**The Phosphorylation Process of CLOCK in the Circadian Clock of the Drosophila Melanogaster**

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Introduction

The circadian clock helps to regulate behavior, metabolism and physiology (Hardin, 2005). Our lab is working to build a mathematical model of this circadian clock in the Drosophila melanogaster. The model is being built using data collected on the molecular structure and reactions of the clock from past studies. Once complete, the model will be tested against wet lab data to determine its accuracy and whether or not further adjustments need to be made. In Drosophila the circadian clock has a protein, CLK, which binds to form a complex with CYC. At some point during the ~24 hour cycle, CLK is phosphorylated by a kinase, which causes a variety of important events to occur in the clock. This study has worked to further determine the processes surrounding the phosphorylation of CLK, from the action kinase to the effects of the phosphorylation, as well as when and where this phosphorylation occurs.

Methods

Data was collected from past studies on the causes, effects, location and timing of CLK phosphorylation. Articles were initially found by searching with the following key terms: CLOCK, phosphorylation, and Drosophila melanogaster. A variety of methods were employed to determine the more likely scenario when multiple were suggested. Evidence was considered to point toward one situation over another when one article cited a past article claiming the other situation and their new experiment provided evidence that refuted the past experiment's claim. A situation was also considered more likely if there were many more articles written within the past couple of years claiming its truth over another situation.

**Potential Kinases of CLOCK Phosphorylation**

**NEMO (NOMO)**
- NMO may phosphorylate CLK (Yu et al., 2011)
- When NMO is taken out of the clock phosphorylation levels of CLK affected

**PER (PERIOD)**
- PER activates a kinase to phosphorylate CLK (Grabos et al., 2013; Yu et al., 2006, Yu et al., 2009)
- When PER isn't expressed in Drosophila phosphorylated CLK isn't present
- When PER and DBT form a complex, CLK phosphorylation is promoted

**DOUBLE-TIME (DBT)**
- Initially DBT proposed to phosphorylate CLK (Yu et al., 2006)
- CLK is NOT phosphorylated by DBT because its catalytic activity isn't needed for CLK phosphorylation (Mahesh et al., 2014)
- DBT recruits another kinase to phosphorylate CLK (Mahesh et al., 2014; Sato et al., 2013; Yu et al., 2009)

**EXTRACELLULAR SIGNAL-RELATED KINASE 2 (ERK2)**
- CLK2 and CaMKII may phosphorylate CLK (Walter et al., 2006)
- When CLK was incubated with ERK2 and CaMKII CLK was phosphorylated

**Products of CLOCK Phosphorylation**

**PER (PERIOD)**
- PER likely activates DBT which in turn activates either CK2 or ERK2 and CaMKII to act as a kinase of CLK phosphorylation
- Phosphorylation cycles from hyperphosphorylated at dawn in the cytoplasm to hypophosphorylated at dusk in the nucleus
- Results in the stabilization of the CLK protein at a certain level of phosphorylation and destabilization when CLK is maximally hyperphosphorylated
- Limits the activity of CLK-CYC complexes
- Regulates the rhythms of the circadian clock to follow cycles in temperature