Modeling the *Drosophila* circadian clock
Circadian rhythms

Monitoring the locomotor activity of flies (Wild Type and perS mutant in DD and in LD)

See animations: droso_activity_animation.mov

Source: http://www.hhmi.org/biointeractive/clocks/animations.html
Circadian rhythms: genetic bases

Locomotor activity

A. normal

B. arrhythmic mutant

C. short-period mutant

D. long-period mutant

Drosophila

Identification of the period (per) gene

Oscillations of *per* mRNA and PER protein in *Drosophila*

- Levels of *per* mRNA and PER proteins oscillates with a circadian period (both in the eye and in the brain of the fly).
- The peak of PER protein occurs a few hours after the peak of mRNA.
- The fact that mRNA level decreases when the level of protein is high (together with other experiments) suggests that the PER protein inhibits the expression of the *per* gene.

Characterization of \textit{per} gene in \textit{Drosophila}

Feedback of the \textit{Drosophila period} gene product on circadian cycling of its messenger RNA levels
Hardin PE, Hall JC, Rosbash M.

Circadian oscillations in \textit{period} gene mRNA levels are transcriptionally regulated
Hardin PE, Hall JC, Rosbash M.

Temporal phosphorylation of the \textit{Drosophila} period protein
Edery I, Zwiebel LJ, Dembinska ME, Rosbash M.
Goldbeter's 5-variable model

Goldbeter's 5-variable model: equations

\[ \frac{d M_P}{dt} = v_s \frac{K_1^n}{K_1^n + P_N^n} - v_m \frac{M_P}{K_m + M_P} \]

\[ \frac{d P_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1} \]

\[ \frac{d P_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2} \]

\[ \frac{d P_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N \]

\[ \frac{d P_N}{dt} = k_1 P_2 - k_2 P_N \]

Goldbeter's 5-variable model: equations

Dynamics of *per* mRNA (*Mₚ*): synthesis

\[
\frac{dM_P}{dt} = v_s \frac{K_I^n}{K_I^n + P_N^n} - v_m \frac{M_P}{K_m + M_P}
\]

Inhibition: Hill function

Cooperativity
Goldbeter's 5-variable model: equations

Dynamics of *per* mRNA ($M_P$): degradation

\[ \frac{d M_P}{dt} = v_s \frac{K^n_I}{K^n_I + P^n_N} - v_m \frac{M_P}{K_m + M_P} \]

Degradation: Michaelis-Menten

\[ E << M \quad \quad k_1, k_-1 >> k_2 \]

\[ E_{tot} = E + ME \]

\[ K_M = \frac{(k_-1 + k_2)}{k_1} \]

\[ v_m = k_2 E_{tot} \]
Goldbeter's 5-variable model: equations

Dynamics of PER protein \((P_0, P_1, P_2, P_N)\)

\[
\frac{dP_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}
\]

**PER synthesis:**
proportional to mRNA

\[
\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}
\]

\[
\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N
\]

\[
\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N
\]
Goldbeter's 5-variable model: equations

Dynamics of PER protein \((P_0, P_1, P_2, P_N)\)

\[
\frac{dP_0}{dt} = k_s M_P - \left( v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1} \right)
\]

PER phosphorylation/dephosphorylation:
Michaelis-Menten

\[
\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - \left( v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2} \right)
\]

PER phosphorylation/dephosphorylation:
Michaelis-Menten

\[
\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N
\]

\[
\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N
\]
Goldbeter's 5-variable model: equations

Dynamics of PER protein \((P_0, P_1, P_2, P_N)\)

\[
\frac{d P_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}
\]

\[
\frac{d P_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}
\]

\[
\frac{d P_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N
\]

PER degradation: Michaelis-Menten

\[
\frac{d P_N}{dt} = k_1 P_2 - k_2 P_N
\]
Goldbeter's 5-variable model: equations

Dynamics of PER protein \((P_0, P_1, P_2, P_N)\)

\[
\frac{dP_0}{dt} = k_s M_p - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}
\]

\[
\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}
\]

\[
\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N
\]

PER nuclear transport: linear

\[
\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N
\]
Goldbeter's 5-variable model: parameters

Parameter values

The values of the kinetic parameters are (usually) not known. They are taken in the physiological range in order to satisfy the constraints (self-sustained oscillations, period of 24h, phase relationship, entrainment by light-dark cycles,...).

*With four parameters, I can fit an elephant, and with five I can make him wiggle trunk.*

(attributed to J. von Neumann by E. Fermi)

Goldbeter's 5-variable model: parameters

How to determine the parameter values?

- **Manually**
  - By trial-errors
  - Using intuition
  - Qualitative assessment (by eye)

- **Automatically**
  - By (computer) trial-errors
  - Using optimization
    - objective function (e.g. look for oscillations with a given period)
    - fitting to time series (often requires pre-processing of the data)
  - Optimization methods
    - simplex / Nelder-Mead
    - genetic algorithm
    - simulated annealing

"objective" function (to be minimized)

multi-dimensional parameter space
## Goldbeter's 5-variable model: parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_s$</td>
<td>maximum transcription rate</td>
<td>0.5 nM/h</td>
</tr>
<tr>
<td>$K_I$</td>
<td>inhibitory constant</td>
<td>2 nM</td>
</tr>
<tr>
<td>$n$</td>
<td>cooperativity degree</td>
<td>4</td>
</tr>
<tr>
<td>$v_m$</td>
<td>maximum mRNA degradation rate</td>
<td>0.3 nM/h</td>
</tr>
<tr>
<td>$K_m$</td>
<td>Michaelis constant for mRNA degradation</td>
<td>0.2 nM</td>
</tr>
<tr>
<td>$k_s$</td>
<td>translation rate</td>
<td>2 /h</td>
</tr>
<tr>
<td>$v_1 = v_3$</td>
<td>maximum phosphorylation rate</td>
<td>6 nM/h</td>
</tr>
<tr>
<td>$K_1 = K_3$</td>
<td>Michaelis constant for phosphorylation</td>
<td>1.5 nM</td>
</tr>
<tr>
<td>$v_2 = v_4$</td>
<td>maximum dephosphorylation rate</td>
<td>3 nM/h</td>
</tr>
<tr>
<td>$K_2 = K_4$</td>
<td>Michaelis constant for dephosphorylation</td>
<td>2 nM</td>
</tr>
<tr>
<td>$k_1$</td>
<td>nuclear transport rate</td>
<td>2 /h</td>
</tr>
<tr>
<td>$k_2$</td>
<td>nuclear export rate</td>
<td>1 /h</td>
</tr>
<tr>
<td>$v_d$</td>
<td>maximum degradation rate</td>
<td>1.5 nM/h</td>
</tr>
<tr>
<td>$K_d$</td>
<td>Michaelis constant for degradation</td>
<td>0.1 nM</td>
</tr>
</tbody>
</table>
Goldbeter's 5-variable model: simulation

Goldbeter's 5-variable model:

\[ \frac{dx}{dt} = \frac{v_3 + \frac{v_4 + \frac{v_5 + \frac{v_6 + \frac{v_7 + \frac{v_8 + \frac{v_9 + \frac{v_{10} + \frac{v_{11} + \frac{v_{12} + \frac{v_{13}}{m}}{m}}{m}}{m}}{m}}{m}}{m}}{m}}{m}}{m} \]

Default integration method: Runge-Kutta with variable step-size

Numerical simulation (XPP-Auto)

XPP-AUTO: http://www.math.pitt.edu/~bard/xpp/xpp.html
Goldbeter's 5-variable model: results

Self-sustained (limit cycle) oscillations

Limit-cycle oscillations are characterized by a fixed amplitude and a fixed period. Such oscillations correspond to a close curve in the phase space, i.e. the space of the variables. They are robust in the sense that if a perturbation is exerted on such a system, the system will automatically come back to its "normal" behavior, i.e. to the limit cycle.

Goldbeter's 5-variable model: results

Self-sustained (limit cycle) oscillations

Goldbeter's 5-variable model: results

**Self-sustained (limit cycle) oscillations**

Mutants:

- *per*\(^l\) long-period mutant
- *per*\(^s\) short-period mutant

The *per* mutants can be explained by a change in the protein degradation rate.
Goldbeter's 5-variable model: results

Bifurcation diagram

Hopf bifurcation
Goldbeter's 5-variable model: results

Bifurcation diagram

- Damped oscillations
- Low-amplitude oscillations
- Large-amplitude oscillations
Effect of light on the circadian clock


Extended model for the *Drosophila* circadian clock

**per mRNA**

\[
\frac{dM_p}{dt} = \nu_{1p} \frac{K_{IP}^a}{K_{IP}^a + G_N^a} - \nu_{mpr} \frac{M_p}{K_{mp} + M_p} - k_4 M_p
\]

**PER protein**

\[
\frac{dP_0}{dt} = k_0 M_p - V_{1p} \frac{P_0}{K_{1p} + P_0} + \nu_{1p} \frac{R_1}{K_{1p} + R_1} - k_4 P_0
\]

\[
\frac{dP_1}{dt} = V_{1p} \frac{P_1}{K_{1p} + P_1} - V_{2p} \frac{P_1}{K_{2p} + P_1} - \nu_{3p} \frac{R_1}{K_{3p} + R_1} + \nu_{4p} \frac{P_2}{K_{4p} + P_2} - k_4 P_1
\]

\[
\frac{dP_2}{dt} = V_{3p} \frac{P_2}{K_{3p} + P_2} - k_5 P_2 T_2 + k_4 C - \nu_{4p} \frac{P_2}{K_{4p} + P_2} - k_4 P_2
\]

**tim mRNA**

\[
\frac{dM_t}{dt} = \nu_{1t} \frac{K_{IT}^a}{K_{IT}^a + G_N^a} - \nu_{mt} \frac{M_t}{K_{mt} + M_t} - k_4 M_t
\]

**TIM protein**

\[
\frac{dT_0}{dt} = k_0 M_t - V_{1t} \frac{T_0}{K_{1t} + T_0} + \nu_{1t} \frac{T_1}{K_{1t} + T_1} - k_4 T_0
\]

\[
\frac{dT_1}{dt} = V_{1t} \frac{T_1}{K_{1t} + T_1} - V_{2t} \frac{T_1}{K_{2t} + T_1} - \nu_{3t} \frac{T_1}{K_{3t} + T_1} + \nu_{4t} \frac{T_2}{K_{4t} + T_2} - k_4 T_1
\]

\[
\frac{dT_2}{dt} = V_{3t} \frac{T_2}{K_{3t} + T_2} - \nu_{4t} \frac{T_2}{K_{4t} + T_2} - k_5 P_2 T_2 + k_4 C - \nu_{4t} \frac{T_2}{K_{4t} + T_2} - k_4 T_2
\]

**PER-TIM complex**

\[
\frac{dC}{dt} = k_5 P_2 T_2 - k_4 C - k_4 C + k_2 G_t - k_{ac} C
\]

\[
\frac{dC_t}{dt} = k_5 C - k_4 C - k_{ac} C_t
\]

Leloup-Goldbeter's 10-variable model

Self-sustained oscillations

Limit cycle
Influence of parameters on the period of oscillations: Bifurcation diagrams

**Long period** (*per^L*) mutant

**Short period** (*per^S*) mutant

\[ k_3 \leftrightarrow \text{PER-TIM complex formation} \]

\[ k_4 \leftrightarrow \text{PER-TIM complex dissociation} \]

\[ k_{dN} \leftrightarrow \text{PER-TIM complex degradation} \]

(a), (b): two different parameter sets
Leloup-Goldbeter's 10-variable model

Modeling the effect of light

The effect of light can be modeled by modulation of parameter $v_{dT}$ (TIM degradation rate). Light-dark cycles can thus be simulated by a periodic variation of $v_{dT}$ (sine or square wave).
Phase shifting by a light pulse

Phase shifting induced by a light pulse: depending on the time (phase) at which the light pulse is given, the oscillations are advance, delayed, or no phase shifted compared the the unperturbed oscillations.

In the model, light affects TIM$_2$ degradation rate, $v_{dT}$. A pulse of light can thus be modeled by a transient increase of $v_{dT}$:

- Pulse duration
- Pulse amplitude (strength)
Leloup-Goldbeter's 10-variable model

Phase shifting by a light pulse

The phase response curve (PRC) gives the phase shift as a function of the phase of the perturbation (pulse).
Phase shifting by a light pulse

Light pulses give phase advances, TIM fails to reaccumulate due to low tim RNA levels

Virtual absence of TIM gives “dead zone” of little or no phase resetting by light

Peak PER and TIM levels further decrease due to turnover without replacement

PER and TIM levels decline due to turnover without replacement

High levels of per and tim RNA allow PER and TIM assembly

Nuclear PER and TIM begin suppressing per and tim RNA accumulation

Light pulses produce phase delays due to reaccumulation of TIM from high tim RNA levels

Phase response curve

Phase shift

Initial phase (h)
Leloup-Goldbeter's 10-variable model

Phase shifting and PRC
comparison with experimental data

Wild type


Short period mutant

Leloup-Goldbeter's 10-variable model

Bifurcation diagram as a function of TIM degradation rate ($v_{dT}$)

![Bifurcation diagram](image)

- Maximum TIM degradation rate, $v_{dT}$ (nM h$^{-1}$)
- TIM protein, $T_2$ (nM)
- Steady state
- Max oscillations
- Min oscillations
- Steady state
Leloup-Goldbeter's 10-variable model

Bifurcation diagram as a function of TIM degradation rate ($v_{dT}$)

Hard excitation = coexistence between a stable steady state and stable oscillations
Leloup-Goldbeter's 10-variable model

Interpretation of the bifurcation diagram

- Stable steady state
- Limit-cycle oscillations
- Maximum TIM degradation rate, $v_{dT}$

When $v_{dT}$ is too small, only damped oscillations can be observed. The system ultimately converges to a steady state.

When $v_{dT}$ is too large, only damped oscillations can be observed. The system ultimately converges to a steady state.
Leloup-Goldbeter's 10-variable model

Interpretation of the bifurcation diagram

- Stable steady state
- Limit-cycle oscillations
- Stable steady state

Maximum TIM degradation rate, \( v_{dT} \)

When \( v_{dT} \) is intermediary, only limit-cycle oscillations can be observed.
In these intermediary regions, the system has the choice: it can either oscillate or converge to a steady state. This choice depends on the initial state of the system. This property is called hard excitation.
Leloup-Goldbeter's 10-variable model

Suppression of oscillations by a light pulse

Depending on the initial conditions, the system can either display self-sustained oscillations or converge to a steady state.

A pulse of light can permanently stop the oscillations or restore the oscillations, but there are conditions on:
- phase of the pulse
- amplitude of the pulse
- duration of the pulse
Leloup-Goldbeter's 10-variable model

Suppression of oscillations by a light pulse
Experiment in Kalanchoe

Goldbeter's 5-variable model

Why are circadian rhythms *circadian*?

Amplitude of circadian oscillations entrained by 24-h light–dark cycles

Gen Kurosawa, Albert Goldbeter


The model predicts that the maximum **amplitude** obtained upon LD entrainment is reached when the free-running period is smaller than 24h.

The model also shows that the **entrainment phase** depends on the free-running period 24h. Since the phase is critical for a proper adaptation to the LD cycle, it is thus likely that the FRP was tuned to "optimize" the phase.
PER and TIM do not repress directly the expression of *per* and *tim* genes (they do not bind themselves to the gene promoter).

They rather block a transcriptional activator, formed by the complex CLOCK-CYCLE (CLK-CYC).


An update scheme of the Drosophila clock

Indirect transcriptional regulation + additional feedback loops

The complex CLK-CYC regulated the transcription of CLK, forming an additional, negative feedback loop.

A more update scheme of the Drosophila clock

Multiple, interlocked feedback loops

The genetic basis of circadian rhythms can be modeled by ordinary differential equations.

Because of the large number of variables and multiple sources of nonlinearities, analytical approaches are very limited and one must resort to numerical simulations (e.g. with xpp-auto).

Simulation of the models shows that the endogenous circadian rhythm (in constant conditions) can be described as limit cycles oscillations.

The effect of mutations on the period length can be modeled by changing specific parameter values.

The light-dark cycle (LD) can be modeled by periodic modulation of the light controlled parameter. The simulations account for the entrainment of the oscillations in various LD conditions (different day lengths, different photoperiods, etc).

Simulations can be used to provide explanation of non-intuitive observations (cf. the example of suppression of oscillations by a light pulse).
Modeling circadian rhythms in Drosophila

References


Modeling circadian rhythms in Drosophila

References


