Modeling the *Drosophila melanogaster* Circadian Oscillator via Phase Optimization

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In this study, we develop a detailed 29-state model of the *Drosophila melanogaster* circadian oscillator that describes circadian phase behavior through mass action kinetics without the explicit use of time delays. This model simulates the transcription, translation, phosphorylation, transport, association, and degradation of 5 unique clock components. The system is comprised of 29-states and 84 parameter, 36 of which are estimated through use of a genetic algorithm.

1 State Equations

The equations below describe the ordinary differential equation model that makes up the 29-state *Drosophila melanogaster* circadian system. Due to space constraints, *per*-related states are abbreviated to *p*, *tim* to *t*, *vri* to *v*, *pdp* to *d*, and *clk* to *c*. Lowercase states reflect mRNA components, while uppercase states denote their protein counterparts. If the lowercase letter *p* appears after the protein name, it reflects an individual phosphate group; *n* reflects the nuclear localization of the circadian gene/protein. Thus, $[Pppn]$ refers to the doubly phosphorylated nuclear PER protein state.

- Transcription:

$$\frac{d[pn]}{dt} = \alpha_{cp} \cdot \frac{[Cpn]^{hcp}}{K_{cap}^{hcp} + [Cpn]^{hcp}} - \beta_p[pn] - \delta_{mp}[pn] \quad (1)$$

$$\frac{d[tn]}{dt} = \alpha_{ct} \cdot \frac{[Cpn]^{hct}}{K_{cat}^{hct} + [Cpn]^{hct}} - \beta_t[tn] - \delta_{mt}[tn] \quad (2)$$

$$\frac{d[vn]}{dt} = \alpha_{cv} \cdot \frac{[Cpn]^{hcv}}{K_{cav}^{hcv} + [Cpn]^{hcv}} - \beta_v[vn] - \delta_{mv}[vn] \quad (3)$$

$$\frac{d[dn]}{dt} = \alpha_{cd} \cdot \frac{[Cpn]^{hcd}}{K_{cad}^{hcd} + [Cpn]^{hcd}} - \beta_d[dn] - \delta_{md}[dn] \quad (4)$$

$$\frac{d[cn]}{dt} = \alpha_{dc} \cdot \frac{[Dpn]^{hdc}}{K_{dac}^{hdc} + [Dpn]^{hdc}} \cdot \frac{K_{vic}^{hvc}}{K_{vic}^{hvc} + [Vpn]^{hvc}} - \beta_c[cn] - \delta_{mc}[cn] \quad (5)$$

- Transport:

$$\frac{d[p]}{dt} = \beta_p[pn] - \delta mp[p] \quad (6)$$

$$\frac{d[t]}{dt} = \beta_t[tn] - \delta mt[t] \quad (7)$$

$$\frac{d[v]}{dt} = \beta_v[vn] - \delta mv[v] \quad (8)$$

$$\frac{d[d]}{dt} = \beta_d[dn] - \delta md[d] \quad (9)$$

$$\frac{d[c]}{dt} = \beta_c[cn] - \delta mc[c] \quad (10)$$

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- Translation:

$$\frac{d[P]}{dt} = \gamma_p[p] - \eta_{pp}[P] + \eta_{pm}[Pn] - \delta_{0p}[P] \quad (11)$$

$$\frac{d[T]}{dt} = \gamma_t[t] - \epsilon_{t1p}[T] + \epsilon_{t1m}[Tp] - (\delta_{0t} + l \cdot G(t))[T] \quad (12)$$

$$\frac{d[V]}{dt} = \gamma_v[v] - \epsilon_{v1p}[V] + \epsilon_{v1m}[Vp] - \delta_{0t}[V] \quad (13)$$

$$\frac{d[D]}{dt} = \gamma_d[d] - \epsilon_{d1p}[D] + \epsilon_{d1m}[Dp] - \delta_{0d}[D] \quad (14)$$

$$\frac{d[C]}{dt} = \gamma_c[c] - \eta_{cp}[C] + \eta_{cm}[Cn] - \delta_{0c}[C] \quad (15)$$

- Phosphorylated Cytoplasmic Proteins:

$$\frac{d[Tp]}{dt} = \epsilon_{t1p}[T] - \epsilon_{t1m}[Tp] - \epsilon_{t2p}[Tp] + \epsilon_{t2m}[Tpp] - (\delta_{1t} + l \cdot G(t))[Tp] \quad (16)$$

$$\frac{d[Tpp]}{dt} = \epsilon_{t2p}[Tp] - \epsilon_{t2m}[Tpp] - \eta_{tp}[Tpp] + \eta_{tm}[Tppn] - (\delta_{2t} + l \cdot G(t))[Tpp] \quad (17)$$

$$\frac{d[Vp]}{dt} = \epsilon_{v1p}[V] - \epsilon_{v1m}[Vp] - \eta_{vp}[Vp] + \eta_{vm}[Vpn] - \delta_{1t}[Vp] \quad (18)$$

$$\frac{d[Dp]}{dt} = \epsilon_{d1p}[D] - \epsilon_{d1m}[Dp] - \eta_{dp}[Dp] + \eta_{dm}[Dpn] - \delta_{1t}[Dp] \quad (19)$$

- Nuclear Protein

$$\frac{d[Pn]}{dt} = \eta_{pp}[P] - \eta_{pm}[Pn] - \epsilon_{p1p}[Pn] + \epsilon_{p1m}[Ppn] - \delta_{0pn}[Pn] \quad (20)$$

$$\frac{d[Ppn]}{dt} = \epsilon_{p1p}[Pn] - \epsilon_{p1m}[Ppn] - \epsilon_{p2p}[Ppn] + \epsilon_{p2m}[Pppn] - \delta_{1pn}[Ppn] \quad (21)$$

$$\frac{d[Pppn]}{dt} = \epsilon_{p2p}[Ppn] - \epsilon_{p2m}[Pppn] - \zeta_p[Pppn][Tppn][Cpn] + \zeta_m[PppTppCpn] - \delta_{2pn}[Pppn] \quad (22)$$

$$\frac{d[Tpn]}{dt} = -\epsilon_{p2p}[Tpn] + \epsilon_{p2m}[Tppn] - \eta_t[Tpn] - (\delta_{1tn} + l \cdot G(t)) * Tpn \quad (23)$$

$$\frac{d[Tppn]}{dt} = \epsilon_{p2p}[Tpn] - \epsilon_{p2m}[Tppn] + \eta_{tp}[Tp] - \eta_{tm}[Tpn] - \zeta_p[Pppn][Tppn][Cpn] + \zeta_m[PppTppCpn] - (\delta_{2tn} + l \cdot G(t))[Tppn] \quad (24)$$

$$\frac{d[Vpn]}{dt} = \eta_{vp}[Vp] - \eta_{vm}[Vpn] - \delta_{1vn}[Vpn] \quad (25)$$

$$\frac{d[Dpn]}{dt} = \eta_{dp}[Dp] - \eta_{dm}[Dpn] - \delta_{1dn}[Dpn] \quad (26)$$

$$\frac{d[Cn]}{dt} = \eta_{cp}[C] - \eta_{cm}[Cn] - \epsilon_{p1p}[Cn] + \epsilon_{p1m}[Cpn] - \delta_{0cn}[Cn] \quad (27)$$

$$\frac{d[Cpn]}{dt} = \epsilon_{p1p}[Cn] - \epsilon_{p1m}[Cpn] - \zeta_p[Pppn][Tppn][Cpn] + \zeta_m[PppTppCpn] - \delta_{1cn}[Cpn] \quad (28)$$

$$\frac{d[PppTppCpn]}{dt} = \zeta_p[Pppn][Tppn][Cpn] - \zeta_m[PppTppCpn] - \delta_{4xn}[PppTppCpn] \quad (29)$$

- State-Dependent Gating Function

$$G(t) \quad = \quad \frac{1 - \left(\frac{[c]^3}{\max([c]^3)} + \frac{[Cn]^5}{\max([Cn]^5)} \right)^4}{\max\left(1 - \left(\frac{[c]^3}{\max([c]^3)} + \frac{[Cn]^5}{\max([Cn]^5)} \right)^4 \right)}$$

2 State Variables

We define the state variables (characterized in nM concentrations) used to model the mathematical representation of the *Drosophila melanogaster* circadian oscillator. The clock components included in the model are *period*, *timeless*, *vrille*, *PAR-domain protein 1*, and *clock* genes and proteins.

	no.	State Variable	Description
mRNA	01	pn	nuclear *period* mRNA
	02	tn	nuclear *timeless* mRNA
	03	vn	nuclear *vrille* mRNA
	04	dn	nuclear *Pdp1* mRNA
	05	cn	nuclear *dClock* mRNA
	06	p	cytoplasmic *period* mRNA
	07	t	cytoplasmic *timeless* mRNA
	08	v	cytoplasmic *vrille* mRNA
	09	d	cytoplasmic *Pdp1* mRNA
	10	c	cytoplasmic *dClock* mRNA
Cytoplasmic Protein	11	P	unphosphorylated *period* protein
	12	T	unphosphorylated *timeless* protein
	13	V	unphosphorylated *vrille* protein
	14	D	unphosphorylated *Pdp1* protein
	15	C	unphosphorylated *dClock* protein
	16	Tp	singly phosphorylated *timeless* protein
	17	Tpp	doubly phosphorylated *timeless* protein
	18	Vp	active (phosphorylated) *vrille* protein
	19	Dp	active (phosphorylated) *Pdp1* protein
Nuclear Protein	20	Pn	unphosphorylated *period* protein
	21	Ppn	singly phosphorylated nuclear *period* protein
	22	Pppn	doubly phosphorylated nuclear *period* protein
	23	Tpn	singly phosphorylated nuclear *timeless* protein
	24	Tppn	doubly phosphorylated nuclear *timeless* protein
	25	Vpn	active (phosphorylated) nuclear *vrille* protein
	26	Ppn	active (phosphorylated) nuclear *Pdp1* protein
	27	Cn	unphosphorylated nuclear *dClock* protein
	28	Cpn	singly phosphorylated nuclear *dClock* protein
Nuclear Protein Complex	29	PppTppCpn	doubly phosphorylated nuclear *period* protein dimerized with doubly phosphorylated nuclear *timeless* protein and unphosphorylated nuclear *dClock* protein

3 Parameter Variables

3.1 A Genetic Algorithm-Based Optimization Strategy

Through use of random trial and error, we identify an initial population of 25 "parent" parameter sets that yield oscillatory behavior. Given that our search space spans 36-dimensions[1], it is important to restrict these parameters within predetermined boundaries. Once the parent parameter vectors and their relative costs are defined, we use a selection operator where only the 11 fittest members are eligible for reproduction (Beyer and Schwefel, 2002; Goldberg, 1989; Michalewicz, 1996). The "genes" (or parameters) of two randomly chosen parents are recombined to form a "child" parameter vector. This child is mutated via a normally distributed random number with a mean of zero and a variance of 10% of the span (the upper bound minus the lower bound) of the given gene. 33 unique children are generated using this procedure. The accuracy of the model with respect to experimental observations is quantified for each child by the cost function, which establishes a means to regulate survival of the fittest. The pool of candidate vectors contains both parents and children, ensuring survival of the most fit parameter set. We run the algorithm three times (using three unique cost functions) for 60+ generations. Many of our resulting parameter sets are similar, suggesting that the optimizer does converge to a minimum.

The upper and lower bounds defining the range of parameter values were based on literature mining. Additional constraints confining parameter estimation relate to the relative magnitude of certain rate constants. DBT stably associates to and phosphorylates PER in both the cytoplasm and the nucleus (Kloss et al., 2001; Ko et al., 2002; Price et al., 1998). Hyperphosphorylated isoforms of PER are targeted by the F-box protein SLIMB (Hardin, 2005; Kim et al., 2007; Ko et al., 2002; Schoning and Staiger, 2005) or CK2 (Akten et al., 2003; Hardin, 2005; Lin et al., 2002), both of which lead to PER ubiquitination and degradation (Cyran et al., 2005; Ko et al., 2002). Given the temporal programming that underlies phosphorylation-dependent protein stability, we assume that PER has a higher chance of being degraded as the number of bound phosphates increases. Therefore, protein degradation rates should increase in magnitude as a result of phosphorylation. We impose this parameter constraint in our estimation problem via δ_{0p}, δ_{1p}, δ_{2p}, and so on, where each subscript reflects the number of phosphates (0, 1, or 2) on a given protein (in this case, p refers to PER). Assuming TIM (via SGG (Martinek et al., 2001)), VRI, PDP, and CLK undergo similar protein regulation, their degradation rates are similarly constrained such that $\delta_{i*} \leq \delta_{j*}$ given $i, j \in [0, 4]$ and $i < j$. Although we acknowledge that TIM stabilizes PER by binding it and thus preventing its degradation, the optimization strategy does not immediately reflect this condition since proteins (and protein complexes) bound to a greater number of phosphates are required to degrade at higher rate constants relative to their unphosphorylated counterparts. We do not establish additional optimization criteria mandating that the protein complex degrade at a lesser rate than the respective independent proteins.

3.2 Resulting Parameter Values

We outline the parameter variables used to describe the 29-state *Drosophila melanogaster* circadian model. The boundary conditions (LB - lower bound; UB - upper bound) used to limit the parameter estimation search, and the resulting parameter values (#) are defined for each variable in a separate column. Parameter values are rounded to the nearest tenths. All parameters are in units of [nM/hr] with the exception of:

i. Hill coefficients [-],

ii. Michaelis-Menten protein activation/inhibition constants [nM], and

iii. light [-].

[1]There are 84 parameters that characterize the proposed 29-state *Drosophila melanogaster* circadian model. Assuming similar rate constants, we lump certain parameters together to reduce the search space from an 84-dimensional problem to a 36-dimensional problem. The gene transcription rate of *per*, *tim*, *vri*, and *pdp*, for instance, are set equal. This method has been successfully adopted by several researchers (Leloup and Goldbeter, 1998; Ueda et al., 2001; Xie and Kulasiri, 2007).

The upper and lower bounds defining the range of parameter values were based on literature mining. We chose the mean value of each gene, or parameter, based on the mode (or most frequently established) values of similar parameters presented in circadian literature. The bounds were defined based on the span of these similar parameters. To further reduce the search space, we discretized the possible parameter space according to the relative sensitivity distributions common to circadian networks (Bagheri et al., 2007). If the state- and phase-based performance of a 10-state and 16-state circadian model was consistently more sensitive to perturbations within a certain parameter group (Bagheri et al., 2007), we allowed the optimizer to estimate the parameter to an accuracy of one hundredths. If the performance of a system is less sensitive to parametric perturbations, the optimizer chooses parameter values rounded to the ones place. In this study, parameters defining transcription or mRNA degradation rates were accurate to the hundredths. Those defining translation, protein degradation, (dis)association, and transport rates were accurate to the tenths. Finally, Hill coefficients, Michaelis-Menten constants and (de)phosphorylation rates are accurate to the ones place.

	no.	Var.	Description	LB	UB	#
Transcription	01	αcp	nuclear *dClock* protein activated *period* Michaelis-Menton (Hill-type) transcription rate	0.2	2.0	0.7
	02	αct	nuclear *dClock* protein activated *timeless* Michaelis-Menton (Hill-type) transcription rate	0.2	2.0	0.7
	03	αcv	nuclear *dClock* protein activated *vrille* Michaelis-Menton (Hill-type) transcription rate	0.2	2.0	1.2
	04	αcd	nuclear *dClock* protein activated *Pdp1* Michaelis-Menton (Hill-type) transcription rate	0.2	2.0	0.6
	05	αdc	nuclear *Pdp1* protein activated *dClock* Michaelis-Menton (Hill-type) transcription rate	0.2	2.0	1.6
	06	hcp	nuclear *dClock* protein activated *period* Hill coefficient	1.0	5.0	5
	07	hct	nuclear *dClock* protein activated *timeless* Hill coefficient	1.0	5.0	5
	08	hcv	nuclear *dClock* protein activated *vrille* Hill coefficient	1.0	5.0	5
	09	hcd	nuclear *dClock* protein activated *Pdp1* Hill coefficient	1.0	5.0	5
	10	hdc	nuclear *Pdp1* protein activated *dClock* Hill coefficient	1.0	5.0	5
	11	hvc	nuclear *vrille* protein inhibited *dClock* Hill coefficient	1.0	5.0	4
	12	$Kcap$	nuclear *dClock* protein activation of *period* expression - Michaelis-Menton (Hill-type) transcription rate	0.2	1.0	1.0
	13	$Kcat$	nuclear *dClock* protein activation of *timeless* expression - Michaelis-Menton (Hill-type) transcription rate	0.2	1.0	1.0
	14	$Kcav$	nuclear *dClock* protein activation of *vrille* expression - Michaelis-Menton (Hill-type) transcription rate	0.2	1.0	1.0
	15	$Kcad$	nuclear *dClock* protein activation of *Pdp1* expression - Michaelis-Menton (Hill-type) transcription rate	0.2	1.0	1.0
	16	$Kpac$	nuclear *Pdp1* protein activation of *dClock* expression - Michaelis-Menton (Hill-type) transcription rate	0.2	1.0	0.2
	17	$Kvic$	nuclear *vrille* protein inhibition of *dClock* expression - Michaelis-Menton (Hill-type) transcription rate	0.2	1.0	0.2
Translation	18	γp	*period* protein translation rate	0.1	2.0	1.2
	19	γt	*timeless* protein translation rate	0.1	2.0	1.2
	20	γv	*vrille* protein translation rate	0.1	2.0	2.0
	21	γd	*Pdp1* protein translation rate	0.1	2.0	1.6

	22	γc	*dClock* protein translation rate	0.1	2.0	1.6
Degradation	23	δmp	*period* mRNA degradation rate	0.1	2.0	0.1
	24	δmt	*timeless* mRNA degradation rate	0.1	2.0	0.1
	25	δmv	*vrille* mRNA degradation rate	0.1	2.0	0.2
	26	δmd	*Pdp1* mRNA degradation rate	0.1	2.0	0.1
	27	δmc	*dClock* mRNA degradation rate	0.1	2.0	0.5
	28	$\delta 0p$	unphosphorylated cytoplasmic *period* protein degradation rate	0.2	3.0	0.2
	29	$\delta 0pn$	unphosphorylated nuclear *period* protein degradation rate	0.2	3.0	0.2
	30	$\delta 1pn$	singly phosphorylated nuclear *period* protein degradation rate	0.2	3.0	0.2
	31	$\delta 2pn$	doubly phosphorylated nuclear *period* protein degradation rate	0.2	3.0	1.5
	32	$\delta 0t$	unphosphorylated cytoplasmic *timeless* protein degradation rate	0.2	3.0	0.2
	33	$\delta 1t$	singly phosphorylated cytoplasmic *timeless* protein degradation rate	0.2	3.0	0.2
	34	$\delta 2t$	doubly phosphorylated cytoplasmic *timeless* protein degradation rate	0.2	3.0	1.5
	35	$\delta 1tn$	singly phosphorylated nuclear *timeless* protein degradation rate	0.2	3.0	0.2
	36	$\delta 2tn$	doubly phosphorylated nuclear *timeless* protein degradation rate	0.2	3.0	1.5
	37	$\delta 0v$	unphosphorylated cytoplasmic *vrille* protein degradation rate	0.2	3.0	0.7
	38	$\delta 1v$	singly phosphorylated cytoplasmic *vrille* protein degradation rate	0.2	3.0	1.4
	39	$\delta 0vn$	unphosphorylated nuclear *vrille* protein degradation rate	0.2	3.0	0.7
	40	$\delta 1vn$	singly phosphorylated nuclear *vrille* protein degradation rate	0.2	3.0	1.4
	41	$\delta 0d$	unphosphorylated cytoplasmic *Pdp1* protein degradation rate	0.2	3.0	0.2
	42	$\delta 1d$	singly phosphorylated cytoplasmic *Pdp1* protein degradation rate	0.2	3.0	0.2
	43	$\delta 0dn$	unphosphorylated nuclear *Pdp1* protein degradation rate	0.2	3.0	0.2
	44	$\delta 1dn$	singly phosphorylated nuclear *Pdp1* protein degradation rate	0.2	3.0	0.2
	45	$\delta 0c$	unphosphorylated cytoplasmic *dClock* protein degradation rate	0.2	3.0	0.2

	46	$\delta 0cn$	unphosphorylated nuclear *dClock* protein degradation rate	0.2	3.0	0.2
	47	$\delta 1cn$	singly phosphorylated nuclear *dClock* protein degradation rate	0.2	3.0	0.2
	48	$\delta 2cn$	doubly phosphorylated nuclear *dClock* protein degradation rate	0.2	3.0	2.1
	49	$\delta 4xn$	degradation rate of nuclear protein complexes with 4 phosphate groups	0.2	3.0	2.4
Phosphorylation	50	$\epsilon p1^+$	unphosphorylated *period* protein phosphorylation rate	0.2	8.0	0.2
	51	$\epsilon p1^-$	singly phosphorylated *period* protein dephosphorylation rate	0.1	4.0	2.0
	52	$\epsilon p2^+$	singly phosphorylated *period* protein phosphorylation rate	0.2	8.0	0.2
	53	$\epsilon p2^-$	doubly phosphorylated *period* protein dephosphorylation rate	0.1	4.0	2.0
	54	$\epsilon t1^+$	unphosphorylated *timeless* protein phosphorylation rate	0.2	8.0	0.2
	55	$\epsilon t1^-$	singly phosphorylated *timeless* protein dephosphorylation rate	0.1	4.0	2.0
	56	$\epsilon t2^+$	singly phosphorylated *timeless* protein phosphorylation rate	0.2	8.0	0.2
	57	$\epsilon t2^-$	doubly phosphorylated *timeless* protein dephosphorylation rate	0.1	4.0	2.0
	58	$\epsilon v1^+$	unphosphorylated *vrille* protein phosphorylation rate	0.2	8.0	0.2
	59	$\epsilon v1^-$	singly phosphorylated *vrille* protein dephosphorylation rate	0.1	4.0	2.0
	60	$\epsilon d1^+$	unphosphorylated *Pdp1* protein phosphorylation rate	0.2	8.0	0.2
	61	$\epsilon d1^-$	singly phosphorylated *Pdp1* protein dephosphorylation rate	0.2	8.0	2.0
	62	$\epsilon c1^+$	unphosphorylated *dClock* protein phosphorylation rate	0.2	8.0	0.2
	63	$\epsilon c1^-$	singly phosphorylated *dClock* protein dephosphorylation rate	0.1	4.0	2.0
	64	$\epsilon c2^+$	singly phosphorylated *dClock* protein phosphorylation rate	0.2	8.0	0.2
	65	$\epsilon c2^-$	doubly phosphorylated *dClock* protein dephosphorylation rate	0.1	4.0	2.0
Dimerization	66	ζ^+	doubly phosphorylated *period* protein, doubly phosphorylated *timeless* protein, and unphosphorylated *dClock* protein association rate	0.2	4.0	3.9
	67	ζ^-	doubly phosphorylated *period* protein, doubly phosphorylated *timeless* protein, and unphosphorylated *dClock* protein disassociation rate	0.1	2.0	0.1

Transport	68	βp	transport of nuclear *period* pre-mRNA to cytoplasmic mRNA	0.1	4.0	1.4
	69	βt	transport of nuclear *timeless* pre-mRNA to cytoplasmic mRNA	0.1	4.0	1.4
	70	βv	transport of nuclear *vrille* pre-mRNA to cytoplasmic mRNA	0.1	4.0	1.4
	71	βd	transport of nuclear *Pdp1* pre-mRNA to cytoplasmic mRNA	0.1	4.0	1.4
	72	βc	transport of nuclear *dClock* pre-mRNA to cytoplasmic mRNA	0.1	4.0	1.4
	73	ηp^+	nuclear transport of unphosphorylated *period* protein	0.2	8.0	2.8
	74	ηp^-	cytoplasmic transport of unphosphorylated *period* protein	0.1	4.0	1.4
	75	ηt	cytoplasmic transport of singly phosphorylated *timeless* protein	0.1	4.0	1.4
	76	ηt^+	nuclear transport of doubly phosphorylated *timeless* protein	0.2	8.0	2.8
	77	ηt^-	cytoplasmic transport of doubly phosphorylated *timeless* protein	0.1	4.0	1.4
	78	ηv^+	nuclear transport of singly phosphorylated *vrille* protein	0.2	8.0	2.8
	79	ηv^-	cytoplasmic transport of singly phosphorylated *vrille* protein	0.1	4.0	1.4
	80	ηd^+	nuclear transport of singly phosphorylated *Pdp1* protein	0.2	8.0	2.8
	81	ηd^-	cytoplasmic transport of singly phosphorylated *Pdp1* protein	0.1	4.0	1.4
	82	ηc^+	nuclear transport of unphosphorylated *dClock* protein	0.2	8.0	2.8
	83	ηc^-	cytoplasmic transport of unphosphorylated *dClock* protein	0.1	4.0	1.4
Light	84	l	light-induced *timeless* protein degradation rate	0.1	2.0	2.0

References

Akten B, Jauch E, Genova GK, Kim EY, Edery I, Raabe T, and Jackson FR (2003) A role for CK2 in the *Drosophila* circadian oscillator. Nat Neurosci 6:251–257.

Bagheri N, Stelling J, and Doyle III F (2007) Quantitative performance metrics for robustness in circadian rhythms. Bioinformatics 23:358–364.

Beyer HG and Schwefel HP (2002) Evolution strategies. Nat Comput 1:3–52.

Cyran SA, Yiannoulos G, Buchsbaum AM, Saez L, Young MW, and Blau J (2005) The double-time protein kinase regulates the subcellular localization of the *Drosophila* clock protein period. J Neurosci 25:5430–5437.

Goldberg DE (1989) Genetic Algorithms in Search, Optimization, and Machine Learning. Addison-Wesley Publishing Company, Inc., Reading MA.

Hardin PE (2005) The circadian timekeeping system of *Drosophila*. Curr Biol 15:714–722.

Kim EY, Ko HW, Yu W, Hardin PE, and Edery I (2007) A DOUBLETIME kinase binding domain on the *Drosophila* PERIOD protein is essential for its hyperphosphorylation, transcriptional repression, and circadian clock function. Mol Cell Biol 27:5014–5028.

Kloss B, Rothenfluh A, Young MW, and Saez L (2001) Phosphorylation of PERIOD is influenced by cycling physical associations of DOUBLE-TIME, PERIOD, and TIMELESS in the *Drosophila* clock. Neuron 30:699–706.

Ko HW, Jiang J, and Edery I (2002) Role for Slimb in the degradation of *Drosophila* Period protein phosphorylated by Doubletime. Nature 420:673–678.

Leloup JC and Goldbeter A (1998) A model for circadian rhythms in *Drosophila* incorporating the formation of a complex between the PER and TIM proteins. J Biol Rhythms 13:70–87.

Lin JM, Kilman VL, Keegan K, Paddock B, Emery-Le M, Rosbash M, and Allada R (2002) A role for casein kinase 2a in the *Drosophila* circadian clock. Nature 420:816–820.

Martinek S, Inonog S, Manoukian AS, and Young MW (2001) A role for the segment polarity gene *shaggy*/gsk-3 in the *Drosophila* circadian clock. Cell 105:769–779.

Michalewicz Z (1996) Genetic Algorithms + Data Structures = Evolution Programs. Springer-Verlag, New York NY, 3rd ed.

Price JL, Blau J, Rothenfluh A, Abodeely M, Kloss B, and Young MW (1998) *double-time* is a novel *Drosophila* clock gene that regulates PERIOD protein accumulation. Cell 94:83–95.

Schoning JC and Staiger D (2005) At the pulse of time: protein interactions determine the pace of circadian clocks. FEBS Letters 579:3246–3252.

Ueda HR, Hagiwara M, and Kitano H (2001) Robust oscillations within the interlocked feedback model of *Drosophila* circdian rhythm. J theor Biol 210:401–406.

Xie Z and Kulasiri D (2007) Modelling of circadian rhythms in *Drosophila* incorporating the interlocked PER/TIM and VRI/PDP1 feedback loops. J theor Biol 245:290–304.