Possible Applications for an Updated Model of the Drosophila Melanogaster Circadian Clock

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Why Circadian Clocks?

All living organisms exhibit circadian rhythms, or clocks, which coordinate a variety of behavioral, physiological, and metabolic processes. For example, clocks have been implicated in sleep, learning, and attention. In humans, some mood disorder treatments are thought to operate by influencing clocks. Due to this pervasive circadian influence, a better understanding of the genetic basis of clocks has many potential applications. The model organism Drosophila melanogaster is often used to study clocks because it reproduces rapidly, is inexpensive to maintain, and has a complex biology that can be studied by many powerful tools of modern genetics.

Hypothesis 1: SGG and TIM

- SGG phosphorylates TIM: GSK3β can bind to TIM in vitro, and GSK3β can be used instead of SGG for some assays.
- SGG influences TIM nuclear accumulation: Overexpression of sgg leads to earlier TIM nuclear accumulation and greater TIM phosphorylation. In per⁻ mutant (without PER and with TIM), sgg overexpression alters TIM. However, sgg overexpression in tim⁻ mutant (with PER and without TIM) did not alter PER nuclear accumulation. Introduction of exogenous sgg into tim⁻ (a sgg null mutant) influenced TIM, but not PER, nuclear accumulation. These results are consistent with the suggestion that SGG directly phosphorylates TIM.
- CRY enhances SGG activity: Activated CRY could promote SGG activity, thereby enhancing TIM phosphorylation and degradation.

Hypothesis 2: SGG and CRY

- SGG stabilizes CRY: CRY stability increased with SGG overexpression, even in light (which normally degrades CRY). Decreased SGG expression reduced CRY levels.
- SGG interacts directly with CRY, not TIM: An immunoprecipitation assay suggested that CRY directly interacts with SGG but did not find a direct interaction between SGG and TIM.
- SGG enhances CRY activity: tim-SSG flies, which overexpress SGG, decrease the clock period to 20.3 hours. tim-SSG flies, which overexpress SGG but have inactive CRY, display a period more like wild-type flies.

Hypothesis 3: SGG and PER

- SGG phosphorylates PER: Some variants of cells transfected with recombinant SGG displayed PER that moved more slowly, including the ssg72A mutant (which has a mutant PER Ser657 residue). Phosphorylation of PER by SGG was significantly decreased in the s661A mutant (mutant PER Ser661 residue), but it was unlikely that SGG phosphorylated PER Ser661. This suggests that SGG phosphorylation of PER Ser657 is primed by PER661 phosphorylation by a different kinase. The identity of this priming kinase is unclear, as is the order of SGG action with respect to other kinases (such as CK2).
- SGG influences PER nuclear accumulation: Assays of the s661A mutant suggested that phosphorylation of PER Ser661 and Ser657 did not influence PER stability but did influence PER nuclear translocation.

Does Shaggy (SGG) impact mood disorders?

Glycogen synthase kinase 3β (GSK3β) and its Drosophila homolog Shaggy (SGG) are important regulatory clock components with potential mental health implications. GSK3β is inhibited by lithium, a medication commonly used to treat bipolar disorder. Serotonin, a neurotransmitter associated with depression, affects SGG. Although both GSK3β and SGG were discovered more than a decade ago, their specific influence and mechanisms remain sources of contention. Here we develop 3 hypotheses on how SGG interacts.

See neighboring boxes

Figure 1. The first of two interlocking loops of the Drosophila clock. The core clock proteins CLK and CYC activate the transcription of an additional transcriptional activator (PDP1) and two transcriptional repressors, CWO and VRI. PDP1, CWO, and VRI create a feedback mechanism which regulates the expression of CLK. The second, more complex clock loop (obscured by the blue box) also involves CLK and CYC. This model builds on the work of Leise and Moin (2007) with the explicit addition of regulatory elements, including SGG, CK2, PP2A, DBT, SLM8, and CRY. Details, including the influence of SGG, remain unclear. The three blue boxes to the left of this diagram represent possible configurations of the second loop.

Figure 2. Standardized concentrations of PER and TIM by cell type and location and time as depicted by Leise and Moin (2007, figure 3P). Zeitgeber time represents subjective day and night, in this case correlating with 0 to 12 hours of light and 12 to 48 hours of darkness. Circles represent data points from Shafer et al (2007), which were used in a coordinate search method to yield the parameters for Leise and Moin (2007) model. Solid lines represent data from the simulation by Leise and Moin (2007). We plan to assess data from each hypothesis in this current experiment by goodness of fit with these time courses.

References

13. Shaggy (SGG) are important regulatory clock components with phosphorylation by a different kinase. The identity of this priming kinase is unclear, as is the order of SGG action with respect to other kinases (such as CK2).
14. SGG influences PER nuclear accumulation: Assays of the s661A mutant suggested that phosphorylation of PER Ser661 and Ser657 did not influence PER stability but did influence PER nuclear translocation.