Circadian control of the daily rhythm of adult emergence by regulation of the timing of ecdysone action in *Drosophila melanogaster*

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ABBREVIATIONS

20E: 20- hydroxyecdysone

BPE: Before prediceted eclosion

CA: *Corpora allata*

clk: clock

CLK: CLOCK

CNS: Central nervous system

cry: *cryptochrome*

CYC: CYCLE

D:D: Constant darkness

E: Ecdysone

EcR: Ecdysone receptor

EcR DN: Ecdysone receptor dominant negative

EH: Eclosion hormone

ETH: Ecdysone Triggering Hormone

FISH: Fluorescent *in situ* hybridization

GC: Glucocorticoid

GR: Glucocorticoid receptor

h: Hour

IHC: Immunohistochemistry

L:D: Light:Dark cycle

mM: milimolar

mRNA: Messenger ribonucleic acid

nl: Nanoliters

NPF: Neuropeptide F

NRs: Nuclear receptors

ORF: Open reading frame

p: Period

per: *period*

PER: PERIOD

Pdp1: *PAR Domain Protein 1*

pdf: pigment dispersing factor

PDF: Pigment dispersing factor neuropeptide

pmol: Picomol

PTMs: Post-translational modifications

PTTH: Prothoracicotropic hormone

RI: Rhythmicity Index

sLNvs: Small lateral ventral neurons

SNC: Suprachiasmatic nucleus

sNPF: small Neuropeptide F

tb: time of bristle pigmentation

te: time of eclosion

tim: timeless

th: time of head eversion

TIM: TIMELESS

tp: Time of prepupal formation

TTFL: Transcription-translation feedback loops

ty: time of yellow eye pigmentation

UAS: Upstream activating sequences

USP: Ultraspiracle

vri: vrille

WP: white-prepupae

ZT: Zeitgeber

In *Drosophila melanogaster*, the circadian clock imposes a daily rhythm to the pattern of adult emergence (eclosion) by a process that has been described as "gating". My research is aimed at identifying the mechanism by which the daily "gating" of the time of emergence occurs.
In this insect, the circadian clock sets the time of emergence through the coupling between the central clock located in the brain and a peripheral clock contained in the Prothoracic Gland (PG), an endocrine gland whose only known function is the production of the molting hormone, ecdysone (E). The levels of E increase to cause the larval molts and metamorphosis, and then drop to signal the end of each molt. Previous work in the insect, *Rhodnius prolixus,* suggests that ecdysone is involved in the gating of eclosion because injections of increasing doses of E delay the time of eclosion to later times ***in a continuous fashion***, suggesting that the titer of E itself is central to the gating mechanism. Moreover, in *Rhodnius*, molting is accompanied by circadian oscillations in ecdysone titers, produced as a consequence of its rhythmic release. Nevertheless, this rhythm of E does not seem to be a general feature among insects. In *Drosophila*, in particular, no circadian oscillations in E have been detected during metamorphosis. However, whether the clock regulates ecdysone signaling through a different mechanism, remains to be explored. Thus, the main goal of my Doctoral thesis is **to use *Drosophila* to explore the role of E in the circadian gating of eclosion** by asking whether **there are elements of the E pathway that are under circadian control and what are the molecular mechanisms used by the clock to impose a daily rhythmicity to adult emergence**. To address this question, in **Chapter 2** I first asked whether the clock regulates the time when flies commit to end metamorphosis and whether the clock can set the time of adult emergence by regulating ecdysone levels. Since my results support a mechanism where the clock acts downstream of E, I also disrupted E signaling in the PG via its receptor, EcR, and evaluated the consequences of these manipulations on the rhythm of eclosion and locomotor activity. Next, in **Chapter 3** I evaluated the role of EcR in the circadian regulation of the rhythm of emergence. For this purpose, I used immunohistochemistry and fluorescent *in situ* hybridization to determine whether the clock regulates the expression of EcR during the course of the day. Moreover, I analyzed whether the clock acts by synchronizing the cellular size within the PG (as a proxy for cellular activity) and, in addition, I determined which are the possible molecular mechanisms used by the clock to regulate EcR action. Finally, in **Chapter 4** I asked whether there are other elements of the E transduction pathway that may be under circadian control. For this I assessed the effects on the rhythm of eclosion of downregulating in the PG candidate genes involved in transducing 20E actions.
Results: I found that the time when flies initiate the final steps of metamorphosis is correlated with the time of emergence, which suggests that the circadian clock gates emergence by controlling the time when the animal commits to complete metamorphosis, and not by simply preventing the emergence of animals that completed metamorphosis before the gate opens (work done in collaboration with Brandon Mark, Guadalupe Cascallares, and Felipe Conejera). Moreover, I found that injecting increasing doses of E prior to eclosion produced a dose-dependent delay in the time of adult emergence but did not disrupt its circadian rhythmicity, which suggests that the clock acts downstream of E. Consistent with this hypothesis, I found that interfering with E signaling in the PG renders arrhythmic the pattern of emergence. In addition, my findings reveal that the circadian clock controls EcR function by regulating its intracellular location as well as its level of expression. Finally, I found that downregulating genes directly induced by ecdysone also eliminated the rhythm of eclosion.
Conclusions: My results suggest that the circadian clock imposes a daily rhythm to the pattern of emergence by regulating the process of E signal transduction in the PG. The study of the pathways linking development and the circadian system will help elucidate how the clock regulates the timing of emergence, in addition to helping in the understanding of how daily steroid hormone rhythms are generated in animals.