Biological Oscillations: Part 2

Prof Rickus
This Lecture

• Circadian Rhythms
  • inherent, stable oscillations
  • period of ‘about a day’

• Period Timeless Model
  • first identified genetic clock
  • biochemical oscillator
  • Exemplifies 4 design principles of biochemical oscillators
    • Feedback
    • Sufficient delay
    • Sufficient non-linearity
    • Tuning / balance of relative rate parameters
Your Biological Day Night Clock

• Why do you think that you sleep at night and are awake during the day?
  • External or Internal Cues?

• What do you think would happen if you were in a cave, in complete darkness?

• If you were in a cave.. Could you track the # of days you were there by the number of times you fell asleep and woke up?
  • i.e. 1 wake – sleep = 1 day = 24 hours?
Internal Clock

• Humans w/o Input or External Cues
  • diurnal (active at day)
  • constant darkness $\rightarrow$ ~25 hour clock (>24 hours)
  • wake up about 1 hour later each day
  • constant light shortens the period

• Rodents
  • nocturnal (active at night)
  • constant darkness $\rightarrow$~23 hour clock period (<24 hours)
  • wake up a little earlier each day
  • constant light lengthens the period
Circadian Rhythms Everywhere!

- actually more rare for a biological factor to not change through-out the 24 hour day

- temperature
- cognition
- learning
- memory
- motor performance
- perception

all cycle through-out the day
The Circadian Clock:

Defined By:

1. Period of ~24 hours

2. synchronized by the environment

3. temperature independent

4. self-sustained (therefore inherent)

*recall our stable limit cycles in previous lecture*
Clock Definitions

• Period (T): time for the rhythm to repeat
Clock Definitions - Phase Shifts

- Phase Advance
- Phase Delay

Period (T) remains the same
External cues can shift the phase
Basis of the Clock? What is the cellular mechanism?

- Self-sustained rhythm
  - Stable limit cycle
  - Looking for a genetic, biochemical, cellular oscillator

- Inherent Period
  - must be some inherent mechanism
  - cells ... proteins ... genes??

- Light Dark Pattern
  - modulates the Phase
  - “sets” the clock (and period slightly)
  - How does light modulate the biochemical/cellular clock?
Genes?

- Drosophila (fruit flies)
  - convenient for genetics
  - mutation – behavior genetic screens
- Screened for flies with altered circadian rhythms
  - some too short
  - some too long
  - some arrhythmic (no repeating pattern at all)
- What genes are mutated?
Seymour Benzer

Purdue Physicist.
Ph.D. from Purdue in 1947
Important role in the invention of the transistor
Mapping phage genomes before the time of genomics or molecular concept of genes

Cal Tech.
• became interested in genes and behavior
• highly original experiments
  1. mutating drosophila
  2. Sort for specific behavior changes or deficits
  3. Search for the underlying gene mutation
• became the father of neurogenetics
• Discovered first genes underlying circadian rhythms
Genes Involved?

- *period (per)*
  - found point mutations in *period*
  - some delayed, some advanced, some arrhythmic
  - found that Period (PER) levels oscillates in single cells with period of 24 hours!
  - Where there clocks in single cells??

Are the oscillations inherent to Period? Or does another gene / protein interact to create the oscillations?
Biochemistry of Period

1. make mRNA
2. mRNA transport into cytosol
3. make protein
4. protein phosphorylation (2Xs)
5. phosphorylated protein travels into nucleus
6. phosphorylated protein inhibits mRNA production
Mathematical Model

• Built a mathematical model based on biochemical, cellular, and gene data
• Asked: Can Period support its own oscillations?
• Model Answer: Yes

**Negative Feedback:**
composite negative autoregulation

**Sufficient Delay.**
Phosphorylation and transport provide sufficient delay on the negative feedback

Stoichiometry on phosphorylation increases nonlinearity

*This model can in fact support 24 hours oscillation of Per protein levels*  
*But no mechanism for entrainment!! How does light interact?*

*Model ... incomplete???
Other genes?

• \textit{timeless} (\textit{tim})
  • timeless mutations were arrhythmic
  • Timeless affected Period
    • Per location
    • Per level
    • Per protein oscillations
    • Per phosphorylation

How do Period and Timeless interact?
How does light interact?
Drosophila PER TIM Model

Ahhh!!
Light induces TIM degradation

fully phosphorylated
PER and TIM
form a complex
that inhibits expression
of both

Can this model simulate the experimental observations of effects of light?

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Mathematical Model of Period / Timeless

\[ \frac{dM_p}{dt} = \frac{v_{ip} K_{ip}^2}{K_{ip}^2 + C_N} - v_{mp} \frac{M_p}{K_{mp} + M_p} - k_d M_p \]  
(1a)

\[ \frac{dP_0}{dt} = k_{sp} M_p - V_{1p} \frac{P_0}{K_{1p} + P_0} + V_{2p} \frac{P_1}{K_{2p} + P_1} - k_d P_0 \]  
(1b)

\[ \frac{dP_1}{dt} = V_{1p} \frac{P_0}{K_{1p} + P_0} - V_{2p} \frac{P_1}{K_{2p} + P_1} - V_{3p} \frac{P_1}{K_{3p} + P_1} + V_{4p} \frac{P_2}{K_{4p} + P_2} - k_d P_1 \]  
(1c)

\[ \frac{dP_2}{dt} = V_{3p} \frac{P_1}{K_{3p} + P_1} - V_{4p} \frac{P_2}{K_{4p} + P_2} - k_d P_2 \]  
(1d)

\[ \frac{dM_T}{dt} = \frac{v_{iT} K_{iT}^2}{K_{iT}^2 + C_N} - v_{mT} \frac{M_T}{K_{mT} + M_T} - k_d M_T \]  
(1e)

\[ \frac{dT_0}{dt} = k_{sT} M_T - V_{1T} \frac{T_0}{K_{1T} + T_0} + V_{2T} \frac{T_1}{K_{2T} + T_1} \]  
(1f)

\[ \frac{dT_1}{dt} = V_{1T} \frac{T_0}{K_{1T} + T_0} - V_{2T} \frac{T_1}{K_{2T} + T_1} - V_{3T} \frac{T_1}{K_{3T} + T_1} + V_{4T} \frac{T_2}{K_{4T} + T_2} - k_d T_1 \]  
(1g)

\[ \frac{dT_2}{dt} = V_{3T} \frac{T_1}{K_{3T} + T_1} - V_{4T} \frac{T_2}{K_{4T} + T_2} - k_d T_2 \]  
(1h)

\[ \frac{dC}{dt} = k_{3p} T_2 - k_d C - k_1 C + k_2 C_N - k_d C \]  
(1i)

\[ \frac{dC_N}{dt} = k_1 C - k_2 C_N - k_d C_N. \]  
(1j)

The total (non-conserved) quantity of PER and TIM proteins, \( P_i \) and \( T_i \), are given by:

\[ P_i = P_0 + P_1 + P_2 + C + C_N \]  
(2)

\[ T_i = T_0 + T_1 + T_2 + C + C_N. \]  
(3)
example of simulation output

Light Entrainment

Effect of light depends on timeless mRNA at time of light exposure

Light Destroys TIM Protein

When tim RNA is high ... it delays the clock (phase delay)
Because mRNA is ready to quickly replace the destroyed TIM

When tim RNA is low ... it advances the clock (phase advance)
Because mRNA is not ready to quickly replace the destroyed TIM

So effect of light depends on the tim mRNA levels

[Diagram: protein oscillations, mRNA oscillations, PER TIM complex oscillations]
For Further Reading

Time, Love & Memory
By Jonathan Weiner
Coming Up

• Translation to Device Engineering
  • Cells as Sensors
  • Cells as Actuators
  • Device Integration