**A model for circadian oscillations in the Drosophila period protein (PER) – Interim Report**

**Project Team:**

Matthew Browe, Eric Chen, Tejas Goculdas, Madan Gopal

**Problem statement:**

Circadian rhythms are set and extremely important metabolic processes that cyclically regulate biological outcomes on a 24-hour cycle. This response to 24-hour light cycles is evolutionarily conserved and observed in all domains of life. The circadian rhythm is a stable oscillatory response under steady state conditions. In this study, we seek to recreate the results of the circadian rhythm model developed by Goldbeter et al [1] involving the *Drosophila* period protein (PER) . The Goldbetter model utilizes the assumptions that the period gene (*per)* mRNA cytosolic concentration (M) is synthesized in the nucleus, transferred to the cytosol at maximum accumulation rate vs, and degraded by an enzyme at a maximum rate vm with Michaelis constant Km. The rate of synthesis of PER from per mRNA is modeled with an apparent first-order rate constant ks. PER is phosphorylated multiple times, and this is accounted for by utilizing three different states of phosphorylation: unphosphorylated (P0), monophosphorylated (P1), and bisphosphorylated (P2) PER, with parameters Vi and Ki (i=1,2,3,4) denoting the maximum rate and Michaelis constant of the kinase(s) and phosphatase(s) involve in reversible phosphorylation of P0 →P1 and P1 →P2.

The bisphosphoylated PER form P2 is degraded by an enzyme of maximum rate vd and Michaelis constant Kd and transported into the nucleus at a rate modeled by first-order rate constant k1. The nuclear biosphosphorylated form of PER, PN, is transported into the cytosol by a process modeled by first-order rate constant k2. The negative feedback exerted by PN of per transcription is described by a Hill type equation in which *n* denotes the degree of cooperativity and KI the threshold constant for repression. Cooperativity regimes are defined as positive (*n*>1), neutral (*n*=1), and negative (*n*<1). The entire scheme of the process is depicted in Figure x.



Figure x. Scheme of the model for circadian oscillations in PER and *per* mRNA

(reproduced from Goldbeter et al)

**Methods and Approach**

To validate the paper and the subsequent models of *Drosophila* circadian cycles, the first effort involved reproducing the Goldbeter results, specifically Figures 1 and 2 which depict PER protein formation over time and PER mRNA concentration versus total PER protein. The model equations governing temporal protein evolution in the Goldbeter model are portrayed in equations x-x. Goldbeter values for the various parameters in the model are depicted in Table x.

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Table x: Parameter values used in the Goldbeter model

|  |  |  |
| --- | --- | --- |
| Parameter | Description | Value |
| vs | Accumulation rate of per mRNA in cytosol | 0.76 um/hr |
| vm | Maximum degradation rate of per mRNA in cytosol | 0.65 um/hr |
| Km | Michaelis constant for cytosolic per mRNA | 0.5 um |
| ks | First-order rate constant for PER synthesis | 0.35 hr-1 |
| vd | Maximum degradation rate of biphosphorylated PER (P2) | 0.95 um/hr |
| k1 | First-order rate constant for P2 transport into nucleus | 1.9 hr-1 |
| k2 | First-order rate constant for PN transport into cytosol | 1.3 hr-1 |
| KI | Threshold constant for repression | 1 um |
| Kd | Michaelis constant for P2 degradation | 0.2 um |
| K1 | Michaelis constants for kinases and phosphatases involved in reverse phosphorylation of P0 into P1 and P1 into P2 | 2 um |
| K2 |
| K3 |
| K4 |
| V1 | Maximum rate for kinases and phosphatases | 3.2 um/hr |
| V2 | 1.58 um/hr |
| V3 | 5 um/hr |
| V4 | 2.5 um/hr |
| n | Degree of cooperativity | 4 |

Stability analysis commenced with constructing the overall Initial Value problem vector and finding solutions to the homogeneous problem. Homogeneous problem solutions are obtained numerically using Newton’s method.

From these solutions, second order dynamics were probed using the Jacobian and each solution’s stability was categorized via Jacobian eigenvalue analysis. Determination of the results of the eigenvalue analysis were related to the temporal behavior of the oscillatory amplitude of the mRNA and nuclear PER proteins. The Jacobian of the system of equations x-x is depicted in equation x

(x)

The eigenvalues λ of the Jacobian are determined from the solution to equation x, where I is the identity matrix of equal size as the Jacobian matrix.

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Analytical solution for the eigenvalues would involve utilization of Kramer’s rule, but in MATLAB, the “eig” function can be utilized to calculate the eigenvalues using the command

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where V is the matrix of eigenvectors and D is the matrix of eigenvalues, presented in the form where constitutive eigenvalues are located on the diagonal terms of the matrix.

A Bifurcation diagram was also constructed based on the system’s parametric dependence on phospine’s putative transcription inhibition.

Kinetic Monte Carlo simulations (Tau leaping algorithm) were conducted to probe ways in which the system crosses from one stable solution to another within a stable subspace. The Tau leaping algorithm was originally proposed by Gillespie et al (Gillespie, J. Chem. Phys 115 (2001) 1716). It involves populating a state vector X(t), describing the number of molecules of each species in a reaction network at time t, in a probabilistic manner. It requires that the leap time τ is small enough that the change in the state during the time interval [t, t+ τ] is so slight that the propensity function will not suffer an appreciable change in its value. The Tau leaping algorithm is defined in equation x

(x)

Where is the stoichiometric vector for reaction j involving a given species and is a Poisson-distributed random variable with mean

Phasic entrainment effects occur in circadian rhythms when there is limited interaction of an organism with its environment for daily restorating of the circadian clock by the amount equal to the error, the difference between oscillatory environmental events and the organism’s circadian rhythm. To probe these phasic entrainment effects, the v PER expression rate parameter (vs) will be turned into a sinusoidal forcing function that will run at various-length cycles (10 - 40 hours) between 0 (total darkness) and the originally stated vs value. This will simulate *Drosophila* exposure to an environmental toxin that causes transcriptional inhibition. We expect that low values of vs will cause dampening of the oscillations into a stable solution. We can also increase transcriptional rates by varying vs to determine the point at which oscillations become unstable. Lastly, upon forcing *Drosophila* to adapt to a 4hr light cycle from a 24hr light cycle, we expect to see fluctuations as the system moves toward a new steady state.

Additionally, Dactinomycin is a drug which is one the most popular inhibitors of transcription in cells. It is made from Streptomyces bacteria and its structure consists of two cyclic peptides linked together through a phenoxazine derivative. It has been shown to inhibit transcription in all three eukaryotic polymerases [DOI 10.4161/trns.2.3.16172]. The drug accomplishes this by preferentially intercalating into GC rich sequences and stabilizing topoisomerase complexes, which prevents RNA progression. For this project, we plan to model the effects of a similar drug which will act as a toxin that inhibits transcription of the mRNA. This inhibitor would reduce the value of the constant vₛ in the reaction 1a.

**Results:**

1. **Replication and validation of Goldbeter model**

Major results from the baseline Goldbetter model have indeed been successfully duplicated in MATLAB and are depicted in Figures 1-3.

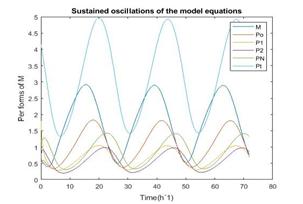


Figure 1: Oscillatory protein levels from *per* mRNA (left, Goldbetter et al)

and reproduced results (right)

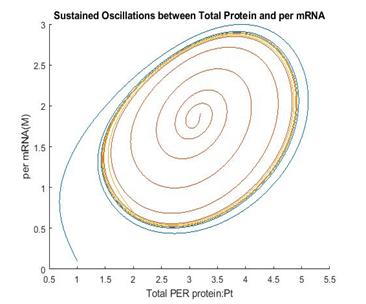
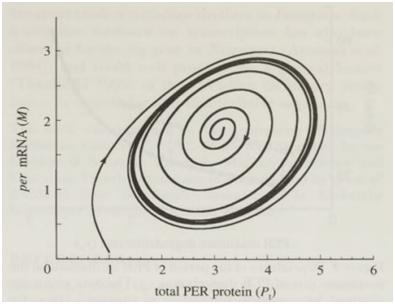


Figure 2: All initial conditions favor tendency towards a limit cycle (left, Goldbetter et al)

and reproduced results (right)

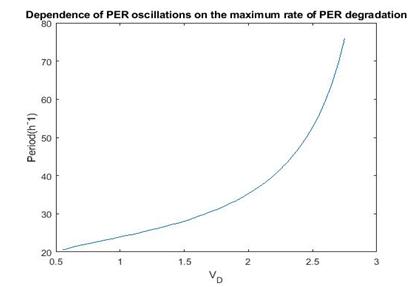
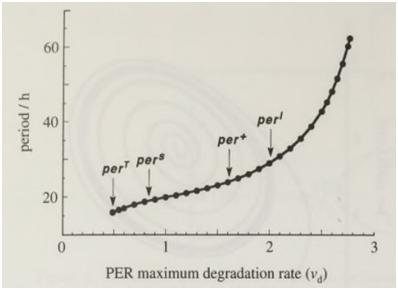


Figure 3. Period for per-induced oscillation versus maximum degradation rate (left, Goldbetter et al)

and reproduced results (right)

1. **Stability Analysis**

Stability analysis will start with constructing the overall Initial Value problem vector and find solutions to the homogeneous problem. From these solutions, we will probe 2nd order dynamics using the Jacobian and categorize each solution’s stability via Jacobian eigenvalue analysis and determination of the results of the eigenvalue analysis as related to the temporal behavior of the oscillatory amplitude of the mRNA and nuclear PER proteins.

The eigenvalues λ of the Jacobian are determined from the solution to equation x

(x)

We will also construct a Bifurcation diagram based on the system’s parametric dependence on phospine’s putative transcription inhibition.

We have derived the Jacobian matrix for the Goldbetter model, defined by equations x-x, in equation x.

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1. **Forcing Function –Phasic Entrainment Effects**

It was originally proposed to probe phasic entrainment effects through modification of the PER expression rate parameter, vs, into a sinusoidal forcing function represented as function f6 in Figure 6. The new system of equations and resulting Jacobian is then thus described by equations x-x.

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1. **Kinetic Monte Carlo Simulations**

**Conclusions**

**References (use Zotero):**

[1] Goldbeter, A. (1995). A model for circadian oscillations in the Drosophila period protein (PER). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *261*(1362), 319-324.

Gillespie, J. Chem. Phys 115 (2001) 1716 – Tau leaping algorithm

DOI 10.4161/trns.2.3.16172

**Appendix**

PER equations 1a through 1e

Jacobian is

Homogeneous problem solutions

Solving for PN

Solving for M

Solving for M

Solving for P0

Solving for P1

Solving for P0

Solving for P1

Assume only addition of square root yields a positive (realistic) number:

Solving for P2

Solving for PN

Solving for P1

Solving for P2

The general solution of

Is

Here,

Solving for P2

Solving for PN

**Homogeneous Problem Summary**

**MATLAB code**