The Role of Clockwork Orange and MicroRNA in the Drosophila Melanogaster Circadian Clock

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The Circadian Clock

The circadian clock exists in many living organisms. It works to maintain homeostasis in animals by adapting to changes in the environment.[2] The clock primarily controls rhythms based on behavior and physiology such as locomotor activity, sleep, and memory. The system consists of an input, a core oscillator, and an output. External cues such as light and temperature are received through the input pathway. The cues ultimately synchronize the core oscillator to the organism's environmental surroundings, and finally sends information from the core to circadian rhythms.[3]

Link to Disease

A wide variety of animal models have been used by scientists to examine the physiological effects and diseases caused by disruptions in circadian rhythms. For example, Filipski et al. (2006) found that the deterioration of the suprachiasmatic nucleus (SCN), a small region of the brain located in the hypothalamus responsible for controlling circadian rhythms, greatly increases the growth of a malignant tumor. There are also health implications, including increased mortality rate, associated with desynchronization of the circadian clock. The clock can be desynchronized by exposure to constant light or by repeated shifting of the light-dark cycle. Furthermore, drastic changes in clock timing is thought to contribute toward a large number of nervous system disorders.[1]

Clockwork Orange

- Transcriptional repressor that regulates many parts of cell physiology and metabolism [2]
- Plays a role in causing high-amplitude transcriptional oscillations in the clock [5]
- Works with CLK to regulate pacemaker amplitude [5]
- Does not directly affect CLK and has little influence on CLK levels [5]

MicroRNA

- Small noncoding RNAs that act as post-transcriptional regulators of gene expression[2]
- Regulate developmental and physiological processes[2]
- Little information on their behavior and how they affect the nervous system [2]
- Play a role in controlling the central pacemaker of the circadian clock [2]

Why *Drosophila melanogaster*?

Drosophila melanogaster, commonly known as the fruit fly, is a well-known model organism with a circadian clock that has been studied for decades. This clock is an oscillator which is governed by positive and negative feedback loops. This study looks to further examine the role of CLOCKWORK ORANGE (CWO) and microRNA (miRNA) by preparing their incorporation for an *in silico* model of the *D. melanogaster* circadian clock.

PER and TIM Feedback Loop

The proteins CLOCK (CLK) and CYCLE (CYC) act as important regulators of circadian rhythms by controlling the transcription of clock genes. The proteins PERIOD (PER) and TIMELESS (TIM) work to repress CLK- and CYC-mediated transcription late in the approximately 24-hour clock cycle.[3]

VRI and PDP1 Feedback Loop

The second feedback loop is controlled by VRILLE (VRI) and PAR Domain Protein 1 (PDP1). This loop influences he expression of *clk* mRNA, which impacts the transcriptional activation governed by CLK. In this way, VRI-and PDP1-induced feedback is a molecular mechanism modulating the rhythmic expression of circadian mRNA and proteins. Expression of VRI and PDP1 is directly affected by CLK and CYC.[4]

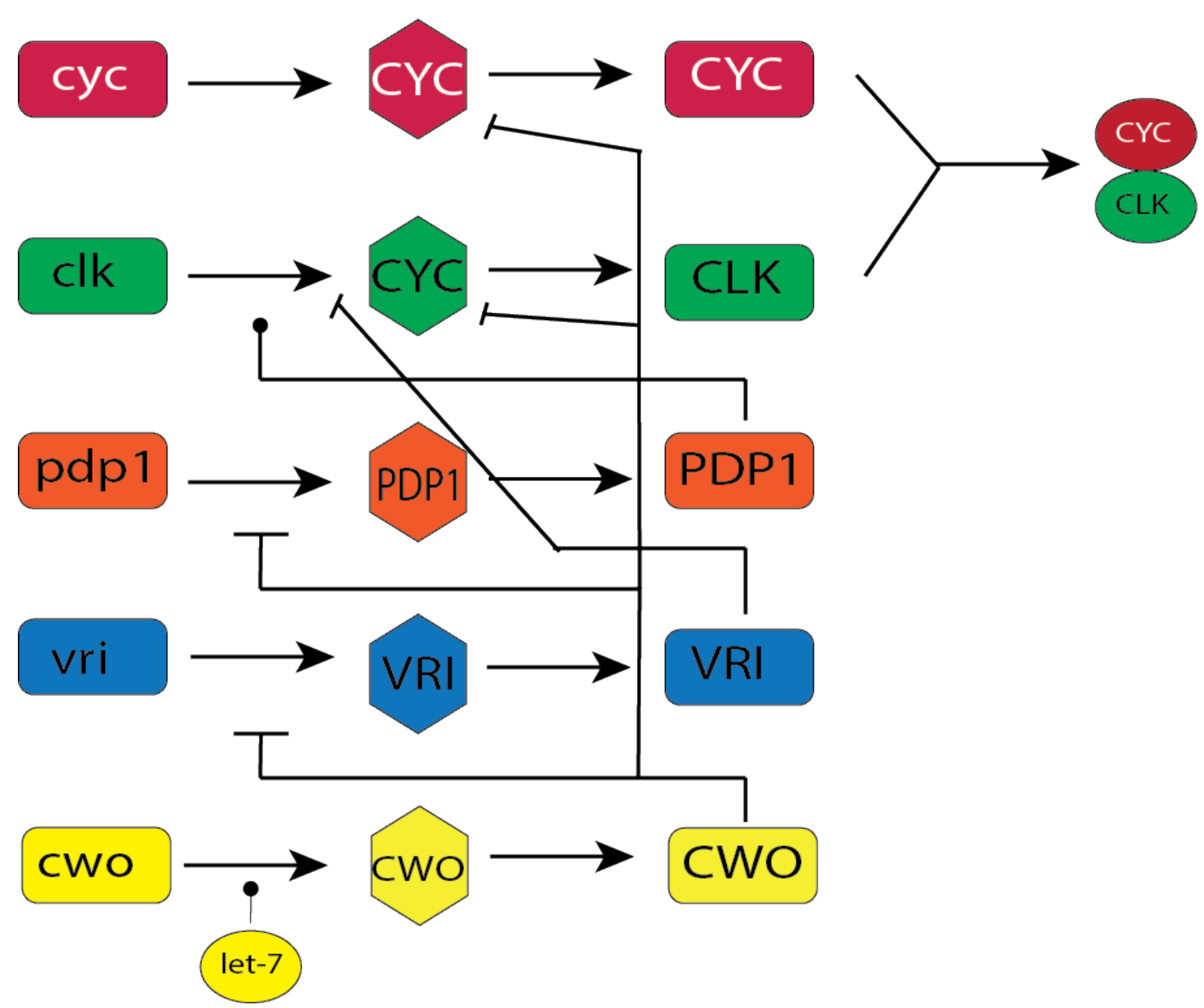

Figure1 : CWO and let-7 play a role in the VRI/PDP1 feedback loop.
This diagram highlights the proposed role of CLOCKWORK ORANGE (CWO) and the micro-RNA let-7 in one loop of the *D. melanogaster* circadian clock.

Let-7

MicroRNA-mediated post-transcriptional regulation is an important process within the circadian clock. The microRNA let-7 serves as a regulator of circadian rhythms through repression of CLOCKWORK ORANGE (CWO). Overexpression of let-7 in *D. melanogaster* increases circadian period, whereas removal of let-7 decreases period and molecular oscillation.[3]

Conclusion

The studies reviewed here provide evidence for the importance of CLOCKWORK ORANGE (CWO) in the *Drosophila melanogaster* circadian clock. Specifically, CWO acts as a transcriptional repressor and works alongside PER to inhibit CLK-mediated transcription of other circadian genes. CWO-induced feedback also helps to regulate behavioral rhythms in *D. melanogaster.*

This model also includes the microRNA let-7, which is important for regulation of core clock components such as the timekeeping mechanism. With our research, we hope to gather more information on the role of let-7 and CWO within *D. melanogaster* to further improve our model.

References: 1. Hansen, K. F., Sakamoto, K., & Obrietan, K. (2011). MicroRNAs: a potential interface between the circadian clock and human health. *Genome Medicine*,*3*(2), 10 2. Kadener, S., Stoleru, D., McDonald, M., Nawathean, P., & Rosbash, M. (2007).*Clockwork Orange* is a transcriptional repressor and a new *Drosophila* circadian pacemaker component. *Genes & Development*, *21*(13), 1675–1686 3. W. Chen, Z. Liu, T. Li, R. Zhang, Y. Xue, Y. Zhong, W. Bai, D. Zhou, Z. Zhao Regulation of Drosophila circadian rhythms by miRNA let-7 is mediated by a regulatory cycle. Nat Commun, 5 (2014) 4. Cyran SA, Buchsbaum AM, Reddy KL, Lin MC, Glossop NR, Hardin PE, Young MW, Storti RV, Blau J (2003) vrille, Pdp1, and dClock form a second feedback loop in the Drosophila circadian clock. Cell 112:329 –341 5. Lim, C., Chung, B. Y., Pitman, J. L., McGill, J. J., Pradhan, S., Lee, J., Keegan, K.P., Choe, J., Allada, R. (2007). clockwork orange encodes a transcriptional repressor important for circadian clock amplitude in Drosophila. Current Biology : CB, 17(12), 1082–1089 6. Filipski, E., Li, X.M., Lévi, F. (2006). Disruption of coordination and malignant growth. Cancer Causes Control 17:509-514

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