Parallel investigation of circadian system influence on developmental cell cycle regulation

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- Circadian system is highly conserved endogenous oscillator found in all life forms
- Believed to have evolved to protect cell division
- Recent links have shown dysregulated cell cycle control to be correlated with irregular circadian rhythms (ie. Cancer patients with circadian disruptions[1, 2], or shift work being considered a carcinogen[3])
- Some molecular links between circadian system and cell cycle regulation have been identified such as the kinase Wee1[4], though relationship between molecular level circadian disruption and developmental cell cycle is not well understood

Given the interconnected nature and bidirectional communication between the circadian system and cell cycle, mutant exhibiting a longer Circadian period (Period Long) should have fewer cell divisions in same time, thus fewer cells, and delayed development. Mutants with a dysfunctional period protein (Period_01 mutants) exhibit normal behavior in 12:12 LD cycles, and when kept in this environment will have similar cell cycles to the standard drosophila model (W1118).

Methodology:

Drosophila development: After 6 hour mating period, specimens monitored for pupal emergence every 2 hours.
- Fully formed pupa placed in infrared Drosophila Activity monitor to determine adult eclosion time (first movement)

Drosophila cell count: Harvested drosophila wings imaged, and FIJIwings program used to determine number of cells, density of cells, and area for intervein region selected

Computational component: Boolean modeling used to create mammalian circadian oscillatory network – highly analogous to *drosophila* framework

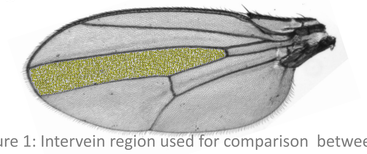


Figure 1: Intervein region used for comparison between genotypes

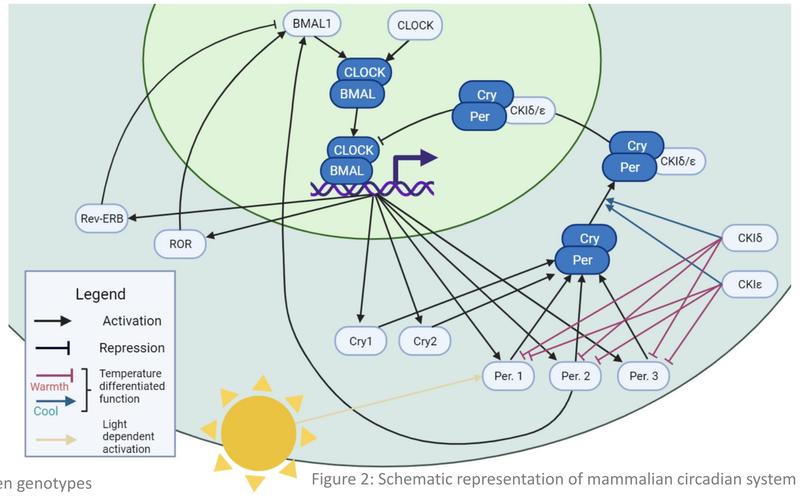


Figure 2: Schematic representation of mammalian circadian system

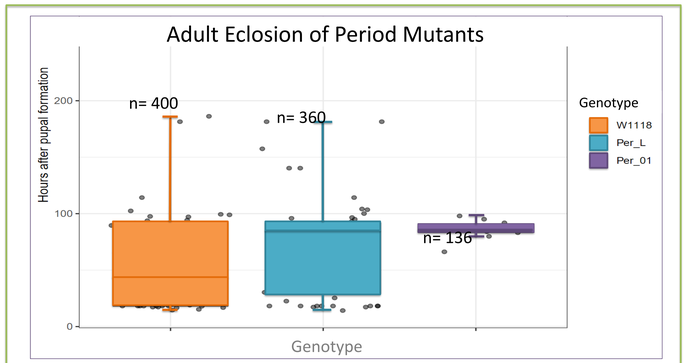
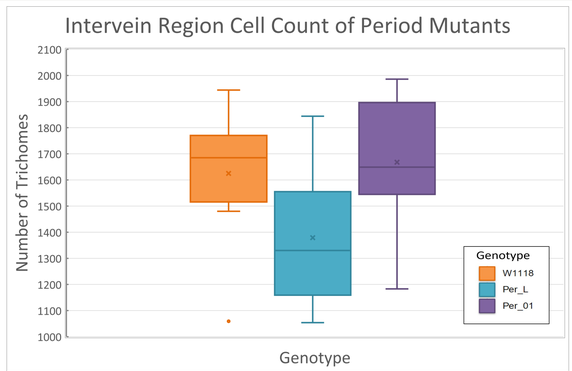
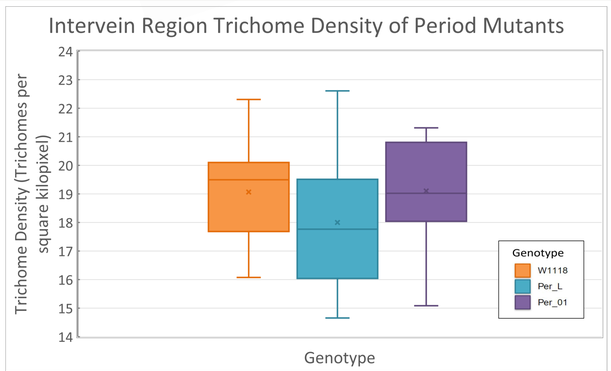
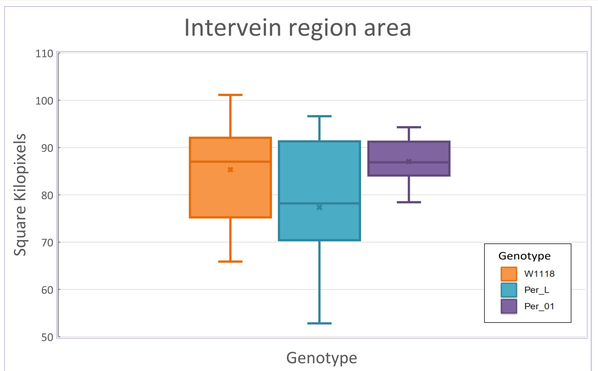


Figure 3: Period Long mutant hatch later than standard W1118 drosophila, consistent with the hypothesis that development will be delayed or extended. Results are complicated by significant differences in survivability of mutant genotypes

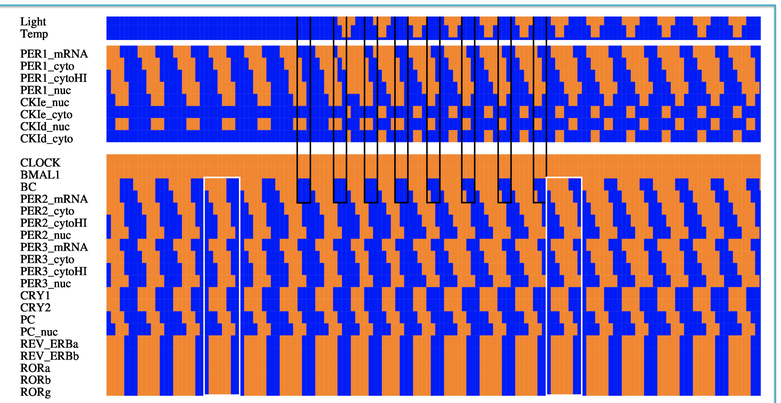






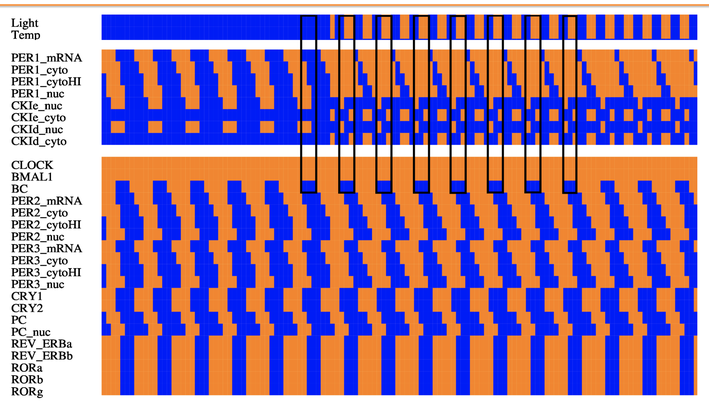
Figures 4-6. : Period Long mutants (Per_L) have fewer total cells (trichomes), supporting the hypothesis that a longer circadian period will influence a longer cell cycle. Area of Period long mutant wings also follow this trend less drastically, and are smaller than wild type. This is reflected in the lower density of trichomes in Period long mutants. There are likely other developmental factors influencing overall size that protect against Period Long mutants having much smaller wings despite such fewer cells.

Normal Entrainment

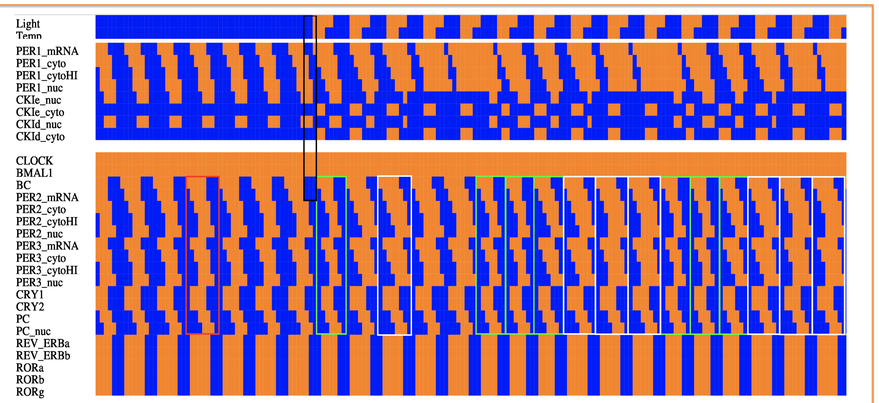


- Model produces a robust rhythmicity that persists without light and temperature input, and can be entrained by the introduction of light and temperature cycles.
- Subjective day is introduced during endogenous “night” in the circadian system, producing a shorted endogenous “day” until entrained with light ques
- “Endogenous night” represented by black boxes,
- Original length circadian period indicated by white boxes

Limits of Entrainment



Left: Too short of external cycle, endogenous oscillator acknowledges every other external “day”



Right: Too long of external cycle, endogenous oscillator cannot settle in extended period length, flips between short cycle (green) and long (white)

Next steps and further questions

- Connecting Circadian model to Cell cycle model
- Simulating a phase shifting and “jet lag”
- Simulating Per_L mutants
- Repeat developmental experiments in D:D to assess Per_01 cell cycle
- Suggestions for statistical analysis

[1] Fu, L., & Kettner, N. M. (2013). The Circadian Clock in Cancer Development and Therapy. *Progress in Molecular Biology and Translational Science*, *119*, 221–282. https://doi.org/10.1016/B978-0-12-396971-2.00009-9
[2] Fuhr, L., Abreu, M., Pett, P., & Relógio, A. (2015). Circadian systems biology: When time matters. *Computational and Structural Biotechnology Journal*, *13*, 417–426. https://doi.org/10.1016/j.csbj.2015.07.001
[3] Khapre, R. V., Samsa, W. E., & Kondratov, R. V. (2010). Circadian regulation of cell cycle: Molecular connections between aging and the circadian clock. *Annals of Medicine*, *42*(6), 404–415. https://doi.org/10.3109/07853890.2010.499134
[4] Zámborszky, J., Hong, C. I., & Csikász Nagy, A. (2007). Computational Analysis of Mammalian Cell Division Gated by a Circadian Clock: Quantized Cell Cycles and Cell Size Control. *Journal of Biological Rhythms*, *22*(6), 542–553. https://doi.org/10.1177/0748730407307225