Circadian Clock Synchronization and the Roles of PDF, cAMP, and PKA in *Drosophila melanogaster*

Devanie Tucker, Kate Scheuer, and Laurence Loewe

Introductory Biology 152, Laboratory of Genetics & Wisconsin Institute for Discovery, University of Wisconsin-Madison

Abstract

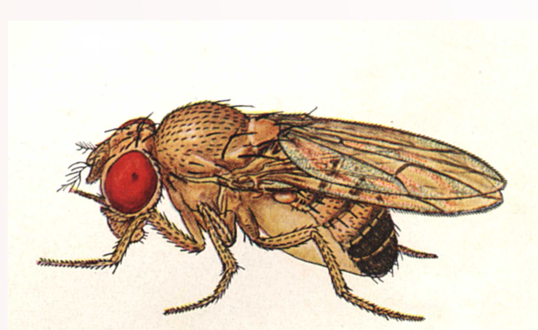
The circadian clock is a ~24 hour rhythm that regulates a variety functions, including sleep, metabolism, and behavior. Due to this widespread influence, circadian rhythms are an important area of research today. The fruit fly *Drosophila melanogaster* is a well-known model organism for the study of circadian rhythms because of its substantial similarities with the human circadian clock, and the reproductive speed, simple manipulation, and genomic and genetic manipulation tools available for this fly. The current study builds off previous research to better define the contributions of PDF, cAMP, and PKA to the clock. Current research suggests that light causes PDF release, which induces activation of cAMP, and signals the cAMP dependent kinase, PKA, to phosphorylate PER and TIM (two proteins crucial to the clock). The phosphorylation of the proteins PER and TIM prolongs their degradation, which is a necessary step in the synchronization of the overall ~24 hour circadian rhythm.

Why circadian clocks?

The circadian clock helps organisms perform functions like regulating metabolism, behavior, and sleep. Our lab is working to build a mathematical model of the circadian clock of *Drosophila melanogaster* that integrates known results. We are building the model based on analysis of previous studies of the *Drosophila* circadian clock. Once complete, the data will be tested against wet lab data to investigate its accuracy and determine how it can be improved by further experiments.

Why *Drosophila melanogaster*?

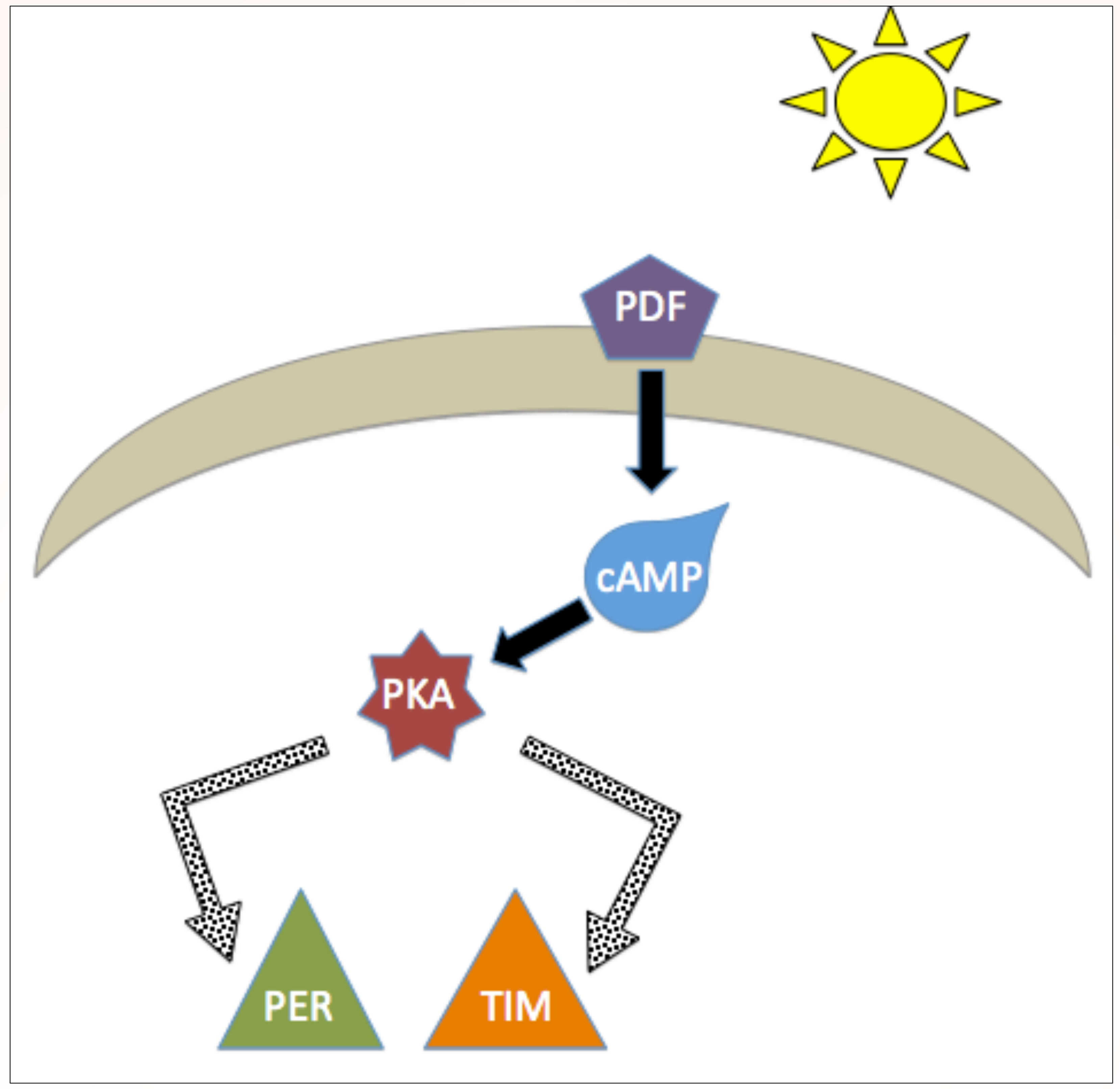
Drosophila melanogaster is a useful model organism for circadian rhythms because of its substantial similarities with the human circadian clock, its ease of maintenance and the vast genomic and genetic manipulation tools available. About 40 years ago, studies to identify the genes involved in circadian regulation were conducted on *Drosophila*. Despite much progress, many aspects of the *D. melanogaster* clock and circadian rhythms in general remain unclear.

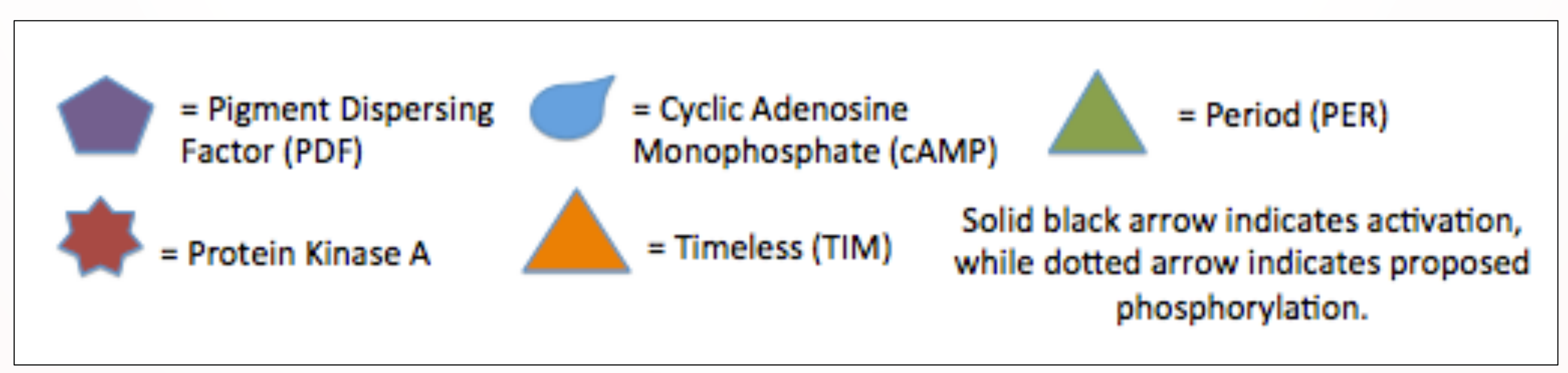


Drosophila Melanogaster

PER and TIM

Two main clock genes regulate the clock, *period* (*per*) and *timeless* (*tim*), which encode the proteins PER and TIM. After their translation in the cytoplasm, they need to eventually dimerize with each other before they can move back into the nucleus, where they inhibit further transcription. This creates a negative feedback loop that is regulated by many other components besides just PER and TIM. This study focuses on the synchronizing neuropeptide *pigment dispersing factor* (PDF) and its role in the pathway of *cyclic adenosine monophosphate* (cAMP) and *protein kinase A* (PKA).





Conclusions

- Light causes PDF release within minutes
- PDF signals cAMP production via G α coupling
- cAMP is used for intracellular signal transduction
- PKA is dependent on the cAMP signal
- Once PKA is activated, it will phosphorylate:
  - PER within hours
  - TIM within hours
- This process stabilizes the synchronization of clock cells and helps keep the circadian rhythm

Methods

Data was collected from past studies surrounding the role of the cAMP/PKA pathway, using Web of Science to find relevant research projects. We analyzed the information gathered to construct a model of the pathway from the information in the primary literature. Keywords of the initial Web of Science search: "cAMP", "PKA", "*Drosophila melanogaster*". Results were screened for articles relevant to circadian clock function. Important information was broken down into "Claims", that were recorded in a text document along with details of the source to enable fast lookup (article reference, claim page number, column, paragraph).
Goal: After a conclusion will be reached about the importance of the cAMP/PKA pathway and the function of all of the parts, these suggested pathways will be added to a mathematical model of the *Drosophila melanogaster* circadian clock.

Results

How is PDF released?

- Light enters the organism and within minutes PDF is released [1]
- Light increases the firing rate of PDF neurons [3]

What signals trigger the production of cAMP?

- PDF binds to its receptor, PDFR
- PDFR activation increases intracellular cAMP, indicating the PDFR is coupled to a G α subunit [1]
- The G alpha subunit binds to and activates an enzyme called adenylate cyclase, which then catalyzes the conversion of ATP into cAMP
- Another study confirms the signaling of PDF through cAMP by activating PDF receptors and seeing an increase in cAMP, and also genetically increasing cAMP and seeing a reduced amplitude of PDF responses [2]

What is PKA's role in the circadian clock?

- Two molecules cAMP bind to each molecule of PKA, activating the enzyme
- PKA phosphorylates and stabilizes PER within hours [3]
  - Rates of PER degradation were increased when a PKA inhibitor was added
  - PER degradation was also decreased by adding amounts of cAMP and PDF, confirming that PDF signals through cAMP, which increases PKA levels and phosphorylates PER
- PKA phosphorylates and stabilizes TIM within hours [4]
  - Decreased amounts of TIM found in flies with PKA inhibitor
  - Decreased amounts of TIM found in flies with reduced PDF levels
  - Indicates that PDF signals through cAMP, which increases PKA levels and phosphorylates TIM

References

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