Using Real-time PCR to Examine Variation of Expression of Detoxifying Enzymes in Drosophila over the Course of a 24-hour Period

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My thesis project involves investigating whether the response of an organism to xenobiotic agents is regulated by the biological clock, a molecular machine which controls important physiological responses such as sleep, feeding, and mating. In particular, I focus on the enzymes involved in detoxifying harmful compounds from the body, asking whether these enzymes are under the control of the biological clock of an organism. To accomplish this, I needed to acquire proficiency in a tool which would allow us to connect physiological response to molecular behavior.

Thanks to the Alan E. Leviton Student Research Award presented by AAAS, Pacific Division, our lab has gained the ability to add a powerful new molecular research tool in the form of quantitative real-time PCR. I applied the award money to the development of a protocol and procedures for utilizing this molecular technique in analyzing gene expression of *Drosophila melanogaster*. As this is a new technique in our laboratory, it required extensive troubleshooting and optimization in order to adapt it to both our project and our organism. With the assistance of another lab within our department, I searched for a group of genes for use as housekeeping reference genes for basal and induced response using traditional published reference genes as well as those listed as possessing minimal oscillation in the database of circadian genes available online. Both automated software and manual methods were utilized to design primers for the detoxifying genes of interest as well as potential genes for use as normalization factors.

I performed several real-time PCR reactions to validate the microarray database results and select a housekeeping gene for use as an endogenous control. I chose a ribosomal protein gene, rpl32, for use as our basal normalization gene. Experimental verification of rpl32 shows minimal variation in RNA expression across the 24 hour circadian day, an important factor in our experiments due to the circadian nature of the genes we are investigating.

I've begun my investigation by examining Canton-S wild-type flies to determine if the enzymes responsible for detoxification display rhythmic oscillation at the mRNA level. Our initial results indicate that relative RNA levels for two P-450 enzymes investigated, cyp6g1 and cyp6a2, show significant decline in the middle of the day. This would suggest that these enzymes may be under the control of the circadian clock.

Because of this research award, our laboratory has gained a powerful new tool to investigate the molecular underpinnings of the physiological observations for my current project as well as for other projects underway throughout the lab. The future direction of my project will be to examine the gene expression of these enzymes in *Drosophila* lines which have had various components of the molecular clock abolished to determine if a specific clock component is responsible for regulating detoxification genes. These lines will also allow us to verify that regular expression of these genes is dependent upon an intact molecular clock.

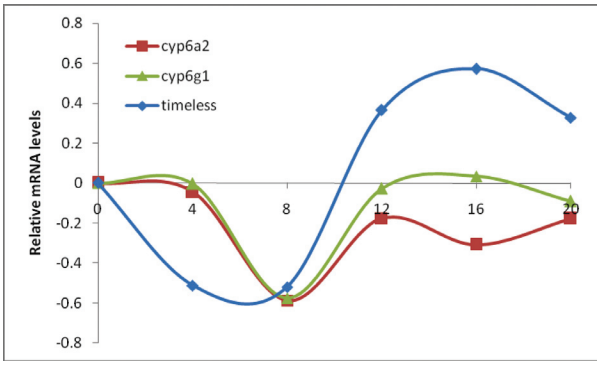

Figure 1. Relative mRNA expression for cyp6a2 and cyp6g1 across the circadian day. Adult flies were collected at different time points during the day in an LD cycle (Zeitgeber time, ZT, where lights-on is at ZT 0 and lights-off is at ZT12). Timeless is included for comparison and served as a positive control with known circadian mRNA expression. All RNA expression was normalized to rpl32 and represents expression relative to ZT 0.