Evidence-driven Hypothesis Modeling 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 the 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 mutants (without PER and with TIM), sgg overexpression alters TIM. However, sgg overexpression in tim mutants (with PER and without TIM) did not alter PER nuclear accumulation. Introduction of exogenous SGG into sgg−/− (A GGG 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 showed that CRY directly interacts with SGG but did not find a direct interaction between SGG and TIM.
- SGG enhances CRY activity: timSGG flies, which overexpress SGG, decrease the clock period to 20.3 hours. timSGG/cry 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 S657A variant (which has a mutant PER Ser657 residue). Phosphorylation of PER by SGG was significantly decreased in the S661A mutant (PER Ser661 residue), but it was unlikely that SGG phosphorylated PER Ser661. This suggests that SGG phosphorylation of PER is primed by PER S661 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 C2K).
- SGG influences PER nuclear accumulation: Assays of the S661A mutant suggested that phosphorylation of PER Ser661 and S657 did not influence PER stability but could influence PER nuclear translocation.

Moving Forward
Each hypothesis will be incorporated into a model variant, and each variation will be compared to wet lab data. The model variation which is most consistent with the data will then provide a theoretical basis for additional wet lab experiments and simulations. Future simulations may incorporate interactions between proteins and psychotropic medication.

References