally robust as determined by the network topology of the oscillatory feedback loop structure, existing models place relatively strong emphasis on transcription regulation, although this is a limitation on robustness. We suggest that more comprehensive modeling including protein phosphorylation, polymerization, and nuclear transport with regard to amplitude sensitivity will be necessary for understanding the light entrainment and temperature compensation of circadian clocks.

Keywords
Systems biology, circadian rhythm, *Drosophila,* mathematical models, cell simulation

1 Introduction

Rhythmical activities are universally observed within terrestrial organisms, in diverse functions and time scales with periods ranging from seconds to years. The biological clock, the internal mechanism controlling the daily 24 hr periodic behavior of living systems, is one of the most fundamental and recognizable features of the various biological rhythms regulating and synchronizing behavior and inner physiology in accordance with the cycles of day and night. Although the biological clock is widely conserved among prokaryotes and eukaryotes to maintain approximately 24 hr periodic cycles, the period is not always precisely 24 hr; for example, the internal period is known to be about 25 hr in humans and 23 hr in mice [26]. In taking account of these variations, the biological clock is commonly referred to as the "circadian" clock (Latin *circa dies* means "around a day") [7].

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The circadian clock plays key roles in the adaptation of organisms to the dramatic changes in the environment introduced by the day-and-night cycle, both by passively responding to and by actively anticipating and preparing physiological processes for diurnal changes. For this purpose, the internal clock mechanism yields self-sustained, persisting 24 hr oscillations even in conditions of constant dark [43]. This self-sustainability is also demonstrated by the circadian oscillations of cyanobacterial clock proteins reconstituted in vitro [25]. Although the autonomous rhythm of this mechanism is constantly reset and entrained to environmental conditions by external stimuli, of which exposure to light is the most important, it is also synchronized by the daily changes in temperature and the circadian clocks of neighboring cells or individuals [17]. On the other hand, since the central clock mechanism is temperature-compensated, the oscillatory period is maintained at 24 hr under a range of environmental temperatures [22, 28]. While perturbations in temperature generally affect intracellular rates of biochemical reactions by altering the catalytic efficiency of enzymes, the periodic output of the circadian clock as a system is stable and robust, and independent of changes in environmental temperature [44].

Since in humans sleep and certain psychological disorders are sometimes attributed to irregularities in circadian rhythms, research on circadian clock mechanisms has been carried out in part from medical perspectives [40]. At the same time, detailed knowledge of the components of this clock has been elucidated using molecular biology approaches, in which circadian rhythm is targeted as a model biological pathway in the study of dynamic and systematic phenomena of living systems characterized by the three major criteria noted above: self-sustainability, light entrainment, and temperature compensation [18]. Upon the introduction of the much-anticipated systems biological approach to understanding of complex biological behaviors as a system, using integrative means as opposed to traditional reductionism, circadian rhythms have been further targeted as a model pathway for the observation of dynamic and complex properties of life. The circadian clock is essentially the result of systematic behavior composed of the intricate interactions of molecular components with multiple feedback mechanisms. However, the dynamic outcomes of systematic interplay of sets of more than three components are nonintuitive, and cannot realistically be studied by in vitro or in vivo methods. Instead, integrative approaches supplemented by computer simulations are required to determine how components and their interactions produce the dynamic behaviors of a system, and in silico experiments are employed as a valid and cost-effective alternative for this purpose [19].

Mathematical models of *Drosophila* enable systematic analyses due to the wealth of available quantitative data, coupled with the knowledge of the molecular components and the complex regulatory features, including protein phosphorylation, dimerization, and nuclear entry. The first molecular component of the circadian clock mechanism, the clock gene *period* (*per*), was identified from the arrhythmic mutant of the model organism *Drosophila* using a molecular genetics approach [20]. Further study revealed that the intracellular concentrations of the mRNA and protein products of the clock genes oscillate with 24 hr periods [13]. Since phases of expression differ among various clock genes, the combined ratios of the concentrations of clock gene products as a whole indicate the biological time of the organism. Clock proteins play essential roles in the reciprocal feedback transcription regulation of the clock genes, and thus generate continuous, self-sustained, diurnal oscillation. In this fashion, the circadian clock of a living system is the sum total of activity of a dynamic reciprocal gene expression regulation network as a system, and mathematical models can be employed to study circadian feedback regulation and simulate the time evolution of mRNA and protein concentrations by modeling reaction networks using differential equations [11]. Current mainstream modeling approaches to the study of feedback regulation using simultaneous ordinary differential equations originate in the Goodwin model of oscillatory behavior in enzyme-catalyzed reactions, published in 1965 [12], and succeeded, for example, by the *per* feedback model of Ruoff et al. [29] and the *frq* (*per* homolog in the filamentous fungus *Neurospora crassa*) feedback model of Smolen et al. [31, 33]. Initially, the circadian pathway of *Drosophila* was thought to be similar to that of *Neurospora*, but since seven more clock genes as well as a circadian *cryptochrome* (*cry*) [9] and several clock-controlled genes were identified in *Drosophila*, while only two more genes (*wc-1* and *wc-2*) were identified in *Neurospora* [8], the *Drosophila* circadian clock appears to be more similar to those of mammals.

Recently, *Drosophila* models have also been used as the basis for mammalian circadian clock models [24].

In computational cell biology, three principal types of differential equations are commonly utilized to model the biochemical kinetics of reaction rates. The first and simplest are mass action differential equations, in which the derivative is the simple product of the reaction constant k and the substrate concentration [S]:

$$\frac{d[\mathrm{P}]}{dt} = k[\mathrm{S}] \tag{1}$$

Henri-Michaelis-Menten (HMM) equations introduce nonlinearity with the Michaelis constant K_m, taking account of catalyst-substrate affinity. In the following equation, [E] is the concentration of the enzyme catalyst, and k_{cat} is the catalytic constant representing enzyme turnover number:

$$\frac{d[\mathrm{P}]}{dt} = \frac{k_{\mathrm{cat}}[\mathrm{E}][\mathrm{S}]}{K_m + [\mathrm{S}]} \tag{2}$$

Since gene expression in eukaryotes is strictly regulated by complex mechanisms involving many transcription factors in diverse combinations, further enhancement of nonlinearity beyond that in HMM equations is obtained with Hill equations (with n the Hill coefficient), which are commonly utilized to model the highly nonlinear nature of processes of mRNA transcription in the circadian pathway. Hill equations are able to represent the rapid and sharp increase in transcription upon activation in response to increase in concentrations of transcription factors, as opposed to the mass action equations, which are limited to linear acceleration:

$$\frac{d[\mathrm{P}]}{dt} = \frac{k_{\mathrm{cat}}[\mathrm{E}][\mathrm{S}]^n}{K_m + [\mathrm{S}]^n} \tag{3}$$

While the majority of the enzyme-catalyzed degradations of mRNA and protein products can be expressed in terms of HMM differential equations, the reaction rate constants usually remain unknown, and the adequacy of reaction equations and rate constants of other reactions is almost entirely uncertain for models of the circadian pathway. Moreover, because molecular biological research has traditionally overlooked the quantitative aspects of biological phenomena, accurate mRNA and protein concentration ratios have not yet been determined comprehensively, resulting in uncertainty regarding the relative level of expression of each clock gene. Accordingly, the quantitative strength of interactions and oscillatory amplitudes exhibited by the models generally lack credibility.

Despite the overall lack of availability of quantitative and detailed information regarding the reaction rates of the circadian clock, the primary objectives of mathematical modeling lie in the study of the remarkable network robustness exhibited by the circadian clock with regard to external stimuli, and also in its ability to oscillate regardless of the details of rate reactions. For *Drosophila*, a multitude of robust mathematical models have been proposed as new clock genes have been identified by molecular biology experiments, adding molecular components and their numerous types of feedback regulation one by one while sustaining a certain degree of network robustness with autonomous 24 hr oscillations. On the other hand, to the best of our knowledge, no comprehensive, comparative study of mathematical models of the circadian clock to elucidate systematic requirements for robustness of the circadian network has yet been performed.

In this study, we focus on mathematical models of the *Drosophila* circadian clock to elucidate how topological and functional network interaction contributes to the robustness of the system, and thus

results in stable circadian oscillations. For this purpose, we compared existing models to examine the parameter sensitivity of individual reactions and how changes in such reactions affect the periodic oscillations of the system, in order to determine the appropriateness of the rate equations and to determine which features are essential to system stability. Similarly, we further examined the parameter sensitivity of the oscillation amplitude, which has not been as carefully examined as that of the period.

2 Model Selection

2.1 Components of the *Drosophila* Circadian Clock

Eight clock genes, *per*, *timeless* (*tim*), *dClock* (*drosophila Clock*, *dClk*), *cycle* (*cyc*), *vrille* (*vri*), *Pdp1* (*PAR domain protein 1*), *doubletime* (*dbt*), and *shaggy* (*sgg*), are currently known to organize the central gene expression regulatory network of the *Drosophila* circadian clock [14]. See Figure 1. (Details are supplied in supplemental information S1.)

2.2 Mathematical Models of the *Drosophila* Circadian Clock

A number of mathematical models of the *Drosophila* circadian clock have been proposed, adding new components and interactions as they have been identified. Each of these models was built with a specific purpose, such as to understand the mechanism of self-sustaining diurnal oscillations composed of simple feedback structures or to examine the temperature compensation of the system. Most of the models aim to reproduce experimental data for phases of expression of the clock genes, in order to demonstrate their own validity, and for this purpose the time difference between peak and trough is especially important.

Some models simply assume that there is a *time delay* in gene expression, so that the process of transcription or translation is delayed according to an algebraic or assignment rule. For example, if a 4 hr delay is assumed for the translation process, translation initiated at time t converts a certain mRNA concentration to a synthesized protein concentration at time $t + 4$. The model by Scheper et al. [30] that contains only two components, an mRNA and a protein, introduces a 4 hr delay in the translation formula to achieve circadian oscillation with the limited number of components. Another model, by Smolen et al. (2004) [34], includes a 3 hr delay in the PDP1 expression equation to reproduce the 3 hr phase difference between VRI and PDP1, since the mechanism is still unknown. However, although four genes (*per*, *tim*, *vri*, and *Pdp1*) are controlled by the dCLK/CYC heterodimer through E-boxes, the phases of *per* and *Pdp1* are delayed by 3 hr compared to those of *tim* and *vri* [44]. It is clearly preferable to avoid the use of time delay whenever possible, since inclusion of it in a model results in treating the underlying mechanism as a black box. Processes related to the translation of mRNA and post-translational modifications, including mRNA and protein degradation and protein dimerization and dissociation, are usually expressed using simple mass action rate equations. A fraction of the processes of degradation of mRNA and protein, nuclear transport and export, and protein phosphorylation are typically modeled using HMM equations based on enzymatic reaction kinetics. Processes related to the transcription of genes are commonly expressed using Hill equations. In all cases, quantitative experimental evidence is scarce, especially for the specific parameters in each of the equations. Details of representative models are supplied in Section S2 of the Web supplement.

In this study, we have selected eight mathematical models of the *Drosophila* circadian clock for comparative examination, namely, five single-feedback-loop models (Goldbeter's 1995 model [10], Tyson et al.'s 1999 model [38], Scheper et al.'s 1999 model [30], Leloup and Goldbeter's 1999 model [23], and Petri and Stengl's 2001 model [27]) and three interlocked feedback loop models (Ueda et al.'s 2001 model [39], Smolen et al.'s 2002 model [32], and Smolen et al.'s 2004 model [34]; see Table 1 for summary and supplementary materials for details of each of the models). All of the models are expressed in terms of simultaneous differential equations to dynamically simulate the time evolution of concentrations of composite molecules. Descriptions of the models are provided below. We have omitted several models that did not meet the following criteria: (1) models should be

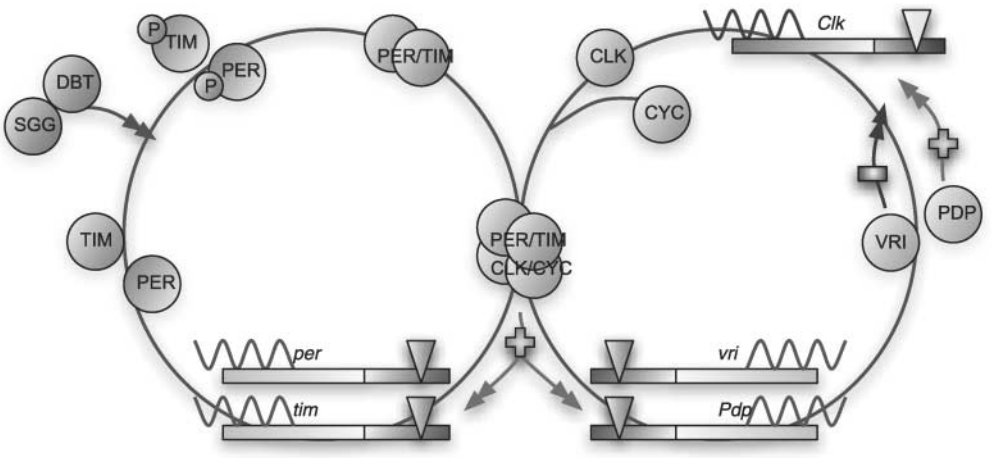

Figure 1. Pathway of regulation of gene expression in the circadian clock of *Drosophila*. Rectangles, wavy lines, and circles represent genes, mRNAs, and proteins, respectively. Levels of expression of clock genes exhibit circadian oscillations, except for *cyc*, *dbt*, and *sgg*, which exhibit constitutive expression. As dCLK proteins accumulate during early morning, dCLK monomers dimerize with CYC monomers. The dCLK/CYC heterodimers then bind to the circadian expression regulatory motif termed the E-box. This subsequently activates expression of circadian clock genes that have an E-box in their upstream region, namely, *per*, *tim*, *vri*, and *Pdp1*. The levels of TIM and VRI proteins reach peak in the early evening, followed by those of PER and PDP1, which peak in mid-evening. VRI inhibits, and PDP1 activates, *dClk* expression. Conversely, undergoing phosphorylation by DBT and SGG phosphoproteins, PER monomer dimerizes with TIM monomers, and thus forms PER/TIM heterodimer, which represses dCLK/CYC activation.

specific to *Drosophila* and not generalized; (2) if multiple models have been provided by the same authors, the most recent and most characteristic model has been chosen; (3) models include only ordinary differential equations, because partial differential equations cannot be simulated using E-Cell Simulation Environment 3 (E-Cell SE 3) [35]; and (4) models should assume a homogeneous distribution of component molecules, and therefore should not include spatial representations including compartments in meshes or diffusion of such molecules.

Table 1. Summary of the eight mathematical models of the *Drosophila* circadian clock considered in this study.

Model	Clock gene	Feedback loop	Phosphorylation	Period
Goldbeter, 1995	*per*	Single	PER	23.91
Scheper et al., 1999	*per*	Single	N/A	24.70
Tyson et al., 1999	*per*, *tim* *[1]	Single	N/A	24.21
Leloup and Goldbeter, 1999	*per*, *tim*	Single	PER,TIM	24.13
Petri and Stengl, 2001	*per*, *tim*	Single	PER,TIM	23.38
Ueda et al., 2001	*per*, *tim*, *dClk*	Double	N/A	24.05
Smolen et al., 2002	*per*, *Clk*	Double	N/A	23.11
Smolen et al., 2004	*per*, *tim*, *Clk*, *vri*, *Pdp1* *	Double	PER	24.91

* *per* and *tim* were treated as one gene.

3 Methods

3.1 Model Implementation

Several options are now available for simulation platforms with mathematical models of intracellular processes with simultaneous ordinary differential equations, including generic numerical analysis systems such as Mathematica. Simulators designed specifically for the purpose of computational cell simulation are also available, with characteristic differences in their policy regarding types of simulations (e.g., dynamic or static, or deterministic or probabilistic) as well as in the inclusion of spatial representation of inner compartments or representation of them as homogeneous. E-Cell SE 3 has advantages over other existing cell simulators, in its enabling of object-oriented modeling and in its ability to handle multiple algorithms (whether dynamic or static, and whether deterministic or probabilistic) and time scales (whether in milliseconds, minutes, or hours) seamlessly in a single simulation model, and can therefore realize intuitive simulations of the nonlinear dynamism of cells [36]. Since modeling in E-Cell SE 3 is object-oriented, a model is not simply a list of differential equations, but is based on an ontological model with biological relevancy. The system is also packaged with model analysis tools, and is distributed as an integrated simulation platform under an open-source license. All simulations were performed using E-Cell SE version 3.1.105, using Euler's method for numerical integration with Euler Stepper.

Only two *Drosophila* circadian clock models, the Leloup and Ueda models, have been implemented in a computer-readable format: the Systems Biology Markup Language (SBML), which is based on Extensible Markup Language (XML) and reposited online for free public access [15]. However, the existing *Drosophila* circadian clock models have basically been implemented on different simulators with various platforms, and are thus not directly comparable. In this study, we have implemented the eight existing models listed above on E-Cell SE 3 for direct comparative study of their behavior on a coherent platform. At the same time, six models that have not yet been reposited to SBML Model Repository were converted to SBML level 2 version 1 format and are available at the E-Cell Developer's Network Web site (http://ecdn.e-cell.org/Projects/Circadian). Wild-type simulation results of these models are supplied in Section S3 of the Web supplement.

3.2 Sensitivity Analysis

All reaction equations included in the models are time-dependent simultaneous differential equations, for the most part modeled with Equations 1–3. (There is one exception in the Smolen 2004 model, which is described in the Section S4 of the Web supplement.) To compute the change in substance concentration at each step of integration, the product of the reaction stoichiometry n_{ij} and velocity v_j is calculated. Positive stoichiometry indicates that the substance is a product in the reaction, while negative stoichiometry indicates that it is a reactant. If the substance is involved in multiple reactions, the net sum is computed as follows:

$$\frac{d[\mathrm{S}_i]}{dt} = \sum_j n_{ij} v_j \tag{4}$$

In order to quantify the sensitivity of the circadian oscillations to the parameters of reaction formulae, the sensitivity coefficients of the kinetic parameters were calculated according to the method described previously by Wolf et al. [41]. Each model was run for 2400 simulated hours to stabilize the oscillatory periods, as in the procedures for wild-type simulation, and the parameters were then altered by 10%, and the new period was calculated as the time difference between the first and second peaks during the next 96 hr. The 10% parameter change here represents an infinitesimal perturbation that does not damp oscillations. As in wild-type simulations, oscillation periods of the PER monomer, which is included in all of the models compared, were used as the periods of the system. For parameters, the reaction rate constants k or k_{cat}, which exhibit linear relationships with

reaction rates, were altered by 90% in normal equations and also in those with time delay. Thus, the sensitivity coefficient $C^{\text{period}}_{\text{parameter}}$ was calculated as the normalized change in period length divided by the normalized change in parameter, as follows:

$$C^{\text{period}}_{\text{parameter}} = \frac{\Delta\text{period}/\text{period}}{\Delta\text{parameter}/\text{parameter}} \tag{5}$$

The oscillation period is the principal feature investigated in the study of circadian rhythms, and the other of the two central measures of oscillation, the amplitude, has thus far been little examined [16]. The sensitivity of the amplitude to parameter changes was calculated with the same procedure as for the period sensitivity. However, since the amplitude differs among components (unlike the period, which is 24 hr for all components, enabling use of PER as reference), the sensitivity of the amplitude was calculated for all components with the following equation:

$$C^{\text{amplitude}}_{\text{parameter}} = \frac{\Delta\text{amplitude}/\text{amplitude}}{\Delta\text{parameter}/\text{parameter}} \tag{6}$$

The sensitivity coefficient for each of the parameters yields a system-dependent value that cannot be directly compared among different models, since the significance of such values is affected by the number of reactions and parameters contained in the model. However, according to the summation theorem of metabolic control analysis, theoretically such control coefficients should sum to 1 in a system when all parameters are considered in the analysis. Based on this summation theorem, the average overall sensitivity of the system can be calculated by normalizing for the number of reactions in the model, as follows [41]:

$$\sigma = \sqrt{\frac{1}{r}\sum_{j=1}^{r}\left(C_j^{\text{period}}\right)^2} \tag{7}$$

σ is commonly treated as the average sensitivity, and r is the number of reactions in the model.

4 Results

4.1 Analysis of the Sensitivity of the Period

The catalytic or rate constants of all reactions in all models were comprehensively perturbed with infinitesimal changes, and sensitivity coefficients were calculated using the PER oscillation period as the reference value (Figure 2). In all models, the sensitivity coefficients were low, implying low sensitivity and high degrees of robustness in terms of parameter insensitivity. The sensitivity coefficient becomes 1 when the relative change in a parameter equals the relative change in the oscillation period, and since, according to the summation theorem, the individual sensitivity coefficients should theoretically be below 1, the relative change in the period must be less than 10%, which is the extent of perturbation applied to the parameters. The results generally accorded with this theory, varying from 22 to 26 hr periods, which were within the 2.4 hr (10%) range of variation in period. This overall robustness can be observed from the average overall sensitivity of the model described by Equation 7. The highest average sensitivity was that of Goldbeter's 1995 model, 0.56, which is still quite low compared to those of other metabolic oscillation models reported in previous work [41] (Table 2).

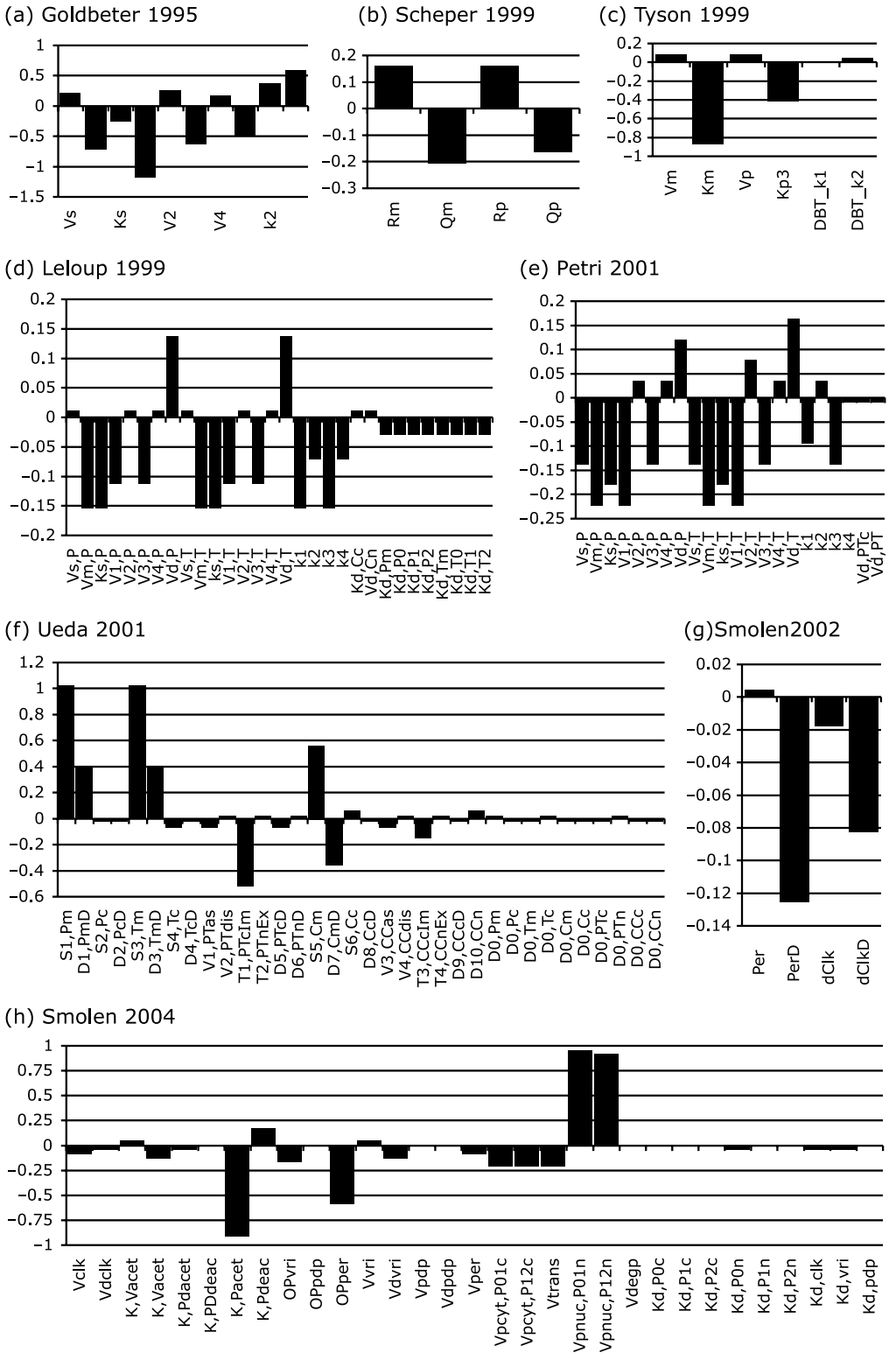

Figure 2. Period sensitivity coefficients of the circadian clock models. All sensitivity coefficients were calculated using the PER monomer oscillation period upon 10% perturbation of all reaction rate constants.

Table 2. Comparison of the overall sensitivity of the circadian models.

Model	Number of variables	Number of reactions	Sensitivity
Goldbeter, 1995	5	10	0.56
Scheper et al., 1999	2	4	0.27
Tyson et al., 1999	2	6	0.39
Leloup and Goldbeter, 1999	10	30	0.09
Petri and Stengl, 2001	10	20	0.14
Ueda et al., 2001	10	30	0.32
Smolen et al., 2002	3	3	0.07
Smolen et al., 2004	9	31	0.31

Models with limited numbers of reactions and with time delay implementation, such as the Scheper 1999 and Smolen 2002 models, exhibited very low sensitivities to all parameters, the largest of which remained within 0.25 in absolute value. Single-feedback-loop models with dual loops of PER and TIM, such as the Leloup 1999 and Petri 2001 models, also exhibited very low sensitivity overall, resulting in highly robust systems. Although other models exhibited similarly low sensitivities for the majority of reactions, several parameters exhibited relatively large effects on the oscillation period. In the Goldbeter 1995 model, the first phosphorylation step had the highest sensitivity, around 1.2, whereas the Tyson 1999 model assigned the highest sensitivity, around 0.9, to the degradation of mRNA. In larger models of interlocked feedback loops, the Ueda 2001 and Smolen 2004 models, several parameters related to the processes of phosphorylation and transcription exhibited high sensitivities of up to about 1, while most other parameters had very low sensitivity coefficients around 0. The Smolen 2004 model omits mRNA from the processes of expression, but includes DNA acetylation. The process of acetylation of the *per* gene exhibited a strongly negative sensitivity coefficient, but since the Hill coefficient for this process is unusually large, with an exponent of 5, while other models for the most part have a exponent of 2, and since periodic oscillation diminishes as soon as the exponent is decreased below 4 (data not shown), this sensitivity is presumably due to the type of equation and choice of parameter. Moreover, the parameter scale varies greatly in this model; for example, the rate constant for expression of PDP1 is 344.088 nM/hr, which is 300 times that of dCLK (1.062 nM/hr). In all models, parameters related to transcription, which was usually expressed using Hill equations, exhibited high absolute sensitivity coefficients, and parameters related to translation, which is usually expressed using mass action kinetics, exhibited low sensitivity coefficients. In this fashion, the factors with greatest sensitivity coefficients in the models can be attributed to the type of equation and the choice of parameters and not to the topology of the network.

At first glance, the sensitivity coefficients for mRNA transcription appear to take positive values while those for mRNA degradation take negative values in most models. However, since both *per* and *tim* mRNA degradations in the Ueda 2001 model took positive values, and the sensitivity coefficients of transcription and degradation were both negative in the Petri 2001 model, the processes related to transcription also depend on the choice of parameter and type of equation used. The Petri 2001 model is based on the Leloup 1999 model, and thus has identical network structure except for the negligible removal of several mass action reactions for the natural degradation of proteins with very small rate constants (0.01 nM/hr), and its parameters are tuned to fit the phases of experimental data. Therefore, the reversal of effects of perturbations on *per* and *tim* transcription

rates, yielding positive and negative sensitivity coefficients, is due to the parameter set used, instead of the number of components or the network topology.

Sensitivity coefficients for the protein degradation reactions exhibited negative values for models without phosphorylation processes, and positive values for models with phosphorylation processes. This seems inconsistent at first glance, since protein phosphorylation is initiated when the non-phosphorylated form is first phosphorylated, and since the protein is only finally degraded when it is doubly phosphorylated; but the gateway for the protein degradation pathway is the first process of phosphorylation, and this results in negative sensitivity coefficients in all models. The process of protein degradation is thus robust by virtue of its network topology, since inclusion of this pathway results in negative sensitivity in all models, regardless of parameters, types of equations, or the number of steps of phosphorylation included.

4.2 Analysis of Amplitude Sensitivity

In order to study the effects of reaction rates on oscillation amplitudes, the rate parameters of each process in the circadian pathway models were perturbed, as in the analysis of period sensitivity, and sensitivity coefficients were calculated for each of the components. Table 3 shows all of the amplitude sensitivity coefficients calculated for 10% perturbations.

Like those for the period sensitivity, the majority of the values were less than 1, indicating that most of the components have little effect on the oscillation amplitudes of other components, which contributes to the high degree of robustness of the system. On the other hand, sensitivity coefficients were generally high for amplitude compared to those for period, for which none of the values exceeded 1.2; and each model contained several reactions with high (>3) sensitivity coefficients, and thus exhibited a high degree of control over almost all amplitudes. Since the amplitudes differ for each of the cellular components (unlike the period, which is around 24 hr throughout), components with high wild-type amplitude tend to have lower sensitivity, while those with low wild-type amplitude tend to have higher sensitivity, and amplitude sensitivity coefficients are therefore more likely to exhibit large variations. Moreover, since the variation of amplitude increases as the number of components increases, the system does not necessarily become more robust as the number of components increases. Parameters with large amplitude sensitivity coefficients were primarily related to the transcription of three central genes, *per*, *tim*, and *dClk*. Again, as for period sensitivity, this is presumably due to the Hill equations employed to model the processes of transcription.

When the *per* transcription rate was increased by 10%, the PER amplitude was increased, whereas the TIM amplitude was decreased. The reverse was true when this rate was decreased by 10% or the *tim* transcription rate was increased by 10%. For models with phosphorylation steps (e.g., the Leloup and Petri models), the effect of amplitude was largest on the doubly phosphorylated forms of proteins. Since *per* and *tim* dual feedback loops exhibit parallel redundancy, perturbation of either of the genes affects the efficiency of heterodimerization, resulting in reciprocal changes in their amplitudes. This effect, which arises from network topology, was also supported by the proportionate change in the amplitude of PER/TIM heterodimer concentration. Of the three key proteins, PER, TIM, and dCLK, in interlocked feedback models, dCLK was the least affected and perturbation of it had the least effect on other components. In the Smolen 2004 model, this robustness of dCLK amplitude to perturbation of the PER/TIM loop was achieved by amplitude buffering by VRI and PDP1, which regulate *dClk* expression.

5 Discussion

To compare the existing mathematical models of *Drosophila* for purposes of elucidating the network requirements contributing to highly robust systems, we first implemented six models (Leloup and Ueda models were already implemented using SBML and published) with the modeling standard format SBML to allow computer simulation on the same platform with uniform integration methods

Table 3. Amplitude sensitivity coefficients of the circadian clock models. All sensitivity coefficients were calculated for the amplitude of all components upon 10% perturbation of all reaction rate constants. Columns indicate the perturbed reaction, while rows indicate the components whose amplitude is observed. Abbreviations are as follows: TC: transcription; TL: translation; EX: expression; D: degradation; D0: natural degradation; mD: mRNA degradation; Pd: protein degradation; as: association, dis: dissociation; Im: nuclear import; Ex: nuclear export; P01: phosphorylation level 0 to 1; AC: acetylation; DAC: deacetylation; OP: opportunity of expression.

(a) Goldbeter 1995

Variable	TC	mRNAd	TL	P01	P10	P12	P21	P2n	Pn2	Vd
M	2.3216	−1.392	−0.3164	−0.4766	0.0994	−0.3117	0.0499	−0.4435	0.3587	0.5406
P0	2.892	−2.5469	0.8693	−3.4365	0.7033	−1.2244	0.2515	−1.1025	0.8357	1.4874
P1	2.3205	−1.8131	0.5288	0.754	−0.2646	−2.2367	0.5591	−0.8798	0.6604	0.5241
P2	2.5483	−2.3363	0.5771	0.4749	−0.1779	0.1073	−0.1603	−1.4075	1.114	−0.4985
Pn	2.5499	−2.3667	0.5658	0.4376	−0.1664	0.0841	−0.1527	−0.2845	0.1977	−0.4783
Pt	2.6916	−2.3285	0.734	−2.2368	0.4454	−1.2565	0.2616	−1.0746	0.8066	1.1506

(b) Scheper 1999

Variable	TC	Md	TL	Pd
M	0.6446	−0.0578	−0.3951	0.8773
P	1.4144	−0.7129	1.4146	1.6319

(c) Tyson 1999

Variable	TC	mRNAD	TL	ProteinD	DBT_k1	DBT_k2
M	0.2651	−0.0536	−0.8163	−0.175	−0.7342	0.0057
P	0.449	−0.9047	0.4489	−0.5074	−0.5151	0.0378

(d) Leloup 1999

Variable	P_TC	PmD	P_TL	P01	P10	P12	P21	P2D	T_TC	TmD
Pm	1.800	−1.266	−0.418	−0.0571	0.0062	−0.062	0.007	0.383	−0.924	0.595
P0	2.409	−1.969	0.879	−1.7258	0.1761	−0.2542	0.027	0.525	−1.563	0.894
P1	2.613	−2.212	0.982	0.0166	−0.0035	−1.6884	0.174	0.297	−2.011	1.084
P2	4.863	−7.296	2.260	0.1085	−0.0147	0.1375	−0.023	−2.118	−9.813	3.044
Pt	3.199	−3.183	1.301	−0.5361	0.0545	−0.5838	0.058	−0.334	−3.671	1.581

Table 3. (continued)

(d) Leloup 1999

Variable	P_TC	PmD	P_TL	P01	P10	P12	P21	P2D	T_TC	TmD
Tm	−0.924	0.595	−0.418	−0.0571	0.0062	−0.062	0.007	0.383	1.800	−1.266
T0	−1.563	0.894	−0.647	−0.0798	0.0084	−0.087	0.010	0.561	2.409	−1.969
T1	−2.011	1.084	−0.799	−0.0939	0.0101	−0.103	0.012	0.662	2.613	−2.212
T2	−9.813	3.044	−2.782	−0.2148	0.0233	−0.2438	0.030	1.741	4.863	−7.296
Tt	−3.671	1.581	−1.271	−0.1321	0.014	−0.1464	0.017	0.937	3.199	−3.183
Cc	1.369	−0.601	0.208	−0.1255	0.0114	−0.1267	0.010	0.072	1.369	−0.601
Cn	1.249	−0.671	0.067	−0.1756	0.0162	−0.1818	0.016	0.127	1.249	−0.671

	T_TL	T01	T10	T12	T21	T2D	as	dis	Im	Ex
Pm	−0.418	−0.057	0.006	−0.062	0.007	0.383	−0.193	0.2274	−0.3102	0.2782
P0	−0.647	−0.080	0.008	−0.087	0.010	0.561	−0.2778	0.3131	−0.4396	0.3798
P1	−0.799	−0.094	0.010	−0.103	0.012	0.662	−0.313	0.3202	−0.4415	0.3721
P2	−2.782	−0.215	0.023	−0.244	0.030	1.741	−0.6893	0.4868	−0.5641	0.4089
Pt	−1.271	−0.132	0.014	−0.146	0.017	0.937	−0.4159	0.3603	−0.4689	0.3784
Tm	−0.418	−0.057	0.006	−0.062	0.007	0.383	−0.193	0.2274	−0.3102	0.2782
T0	0.879	−1.726	0.176	−0.254	0.027	0.525	−0.2778	0.3131	−0.4396	0.3798
T1	0.982	0.017	−0.004	−1.688	0.174	0.297	−0.313	0.3202	−0.4415	0.3721
T2	2.260	0.109	−0.015	0.138	−0.023	−2.118	−0.6893	0.4868	−0.5641	0.4089
Tt	1.301	−0.536	0.055	−0.584	0.058	−0.334	−0.4159	0.3603	−0.4689	0.3784
Cc	0.208	−0.126	0.011	−0.127	0.010	0.072	−0.2855	0.4994	−1.6544	0.9443
Cn	0.067	−0.176	0.016	−0.182	0.016	0.127	−0.3411	0.502	−0.616	0.6089

	PmD0	P0D0	P1D0	P2D0	TmD0	T0D0	T1D0	T2D0	CcD0	CnD0
Pm	−0.040	0.0015	0.002	0.001	0.009	0.002	0.002	0.001	0.0048	0.0312
P0	−0.057	−0.0037	0.002	0.002	0.016	0.002	0.002	0.002	0.0068	0.0428
P1	−0.061	−0.0041	−0.004	0.001	0.022	0.003	0.003	0.003	0.0066	0.0417

Table 3. (continued)

(d) Leloup 1999

	PmD0	P0D0	P1D0	P2D0	TmD0	T0D0	T1D0	T2D0	CcD0	CnD0
P2	−0.132	−0.0098	−0.010	−0.010	0.092	0.009	0.009	0.009	0.0049	0.0416
Pt	−0.076	−0.0054	−0.004	−0.002	0.037	0.004	0.004	0.004	0.0063	0.0418
Tm	0.009	0.0015	0.002	0.001	−0.040	0.002	0.002	0.001	0.0048	0.0312
T0	0.016	0.0023	0.002	0.002	−0.057	−0.004	0.002	0.002	0.0068	0.0428
T1	0.022	0.0027	0.003	0.003	−0.061	−0.004	−0.004	0.001	0.0066	0.0417
T2	0.092	0.0089	0.009	0.009	−0.132	−0.010	−0.010	−0.010	0.0049	0.0416
Tt	0.037	0.0042	0.004	0.004	−0.076	−0.005	−0.004	−0.002	0.0063	0.0418
Cc	−0.039	−0.0009	−0.001	−0.002	−0.039	−0.001	−0.001	−0.002	0.0012	0.0823
Cn	−0.043	−0.0008	−0.001	−0.002	−0.043	−0.001	−0.001	−0.002	0.0011	0.0625

(e) Petri 2001

Variable	P_TC	PmD	P_TL	P01	P10	P12	P21	P2D	T_TC	TmD	T_TL
Pm	1.905	−1.125	−0.282	−0.2108	0.0529	−0.1514	0.0338	0.361	0.024	0.136	−0.282
P0	2.542	−1.896	1.060	−1.8551	0.4515	−0.5481	0.1247	0.540	−0.589	0.417	−0.612
P1	2.421	−1.781	1.062	0.2519	−0.0883	−1.452	0.3382	0.178	−0.895	0.459	−0.758
P2	3.989	−5.399	1.925	0.2479	−0.1107	0.1653	−0.098	−1.177	−5.165	2.002	−2.106
Pt	2.603	−1.999	1.143	−0.9722	0.2303	−0.6724	0.1477	0.279	−0.999	0.536	−0.803
Tm	0.024	0.136	−0.282	−0.2108	0.0529	−0.1514	0.0338	0.361	1.905	−1.125	−0.282
T0	−0.589	0.417	−0.612	−0.3364	0.0843	−0.2388	0.0557	0.619	2.542	−1.896	1.060
T1	−0.895	0.459	−0.758	−0.4123	0.1052	−0.2876	0.0714	0.658	2.421	−1.781	1.062
T2	−5.165	2.002	−2.106	−0.5942	0.1548	−0.3929	0.1106	1.423	3.989	−5.399	1.925
Tt	−0.999	0.536	−0.803	−0.4133	0.105	−0.2894	0.0717	0.706	2.603	−1.999	1.143
PTc	1.335	−0.784	0.155	−0.2716	0.0591	−0.2	0.0325	0.184	1.335	−0.784	0.155
PTn	1.332	−0.793	0.146	−0.2803	0.0614	−0.2056	0.0343	0.190	1.332	−0.793	0.146

Table 3. (continued)

(e) Petri 2001

	T01	T10	T12	T21	T2D	PTcD	PTnD	as	dis	Im	Ex
Pm	−0.211	0.053	−0.151	0.034	0.361	0.000	0.000	−0.277	0.308	−0.3002	0.284
P0	−0.336	0.084	−0.239	0.056	0.619	0.000	0.000	−0.447	0.426	−0.452	0.407
P1	−0.412	0.105	−0.288	0.071	0.658	0.000	0.000	−0.470	0.356	−0.366	0.313
P2	−0.594	0.155	−0.393	0.111	1.423	0.000	0.000	−0.805	0.490	−0.4779	0.367
Pt	−0.413	0.105	−0.289	0.072	0.706	0.000	0.000	−0.498	0.392	−0.4067	0.351
Tm	−0.211	0.053	−0.151	0.034	0.361	0.000	0.000	−0.277	0.308	−0.3002	0.284
T0	−1.855	0.452	−0.548	0.125	0.540	0.000	0.000	−0.447	0.426	−0.452	0.407
T1	0.252	−0.088	−1.452	0.338	0.178	0.000	0.000	−0.470	0.356	−0.366	0.313
T2	0.248	−0.111	0.165	−0.098	−1.177	0.000	0.000	−0.805	0.490	−0.4779	0.367
Tt	−0.972	0.230	−0.672	0.148	0.279	0.000	0.000	−0.498	0.392	−0.4067	0.351
PTc	−0.272	0.059	−0.200	0.033	0.184	0.000	0.000	−0.300	0.459	−1.4946	1.268
PTn	−0.280	0.061	−0.206	0.034	0.190	0.001	0.001	−0.306	0.462	−0.3511	0.350

(f) Ueda 2001

Variable	PERm	PERmD	PERc	PERcD	TIMm	TIMmD	TIMc	TIMcD	PTas	PTdis
PERm	3.083	−0.723	−0.302	0.087	0.939	0.942	−0.302	0.087	−0.087	0.047
PERc	7.757	−3.937	3.264	−0.400	−3.333	5.562	−2.797	0.735	−0.596	0.358
TIMm	0.939	0.942	−0.302	0.087	3.083	−0.723	−0.302	0.087	−0.087	0.047
TIMc	−3.333	5.562	−2.797	0.735	7.757	−3.937	3.264	−0.400	−0.596	0.358
PTn	4.379	0.947	0.469	−0.012	4.379	0.947	0.469	−0.012	−0.121	0.026
PTc	4.451	0.875	0.628	−0.011	4.451	0.875	0.628	−0.011	−0.104	0.010
dCLKm	1.532	0.464	0.014	0.011	1.532	0.464	0.014	0.011	−0.061	0.025
dCLKc	2.306	0.741	0.011	0.014	2.306	0.741	0.011	0.014	−0.114	0.046
CCn	2.865	0.968	−0.011	0.019	2.865	0.968	−0.011	0.019	−0.155	0.063
CCc	2.687	0.879	0.013	0.017	2.687	0.879	0.013	0.017	−0.137	0.055

Table 3. (continued)

(f) Ueda 2001

	PTcIm	PTnEx	PTcD	PTnD	dCLKm	dCLKmD	dCLKc	dCLKcD	CCas	CCdis
PERm	−0.533	0.091	0.110	0.176	0.605	−0.101	0.227	−0.030	0.029	−0.027
PERc	−0.953	0.078	−0.017	0.144	0.823	−0.228	0.250	−0.039	−0.003	−0.013
TIMm	−0.533	0.091	0.110	0.176	0.605	−0.101	0.227	−0.030	0.029	−0.027
TIMc	−0.953	0.078	−0.017	0.144	0.823	−0.228	0.250	−0.039	−0.003	−0.013
PTn	−0.329	−0.121	−0.191	−0.048	1.596	−0.486	0.502	−0.088	−0.002	−0.023
PTc	−2.699	0.547	−0.196	0.419	1.537	−0.395	0.534	−0.085	0.032	−0.043
dCLKm	−0.409	0.033	−0.042	0.030	1.065	−0.059	−0.368	0.158	−0.045	0.028
dCLKc	−0.800	0.045	−0.085	0.025	1.684	−0.243	0.950	−0.094	−0.889	0.583
CCn	−1.118	0.066	−0.111	0.033	2.061	−0.543	0.947	−0.109	0.100	−0.124
CCc	−0.967	0.054	−0.102	0.030	1.983	−0.304	1.115	−0.120	0.236	−0.226

	CCnIm	CCcEx	CCcD	CCnD	PERmD0	PERcD0	TIMmD0	TIMcD0	PTcD0	PTnD0
PERm	0.183	−0.0242	−0.027	0.004	−0.066	0.002	0.002	0.002	0.000	−0.001
PERc	0.134	−0.0168	−0.035	0.036	−0.185	−0.030	0.118	0.025	−0.011	−0.007
TIMm	0.183	−0.0242	−0.027	0.004	0.002	0.002	−0.066	0.002	0.000	−0.001
TIMc	0.134	−0.0168	−0.035	0.036	0.118	0.025	−0.185	−0.030	−0.011	−0.007
PTn	0.242	−0.0266	−0.081	0.057	−0.068	−0.006	−0.068	−0.006	−0.027	−0.052
PTc	0.331	−0.0381	−0.077	0.038	−0.072	−0.007	−0.072	−0.007	−0.030	−0.001
dCLKm	−0.052	0.0843	0.162	0.227	−0.017	−0.001	−0.017	−0.001	−0.007	−0.012
dCLKc	−0.757	0.2902	0.016	0.470	−0.033	−0.002	−0.033	−0.002	−0.014	−0.024
CCn	0.771	−0.0843	−0.089	0.148	−0.043	−0.003	−0.043	−0.003	−0.018	−0.032
CCc	−1.106	0.3953	−0.095	0.559	−0.039	−0.003	−0.039	−0.003	−0.016	−0.029

	dCLKmD0	dCLKcD0	CCcD0	CCnD0
PERm	−0.014	−0.003	−0.0024	−0.005
PERc	−0.028	−0.005	−0.0038	−0.008

Table 3. (continued)

(f) Ueda 2001

	dCLKmD0	dCLKcD0	CCcD0	CCnD0
TIMm	−0.014	−0.003	−0.0024	−0.005
TIMc	−0.028	−0.005	−0.0038	−0.008
PTn	−0.056	−0.010	−0.0076	−0.015
PTc	−0.048	−0.009	−0.0071	−0.014
dCLKm	−0.014	0.006	0.0042	0.014
dCLKc	−0.036	−0.013	−0.0042	0.029
CCn	−0.068	−0.016	−0.0126	−0.024
CCc	−0.045	−0.015	−0.0118	0.036

(g) Smolen 2002

Variable	Per	PerD	dClk	dClkD
Per	1.001	−0.9839	0.407	−0.3798
dClk	0.002	0.0082	1.168	−1.2061
dClkF	0.000	0.0287	1.000	−1.0877

(h) Smolen 2004

Variable	ACpdp_F	DACpdp	OPpdp	EXpdp	Dpdp_k	Dpdp_v	ACvri_F	DACvri	OPvri
CLK	0.5043	−0.5419	−0.4762	0.1773	−0.0039	−0.622	−0.8388	0.9766	0.6932
PDP	2.6158	−2.2017	0.5374	0.9719	−0.0052	−0.8021	0.4233	−0.6736	−0.5627
VRI	−0.1065	0.0919	0.0703	−0.0351	0.0013	0.0914	2.3354	−2.3231	0.1687
PER	−0.0603	0.0413	0.0235	−0.0192	0.0005	0.0224	0.2526	−0.3745	−0.3299

	EXvri_V	Dvri_k	Dvri_v	EXclk_V	Dclk_k	Dclk_v	ACper_F	DACper	OPper
CLK	−0.2908	0.0064	0.8196	0.8537	−0.0094	−0.4033	−2.0328	0.7669	−0.8294
PDP	0.2273	−0.0035	−0.5855	−0.2746	0.0025	0.109	−12.6546	4.4707	−1.3273
VRI	1.1598	−0.008	−1.3221	−0.2516	0.0043	0.1663	−4.6248	2.6995	−0.6758
PER	0.1332	−0.002	−0.3341	−0.1605	0.0008	0.0405	−2.0725	0.9474	−0.5559

Table 3. (continued)

(h) Smolen 2004

	EXp0_V	P0Ic	P12c	PerIm	P0In	P12n	P0cD	P1cD	P2cD
CLK	−0.2861	−0.3363	−0.3376	−0.3386	2.7435	2.7337	0.0002	0.0004	0.0006
PDP	−3.4205	−0.4524	−0.4531	−0.4541	4.8055	4.8097	0.0032	0.0035	0.0036
VRI	−1.5859	−0.2385	−0.2389	−0.2396	2.7153	2.7196	0.0016	0.0018	0.002
PER	−0.2459	−0.3691	−0.3708	−0.372	2.9789	2.976	−0.0004	−0.0002	0

	P0nD	P1nD	P2nD	DP2n
CLK	−0.012	0.0065	0.0009	0.001
PDP	0.0542	0.0138	0.0005	0.0006
VRI	0.0258	0.007	0.0006	0.0007
PER	0.0134	0.0072	0.0003	0.0003

using E-Cell SE 3. SBML provides the standard means for description of mathematical cell models, and it does not specify the types of solvers or methods of integration; it is thus essential to prepare models using the same modeling language, and then simulate on the same platform, if comparative systems biology analyses of the models are to be performed. All of the eight models in both SBML and EML formats (including the methods of simulation) are available at our Web site for download. Using these models, we have performed simulation experiments to calculate the period and amplitude sensitivity coefficients of all rate constants by applying 10% perturbations to the parameters.

Although their assumptions, objectives, and network structures vary, all models compared in this study reproduced stable circadian oscillations. All models exhibited a high degree of robustness overall, compared to metabolic oscillation models presented in previous studies [41], regardless of the parameter values and the types of equations employed. The simple feedback loop structure of the models contributes to system stability, and thus yields a robust network even with simplistic representation of reactions by mass action equations. Comparing the period and amplitude sensitivity coefficients in each of the models, processes related to transcription were found generally to be influential, mostly due to the highly nonlinear nature of the Hill equations incorporated in the representation of these processes, and sensitivity thus cannot be attributed to the importance of control of the oscillation period by processes of transcription. Recent experimental evidence also suggests that post-transcriptional modifications may have greater control over periods of oscillation than transcriptional regulation itself, by regulating pre-mRNA splicing or by stabilizing mRNA; for example, *per* accumulation is decreased by as much as five-fold in the *tim0* mutant even though the *per* transcription rate is the same as that of the wild type [4, 42]. Nevertheless, all models exhibited high degrees of robustness for the entire set of parameters in terms of period sensitivity, presumably due to the only property common to all models, the network topology of *Drosophila* circadian models composed of multiple feedback loops.

Since the circadian clock of higher eukaryotes has traditionally been modeled as a transcription feedback oscillator, transcription was considered the key process in each of the models examined and was mostly implemented using Hill-type equations. This focus on transcription in regulation was also observed in sensitivity analysis, in which the processes of transcription were found to exhibit strong

control of both periods and amplitudes. The sensitivity of the oscillation amplitude, which has thus far been less well documented than that of the period, was strongly influenced by the processes of transcription in the eight models examined in this study. However, since the oscillation amplitude is known to have physiological implications such as the 24 hr sleep-wake rhythm and hormone secretion in humans, oscillation amplitudes should also be stable and robust to perturbations [5, 6, 16]. In terms of network topology, key requirements for achievement of robust amplitude with the assumption of strong transcriptional control are post-transcriptional and translational modifications in phosphorylation, polymerization, and nuclear transport [21]. For example, the Goldbeter, Leloup, and Petri models, which include phosphorylation steps, distributed the amplitude sensitivity among the phosphorylated forms of the proteins, resulting in relatively small sensitivity coefficients of the PER/TIM heterodimer and low sensitivity values for the phosphorylation steps. On the other hand, the Ueda model, which does not include phosphorylation steps, has relatively high sensitivity values for the heterodimer. Recent findings have shown that the central circadian mechanism is composed of protein phosphorylation cycles alone in the photosynthetic cyanobacteria [37], and phosphorylation may also play key roles in the eukaryotic circadian clock, along with transcriptional regulation.

Of the two feedback loops, the dCLK loop is less sensitive to perturbations in terms of amplitude. Experimental evidence suggests that since TIM is related to light entrainment and PER to temperature compensation [1], the PER/TIM loop is responsible for the reception of external stimuli, and the dCLK/CYC loop for maintenance of stable oscillations. The Leloup and Petri models, which are based on the Goldbeter model with the addition of a TIM loop, are more robust, as indicated by lower values of σ. This parallel redundancy of the PER/TIM loop presumably contributes to temperature compensation, even though TIM is degraded by CRY in light-dependent fashion. As shown by the reciprocal effects on amplitudes by perturbations of *per* and *tim* transcription, external stimuli are buffered by the PER/TIM heterodimerization process. Since this heterodimer inhibits transcriptional regulation by dCLK/CYC through interaction with the dCLK/CYC complex and inhibition of dCLK/CYC nuclear transport [14], polymerization of proteins and nuclear transport should be considered in future mathematical models of the *Drosophila* circadian clock, along with protein phosphorylation and transcription regulation. Use of an interlocked feedback loop model with parallel PER and TIM loops will be essential for understanding the mechanisms of light entrainment and temperature compensation.

Modeling and simulation approaches are useful not only for understanding biological systems intuitively, but also for designing experiments by limiting the range of the experimental search space. Therefore, if a certain characteristic is observed throughout many models created under different assumptions and objectives, it will be a good target for experimental validation. For example, temperature compensation is one of the most important features of a robust circadian oscillatory network, and results of analyses of all models in this study suggested that transcription reactions are the most sensitive part of such models, providing a clue to the mechanism of temperature compensation and possibly enabling experimental validation.

Quantitative mathematical models enable simulation of dynamic behavior in time-course progression, and are thus useful for understanding the dynamic nature of oscillatory systems, as demonstrated by the sensitivity analyses described in this study. In addition, qualitative evaluation through topological analyses can complement quantitative analyses, especially for circadian pathways, for which most parameters lack experimental evidence. Topological network analysis of mathematical models based on network structures without quantitative parameters can predict system behavior to a certain extent, as described elsewhere [2, 3]. We believe the availability of these mathematical models in the standard SBML format created in this study will facilitate both quantitative and qualitative research on the circadian rhythm of *Drosophila*.

Acknowledgments

We would like to thank Tatsuya Ishida and Tomoya Kitayama for technical support regarding E-Cell SE 3. This research was supported in part by the Japan Society for the Promotion of Science (JSPS).

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