PER/TIM-mediated amplification, gene dosage effects and temperature compensation in an interlocking-feedback loop model of the *Drosophila* circadian clock

Peter Ruoff[a,*], Melinda K. Christensen[a], Vijay K. Sharma[b]

[a]*Faculty of Science and Technology, University of Stavanger, N-4036 Stavanger, Norway*
[b]*Chronobiology Laboratory, Evolutionary & Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, P.O. Box. 6436, Jakkur, Bangalore 560064, Karnataka, India*

Abstract

We have analysed a first-order kinetic representation of a interlocking-feedback loop model for the *Drosophila* circadian clock. In this model, the transcription factor *Drosophila* CLOCK (dCLK) which activates the clock genes *period* (*per*) and *timeless* (*tim*) is subjected to positive and negative regulations by the proteins 'PAR Domain Protein 1' (PDP1) and VRILLE (VRI), whose transcription is activated by dCLK. The PER/TIM complex binds to dCLK and in this way reduces the activity of dCLK. The results of our simulations suggest that the positive and negative feedback loops of *Pdp1* and *vri* are essential for the overall oscillations. Although self sustained oscillations can be obtained without *per/tim*, the model shows that the PER/TIM complex plays an important role in amplification and stabilization of the oscillations generated by the *Pdp1/vri* positive/negative feedback loops. We further show that in contrast to a single (*per/tim*) negative feedback loop oscillator, the interlocking-feedback loop model can readily account for the effect of gene dosages of *per*, *vri*, and *Pdp1* on the period length. Calculations of phase resetting on a temperature compensated version of the model shows good agreement with experimental phase response curves for high and low temperature pulses. Also, the partial losses of temperature compensation in *per*^{S} and *per*^{L} mutants can be described, which are related to decreased stabilities of the PER/TIM complex in *per*^{S} and the stronger/more stable inhibitory complex between dCLK and PER/TIM in *per*^{L}, respectively. The model shows (somewhat surprisingly) poor entrainment properties, especially under extended light/dark (L/D) cycles, which suggests that parts of the L/D tracking or sensing system are not well represented.
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1. Introduction

Circadian rhythms (Bünning, 1963; Dunlap et al., 2003; Edmunds, 1988) play important roles in the adaptation of organisms to their environments. They act as physiological clocks and exhibit homeostasis of the circadian period against environmental variations such as in temperature, pH, or nutrients (Pittendrigh, 1993; Pittendrigh and Caldarola, 1973). The use of molecular genetic tools have helped to identify clock genes such as *period* (*per*) (Konopka and Benzer, 1971; Rosbash et al., 2003) and *frequency* (*frq*) (Feldman and Hoyle, 1973; Froehlich et al., 2003) in *Drosophila* and *Neurospora*, respectively.

A common element in the mechanisms of circadian rhythms is the presence of negative feedback loops (Dunlap, 1999). Recently, however, positive feedback loops have also been identified (Cyran et al., 2003; Lee et al., 2000), which points to the possibility that, similar

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*Corresponding author. Tel.: +47 51 83 1887; fax: +47 51 83 1750.
  E-mail address: peter.ruoff@uis.no (P. Ruoff).

to chemical oscillators (Franck, 1980; Higgins, 1967), positive *and* negative feedback loops are both important for the generation and stability of circadian rhythms.

Computational models (Goldbeter, 2002) have the potential to provide insights into the cellular processes, environmental influences (such as temperature, light, pH, nutritional conditions), and other yet unexplored aspects of the circadian oscillator. Such models are capable of making (quantitative) predictions which can be tested experimentally. In recent years a variety of reaction kinetic models have been developed for model organisms such as *Drosophila* (Hong and Tyson, 1997; Leloup and Goldbeter, 2000; Smolen et al., 2004; Ueda et al., 2001), *Neurospora* (Gonze et al., 2000; Ruoff et al., 1999a, b, 2001; Smolen et al., 2003), mammals (Forger and Peskin, 2003; Leloup and Goldbeter, 2003), plants (Johnsson et al., 1973; Lillo and Ruoff, 1984; Luttge, 2000; Neff et al., 1998), and other insects except *Drosophila* (Lewis, 1994). Among these the *Drosophila* circadian clock is most intensively studied: the basic mechanism involves the expression of the PERIOD (PER) and TIMELESS (TIM) proteins and the formation of a heterodimer (PER/TIM), which is then transported into the nucleus where it inhibits the transcription of PER and TIM by binding to their transcription factor dCLK.CYC, a heterodimer between dCLOCK and CYCLE (Lee et al., 1999). The CYC concentrations were found to be in excess over the dCLK concentrations suggesting that the activity of dCLK.CYC is determined by the amount of dCLK (Bae et al., 2000).

Recently, two additional feedback loops were identified as parts of the core circadian pacemaker in *Drosophila*. In one of the loops, VRILLE (VRI), a protein which is activated by dCLK.CYC was found to repress transcription of the *dClk* gene forming a negative feedback loop, while in another positive feedback loop the protein 'PAR Domain Protein 1' (PDP1) activates the transcription of *dClk*, which itself activates the transcription of *Pdp1* (Blau and Young, 1999; Glossop et al., 2003). Because of the presence of both positive (activatory) and negative (inhibitory) feedbacks which deviate from previously studied single negative feedback models, we were interested in investigating the possible roles of the positive and negative feedback loops in a model with respect to the generation and stabilization of circadian oscillations in *Drosophila*.

Here we show that a representation of the negative and positive feedback loops by (mostly) first-order processes suggests that the PER/TIM heterodimer with support from the *Pdp1* mediated positive feedback loop, acts as an amplifier and stabilizer for the *vri/Pdp1*-generated oscillations. In contrast to a single negative feedback oscillator, the interlocking-feedback loop model can easily account for *per* and *vri* gene dosage effects on the circadian period. While our work was in progress, results from a corresponding model appeared (Smolen et al., 2004). Although our calculations agree in many aspects with those of Smolen et al. (2004), for example in *per* and *vri* gene dosage effects on the period, a significant difference exists with respect to the role of the PER/TIM complex as an oscillation amplifier and the importance of the *Pdp1*-based positive feedback loop for the stabilization of the oscillations.

2. Computational method

2. 1. The model

Analogous to the Goodwin oscillator (Goodwin, 1965; Ruoff et al., 1996), transcription and translation processes in our model are represented as first-order processes (Eqs. (1)–(12)), with exception of reactions (1) and (17) (Fig. 1). The representation of the (enzyme-catalysed) processes by first-order reactions complies with the view that many enzymes in vivo are present in low concentrations and work in the first-order range of their respective substrates (Dixon et al., 1979). Non-linear terms are included only for the activation of *dClk* transcription (positive feedback) by PDP1 and the inhibition of *dClk* transcription (negative feedback) by VRI (Fig. 1). The respective cooperativities (Eq. (1)) are described by numbers m ($0 \leqslant m \leqslant 1$) and n ($1 \leqslant n \leqslant 6$). For the sake of simplicity, *per* and *tim* are described as one variable (*per/tim*) and a distinction between the cytosolic and nuclear forms of PER/TIM have not been made. The active nuclear form of VRI (VRI_n^*) inhibits the transcription of *dClk* while the active nuclear form of PDP1 ($PDP1_n^*$) activates *dClk* transcription. Because CYC is always present at much higher concentrations than dCLK (Bae et al., 2000), the transcription factor dCLK.CYC is represented by a active nuclear form of dCLK, i.e. $dCLK_n^*$. The genes *vri*, *Pdp1*, and *per/tim* are activated by $dCLK_n^*$, while $dCLK_n^*$ becomes inactive after binding to PER/TIM (Fig. 1). Because we consider in our model only nuclear proteins in their active forms (i.e. VRI_n^*, $PDP1_n^*$, and $dCLK_n^*$) no explicit mass balance between nuclear forms (which should include active and inactive species) and cytosolic forms is formulated. This is an analogous approach as taken earlier in the Goodwin model (Ruoff and Rensing, 1996). The model's rate equations are as follows:

$$\frac{\mathrm{d}[dClk - mRNA]}{\mathrm{d}t} = k_1 [PDP1_n^*]^m \frac{K_d}{K_d + [VRI_n^*]^n} - k_9 [dClk - mRNA], \qquad (1)$$

$$\frac{\mathrm{d}[dCLK_c]}{\mathrm{d}t} = k_2 [dClk - mRNA] - k_{23}[dCLK_c], \qquad (2)$$