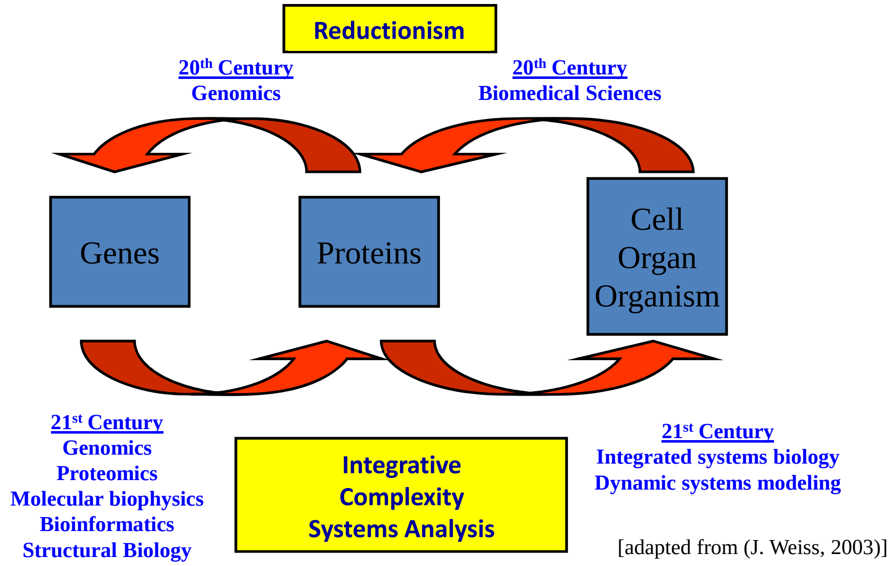
# Trends in Scientific Investigation




[adapted from (J. Weiss, 2003)]

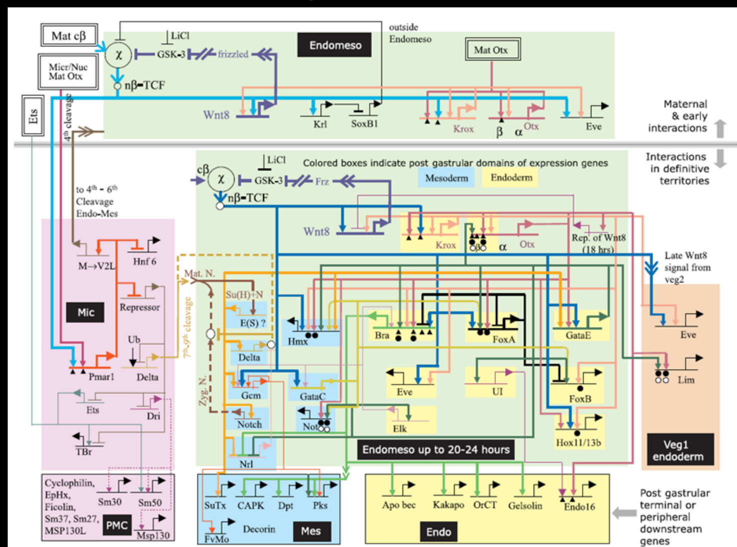


# What is "Systems Biology"?

[WTEC Benchmark Study (2005): M. Cassman, A. Arkin, F. Doyle, F. Katagiri, D. Lauffenburger, C. Stokes]
[also: *Nature*, Dec 22, 2005]

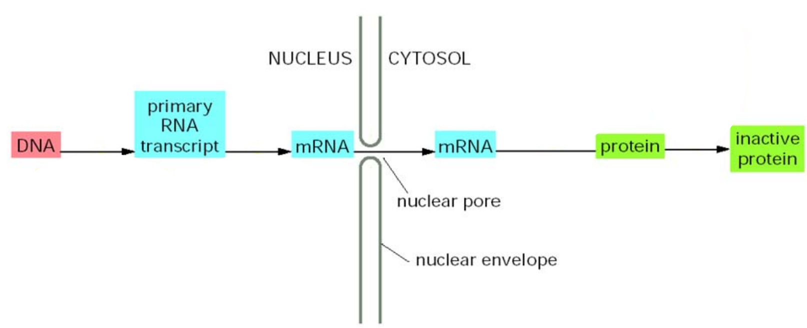
- **Primary Definition**: *The understanding of biological* ***network*** *behavior through the application of modeling and simulation, tightly linked to experiment*
- **Related Ideas**
  - Identification and validation of networks
  - Creation of appropriate datasets
  - Development of tools for data acquisition and software
- **Motivation:** Phenotype is governed by the behavior of **networks**, rather than the operation of single genes. Understanding the dynamics of even the simplest biological networks requires the application of modeling and simulation.

# A Simple Network






# Central Dogma and Levels of Control




[Alberts *et al.*, **Essential Cell Biology**, 1998]

# Circadian Rhythms

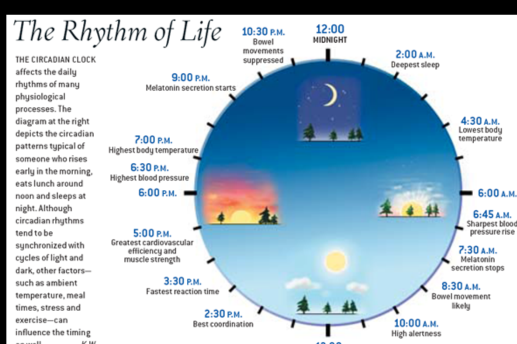
**Circadian rhythms** = self-sustained biological rhythms characterized by a *free-running period* of about 24h (*circa diem*)

**Circadian rhythms characteristics:**

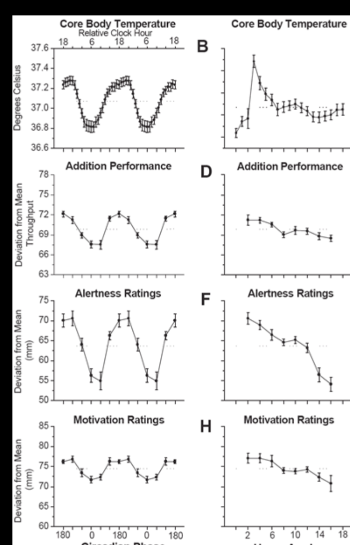
- General – bacteria, fungi, plants, flies, fish, mice, humans, etc.
- Entrainment by light-dark cycles (**zeitgeber**)
- Phase shifting by light pulses
- Temperature compensation

**Circadian rhythms emerge at the molecular level but are reinforced at cellular and tissue scales**

# Circadian Component to Performance



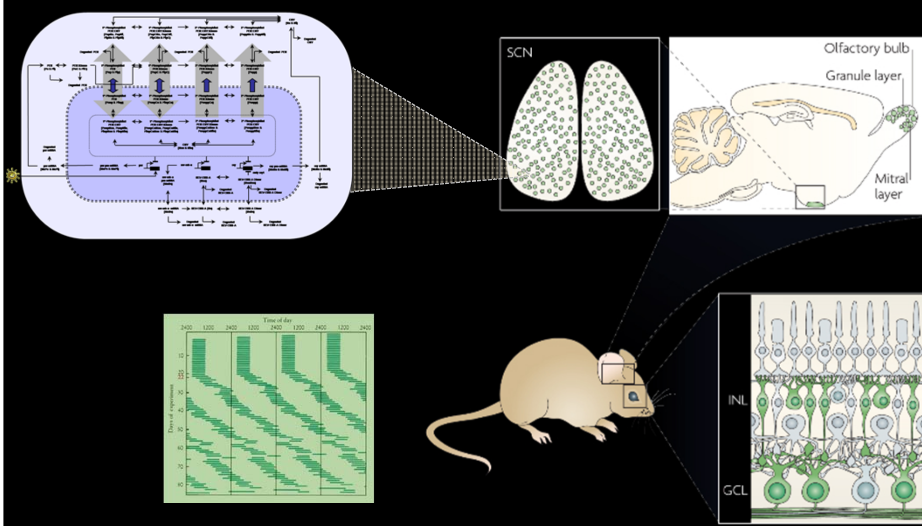

[The Body Clock Guide to Better Health, 2000]




[Hull et al., 2003]

# Multi-scale Networks in Circadian Rhythms

[adapted from Herzog, 2007]

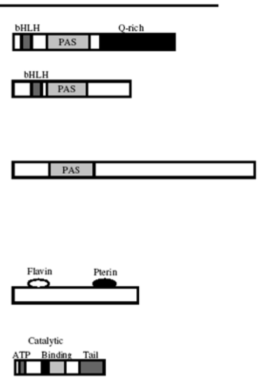



# Molecular Components of Circadian Clock

[Forger et al., 2003]

## Clock Protein Families

| Protein | Features |
|---|---|
| CLOCK<br>MOP4<br>BMAL1<br>MOP9 | |
| PER1<br>PER2<br>PER3 | |
| CRY1<br>CRY2 | |
| CKIε<br>CKIδ | |




## Clock Gene Mutations

| Gene | Type | Behavioral Phenotype |
|---|---|---|
| *Clock* | Deletion | Long period to arrhythmic |
| *BMAL1* | Null | Arrhythmic |
| *Per1* | Null | Var. period to arrhythmic |
| *Per2* | Null | Var. period to arrhythmic |
| *Per3* | Null | Short period |
| *Per1/2* | Null/Null | Arrhythmic |
| *Cry1* | Null | Short period |
| *Cry2* | Null | Long period |
| *Cry1/2* | Null/Null | Arrhythmic |
| *CKIε* | Missense | Short period ( *tau* hamster) |
| *CKIδ* | Not Done | Unknown |

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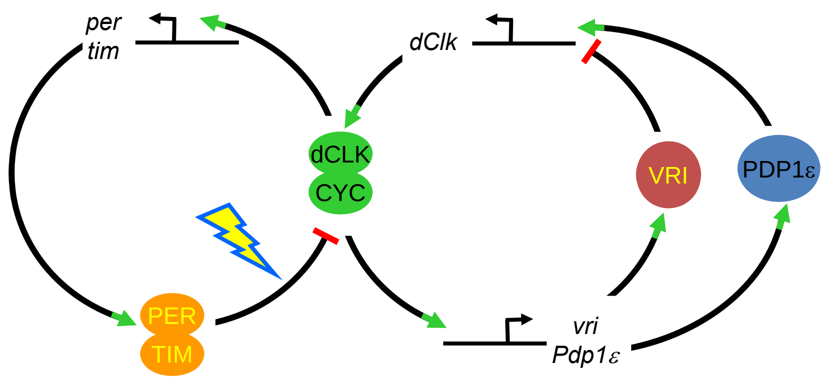
## Circadian Rhythm Gene Network
*[Drosophila]*




adapted from [Cryan *et al.*, *Cell*, 2003]

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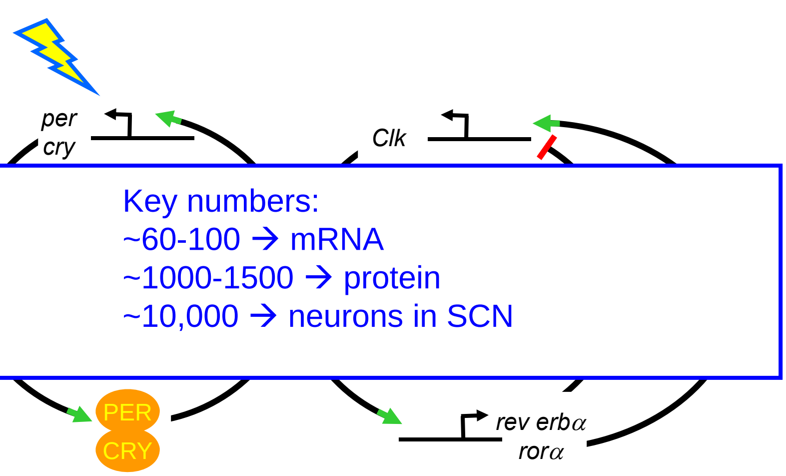
## Circadian Rhythm Gene Network
*[Mouse]*




Key numbers:
~60-100 → mRNA
~1000-1500 → protein
~10,000 → neurons in SCN

adapted from [Cryan *et al.*, *Cell*, 2003]

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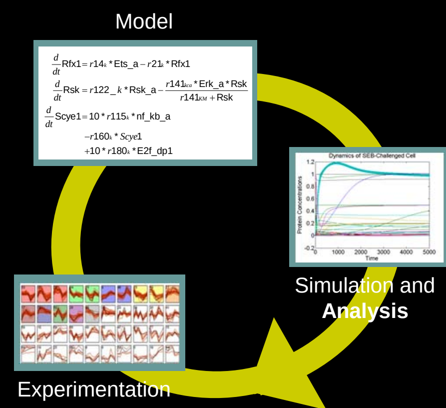
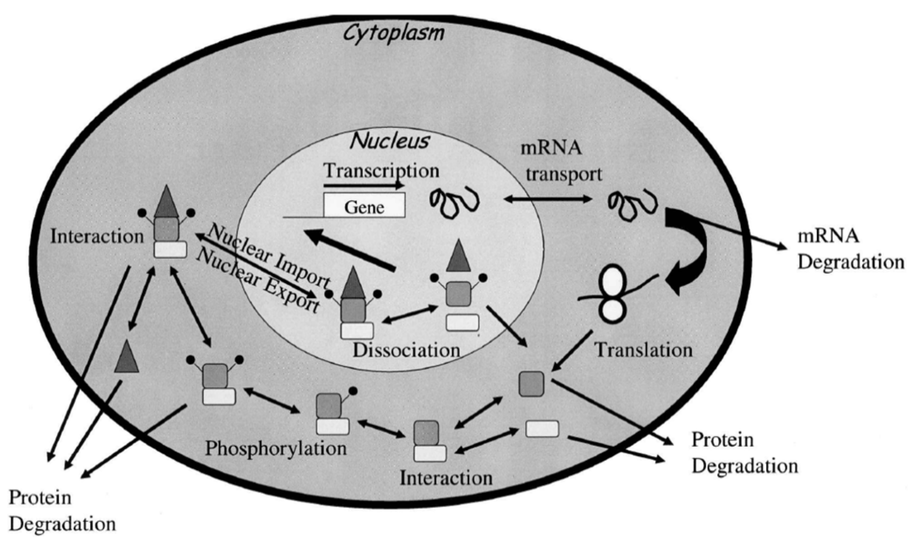
## Model Analysis

- Test hypotheses
- *In silico* clinical trials
- Refine design of experiment
- Probe for understanding
- Determine best control input




## Generic Model

[Forger et al., 2003]

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## Model Validation Criteria

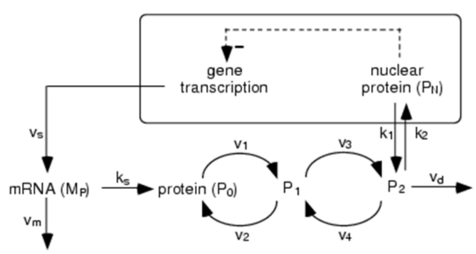
[Forger et al., 2003]

| *Criterion* | *Expected model performance* |
|---|---|
| 1. Period | Molecular oscillations occur with a free-running period (tau) of approximately 24 h. |
| 2. Phase | (a) Molecular oscillations occur with appropriate phase relationships to each other in free-running conditions (appropriate = consistent with experimental data or plausible).<br>(b) Molecular oscillations occur with appropriate phase relationships to each other and to the light-dark cycle. |
| 3. Entrainment | (a) Input repeated at 24-h intervals results in 24-h periodicity of the molecular oscillations.<br>(b) The molecular basis for the input to influence molecular oscillations should be based on experimental data. |
| 4. Phase response | (a) Single stimuli lead to alterations in the phase of molecular oscillation.<br>(b) The response to a stimulus depends on the phase at which it is administered.<br>(c) The molecular basis for the input to influence molecular oscillations should be based on experimental data. |
| 5. Mutations | Mutations affecting the level or activity of circadian-relevant genes *in vivo* should produce similar effects on oscillations *in silico*. |



## 5-State Model

[Goldbeter, 1996; Gonze *et al.*, 2002]




5 states, 18 parameters

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

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

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

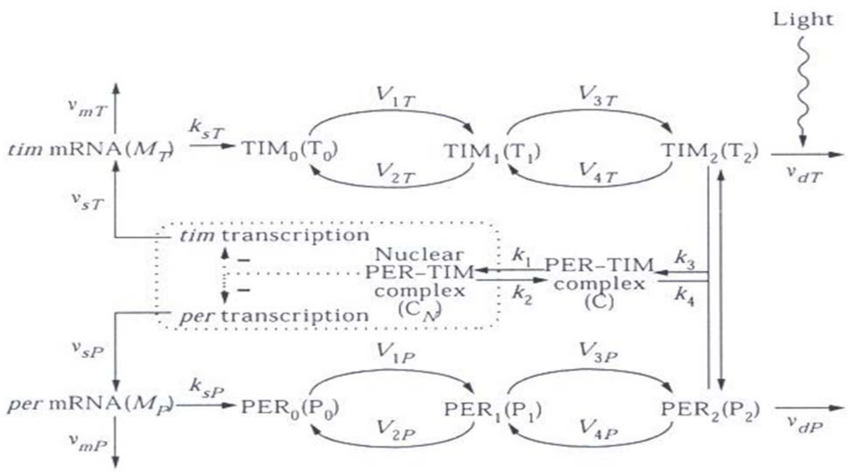
$$\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N$$

$$\frac{dP_2}{dt} = k_1 P_2 - k_2 P_N$$

## 10-state Model

[Gonze *et al.*, 2002]

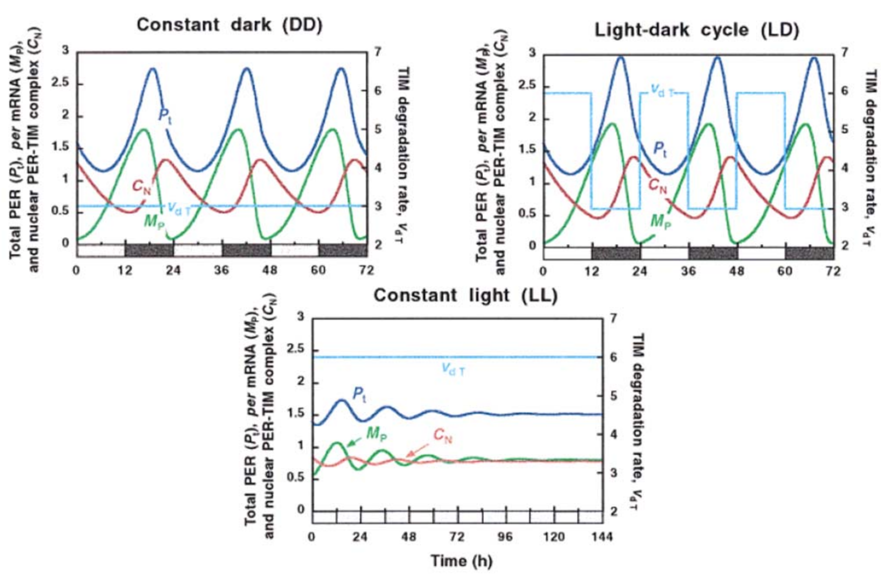
10 states, 38 parameters



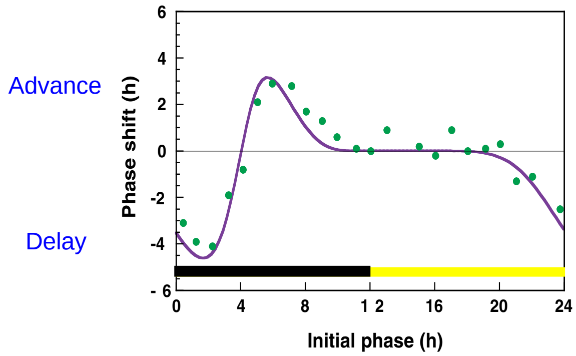



## Entrainment Behavior

[Leloup & Goldbeter]



## Light as a Zeitgeber



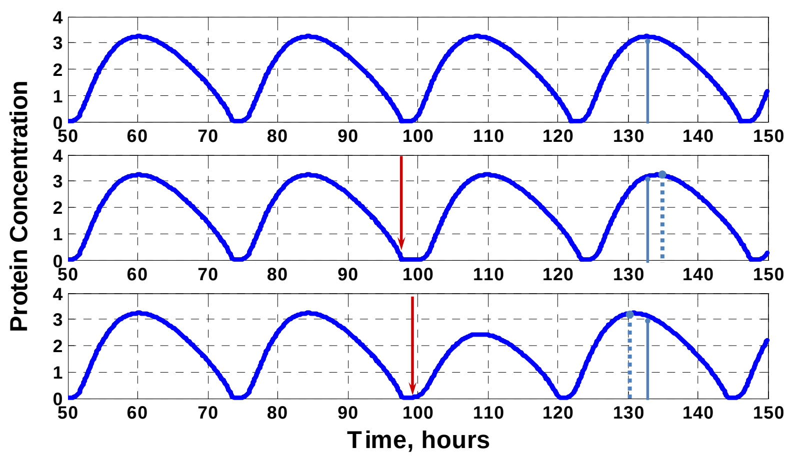

● Experimental data [Hall & Rosbash, 1987]

– Theoretical phase response curve [LeLoup]



## Influence of Light Pulses

[Bagheri et al., 2004]



# Computational Models of Chemical Reacting Systems

- **Continuous and deterministic** – (*rate equations*) Described by ordinary differential equations (ODE). Huge numbers of molecules.
- **Continuous and stochastic** – (*Langevin regime*) Valid under certain conditions. Described by Stochastic Differential Equations (SDE). Large numbers of molecules.
- **Discrete and stochastic** – Finest scale of representation for well stirred molecules. Exact description via Stochastic Simulation Algorithm (SSA) [Gillespie, 1977]. The *only* algorithm for small numbers of molecules.



# Enzymatic Degradation of mRNA

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

$v_m = 0.3 \text{ nMh}^{-1}$
$K_m = 0.2 \text{ nM}$

$$\text{Mp} \longrightarrow \qquad w_3 = (v_m \Omega) \frac{M_P}{(K_m \Omega) + M_P} \qquad M_P \longrightarrow M_P - 1$$

$v_m = 0.3 \text{ mol h}^{-1}$
$K_m = 0.2 \text{ mol}$

| | |
|---|---|
| $\text{Mp} + \text{E}_\text{m} \xrightarrow{k_{m1}} \text{C}_\text{m}$ | $w_{10} = k_{m1} \times M_P \times E_m / \Omega$ |
| $\text{C}_\text{m} \xrightarrow{k_{m2}} \text{Mp} + \text{E}_\text{m}$ | $w_{11} = k_{m2} \times C_m$ |
| $\text{C}_\text{m} \xrightarrow{k_{m3}} \text{E}_\text{m}$ | $w_{12} = k_{m3} \times C_m$ |

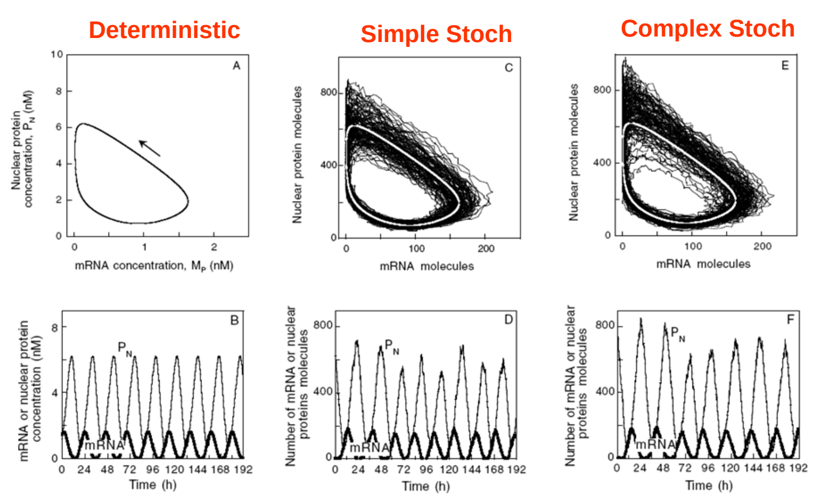
$k_{m1} = 165 \text{ mol}^{-1} \text{ h}^{-1}$,
$k_{m2} = 30 \text{ h}^{-1}$, $k_{m3} = 3 \text{ h}^{-1}$,
$E_{mtot} = E_m + C_m = (0.1 \times \Omega) \text{ mol}$

| Reaction number | Reaction step | Probability of reaction step |
|---|---|---|
| 1a | $G + P_N \xrightarrow{a_1} GP_N$ | $w_1 = a_1 \times G \times P_N/\Omega$ |
| 1b | $GP_N \xrightarrow{d_1} G + P_N$ | $w_2 = d_1 \times GP_N$ |
| 1c | $GP_N + P_N \xrightarrow{a_2} GP_{N2}$ | $w_3 = a_2 \times GP_N \times P_N/\Omega$ |
| 1d | $GP_{N2} \xrightarrow{d_2} GP_N + P_N$ | $w_4 = d_2 \times GP_{N2}$ |
| 1e | $GP_{N2} + P_N \xrightarrow{a_3} GP_{N3}$ | $w_5 = a_3 \times GP_{N2} \times P_N/\Omega$ |
| 1f | $GP_{N3} \xrightarrow{d_3} GP_{N2} + P_N$ | $w_6 = d_3 \times GP_{N3}$ |
| 1g | $GP_{N3} + P_N \xrightarrow{a_4} GP_{N4}$ | $w_7 = a_4 \times GP_{N3} \times P_N/\Omega$ |
| 1h | $GP_{N4} \xrightarrow{d_4} GP_{N3} + P_N$ | $w_8 = d_4 \times GP_{N4}$ |
| 1i | $[G, GP_N, GP_{N2}, GP_{N3}] \xrightarrow{v_s} Mp$ | $w_9 = v_s \times (G + GP_N + GP_{N2} + GP_{N3})$ |
| 2a | $Mp + E_m \xrightarrow{k_{m1}} C_m$ | $w_{10} = k_{m1} \times M_P \times E_m/\Omega$ |
| 2b | $C_m \xrightarrow{k_{m2}} Mp + E_m$ | $w_{11} = k_{m2} \times C_m$ |
| 2c | $C_m \xrightarrow{k_{m3}} E_m$ | $w_{12} = k_{m3} \times C_m$ |
| 3 | $Mp \xrightarrow{k_s} Mp + P_0$ | $w_{13} = k_s \times M_P$ |
| 4a | $P_0 + E_1 \xrightarrow{k_{11}} C_1$ | $w_{14} = k_{11} \times P_0 \times E_1/\Omega$ |
| 4b | $C_1 \xrightarrow{k_{12}} P_0 + E_1$ | $w_{15} = k_{12} \times C_1$ |
| 4c | $C_1 \xrightarrow{k_{13}} P_1 + E_1$ | $w_{16} = k_{13} \times C_1$ |
| 5a | $P_1 + E_2 \xrightarrow{k_{21}} C_2$ | $w_{17} = k_{21} \times P_1 \times E_2/\Omega$ |
| 5b | $C_2 \xrightarrow{k_{22}} P_1 + E_2$ | $w_{18} = k_{22} \times C_2$ |
| 5c | $C_2 \xrightarrow{k_{23}} P_0 + E_2$ | $w_{19} = k_{23} \times C_2$ |
| 6a | $P_1 + E_3 \xrightarrow{k_{31}} C_3$ | $w_{20} = k_{31} \times P_1 \times E_3/\Omega$ |

| Reaction number | Reaction step | Probability of reaction step |
|---|---|---|
| 6b | $C_3 \xrightarrow{k_{32}} P_1 + E_3$ | $w_{21} = k_{32} \times C_3$ |
| 6c | $C_3 \xrightarrow{k_{33}} P_2 + E_3$ | $w_{22} = k_{33} \times C_3$ |
| 7a | $P_2 + E_4 \xrightarrow{k_{41}} C_4$ | $w_{23} = k_{41} \times P_2 \times E_4/\Omega$ |
| 7b | $C_4 \xrightarrow{k_{42}} P_2 + E_4$ | $w_{24} = k_{42} \times C_4$ |
| 7c | $C_4 \xrightarrow{k_{43}} P_1 + E_4$ | $w_{25} = k_{43} \times C_4$ |
| 8a | $P_2 + E_d \xrightarrow{k_{d1}} C_d$ | $w_{26} = k_{d1} \times P_2 \times E_d/\Omega$ |
| 8b | $C_d \xrightarrow{k_{d2}} P_2 + E_d$ | $w_{27} = k_{d2} \times C_d$ |
| 8c | $C_d \xrightarrow{k_{d3}} E_d$ | $w_{28} = k_{d3} \times C_d$ |
| 9 | $P_2 \xrightarrow{k_1} P_N$ | $w_{29} = k_1 \times P_2$ |
| 10 | $P_N \xrightarrow{k_2} P_2$ | $w_{30} = k_2 \times P_N$ |

# Stochastic Behavior

[Ω=100]

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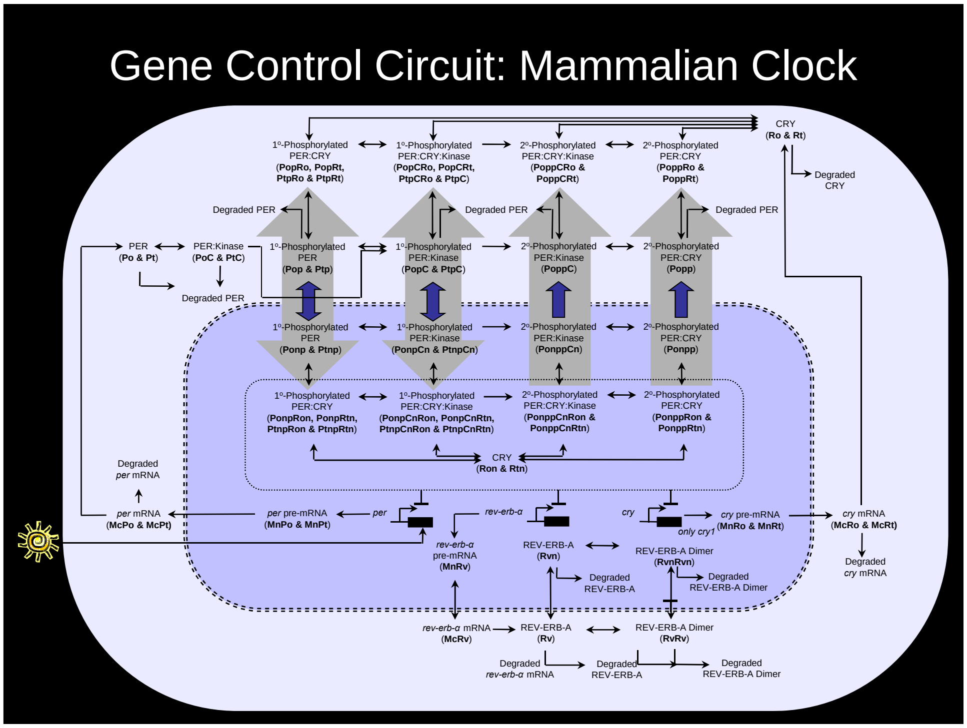
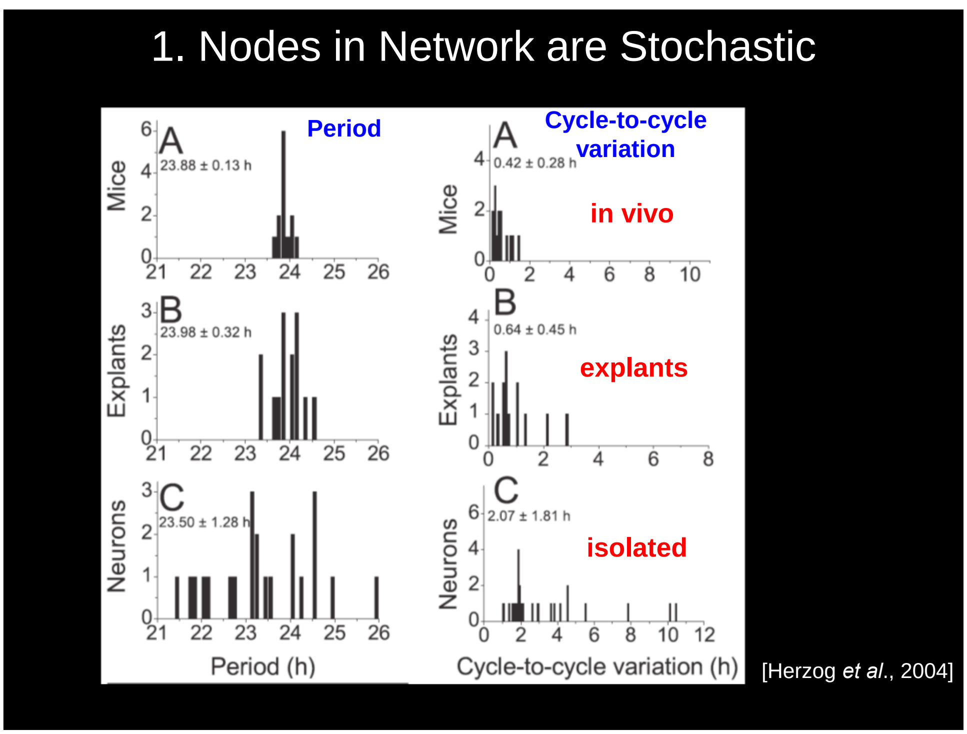

Gene Control Circuit: Mammalian Clock
CRY (Ro & Rt)
Degraded CRY
1°-Phosphorylated PER:CRY (PopRo, PopRt, PtpRo & PtpRt)
1°-Phosphorylated PER:CRY:Kinase (PopCRo, PopCRt, PtpCRo & PtpC)
2°-Phosphorylated PER:CRY:Kinase (PoppCRo & PoppCRt)
2°-Phosphorylated PER:CRY (PoppRo & PoppRt)
Degraded PER
PER (Po & Pt)
PER:Kinase (PoC & PtC)
1°-Phosphorylated PER (Pop & Ptp)
1°-Phosphorylated PER:Kinase (PopC & PtpC)
2°-Phosphorylated PER:Kinase (PoppC)
2°-Phosphorylated PER:CRY (Popp)
1°-Phosphorylated PER (Ponp & Ptnp)
1°-Phosphorylated PER:Kinase (PonpCn & PtnpCn)
2°-Phosphorylated PER:Kinase (PonppCn)
2°-Phosphorylated PER:CRY (Ponpp)
1°-Phosphorylated PER:CRY (PonpRon, PonpRtn, PtnpRon & PtnpRtn)
1°-Phosphorylated PER:CRY:Kinase (PonpCnRon, PonpCnRtn, PtnpCnRon & PtnpCnRtn)
2°-Phosphorylated PER:CRY:Kinase (PonppCnRon & PonppCnRtn)
2°-Phosphorylated PER:CRY (PonppRon & PonppRtn)
CRY (Ron & Rtn)
Degraded per mRNA
per mRNA (McPo & McPt)
per pre-mRNA (MnPo & MnPt)
per
rev-erb-α
cry
cry pre-mRNA (MnRo & MnRt)
only cry1
cry mRNA (McRo & McRt)
Degraded cry mRNA
rev-erb-α pre-mRNA (MnRv)
REV-ERB-A (Rvn)
REV-ERB-A Dimer (RvnRvn)
Degraded REV-ERB-A
Degraded REV-ERB-A Dimer
rev-erb-α mRNA (McRv)
REV-ERB-A (Rv)
REV-ERB-A Dimer (RvRv)
Degraded rev-erb-α mRNA

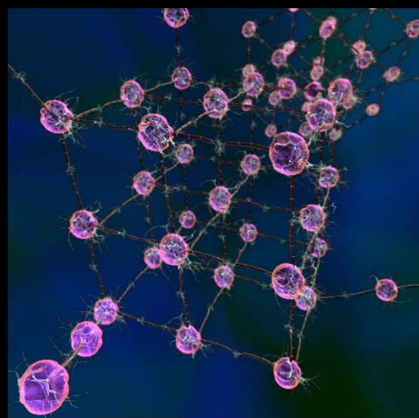

1. Nodes in Network are Stochastic
Period
Cycle-to-cycle variation
in vivo
explants
isolated
Mice
Explants
Neurons
A 23.88 ± 0.13 h
B 23.98 ± 0.32 h
C 23.50 ± 1.28 h
A 0.42 ± 0.28 h
B 0.64 ± 0.45 h
C 2.07 ± 1.81 h
21 22 23 24 25 26
Period (h)
Cycle-to-cycle variation (h)
[Herzog et al., 2004]

# Stochastic Cellular Network Model

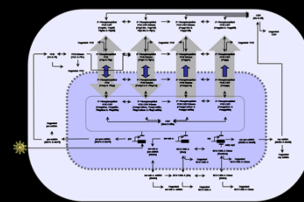
Node: ~25 nonlinear ODES

Network: 20x20 grid

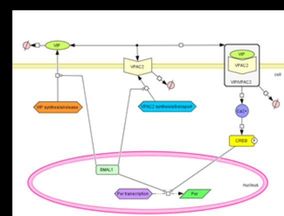
Simulation: DASPK



Gene regulation model

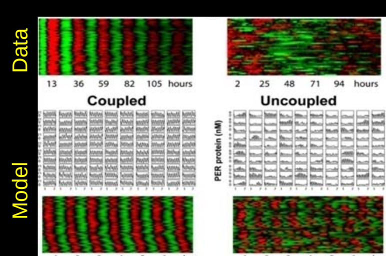


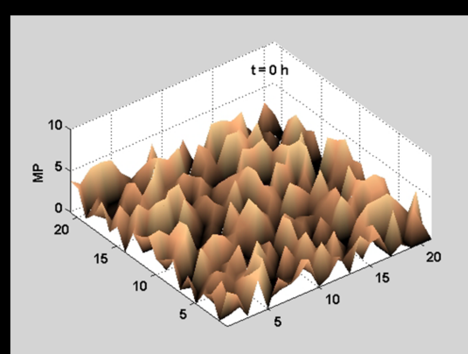
Signaling/coupling model



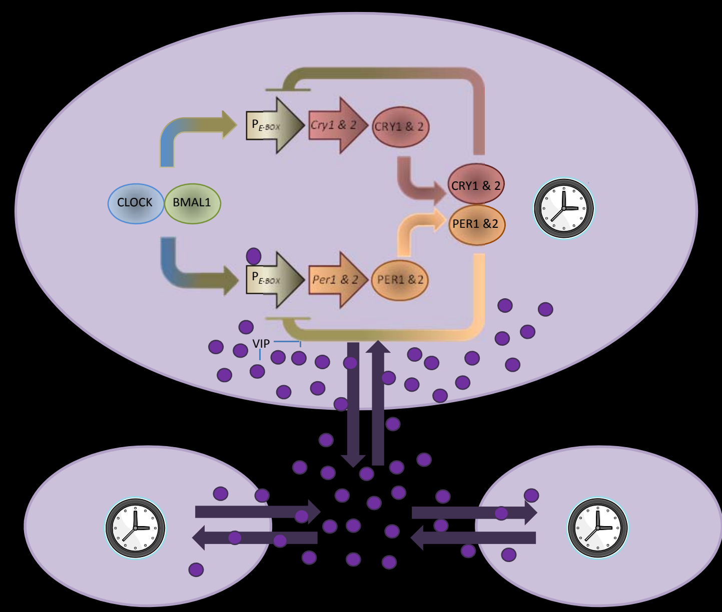
# Stochastic Network Model

[Liu *et al., Cell, 2007*]



## Cellular Network Model - Interactions




## Implications from Systems Biology Studies

- Robustness characteristics of feedback architecture under stochastic uncertainty
- Nature of entrainment, and systems characterization
- Possible therapeutic ramifications (sleep disorders, jet lag, shift work performance, etc.)
- General biological oscillator insights

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