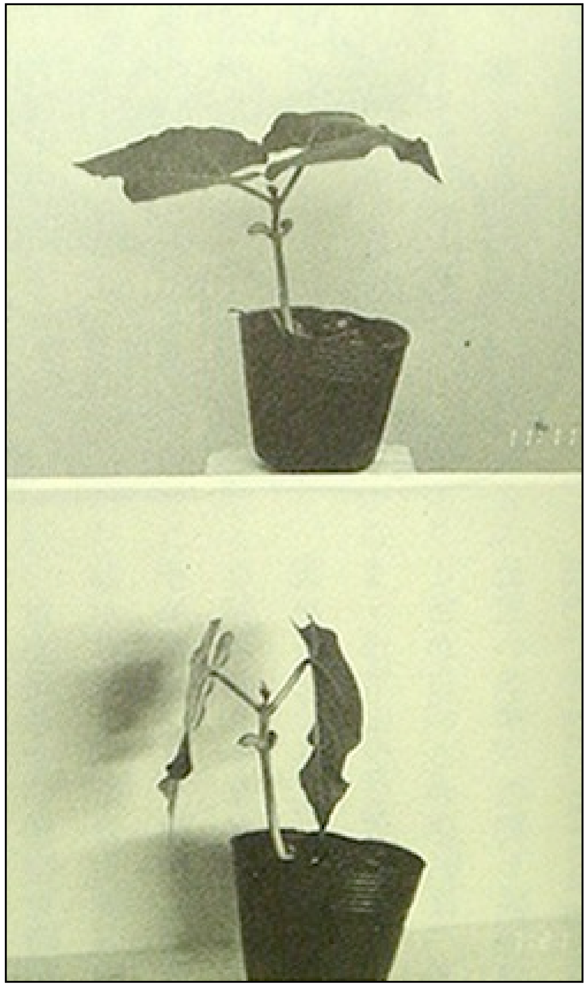
Stochastic simulations

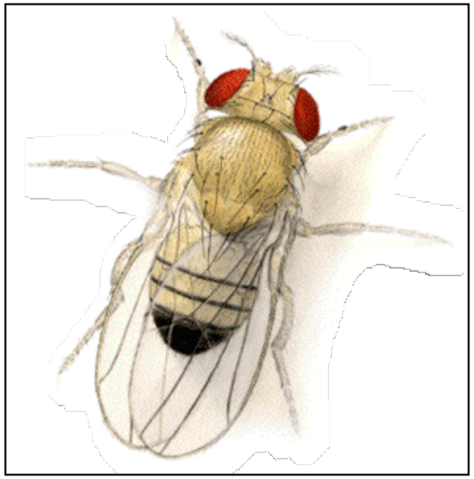
Application to circadian clocks

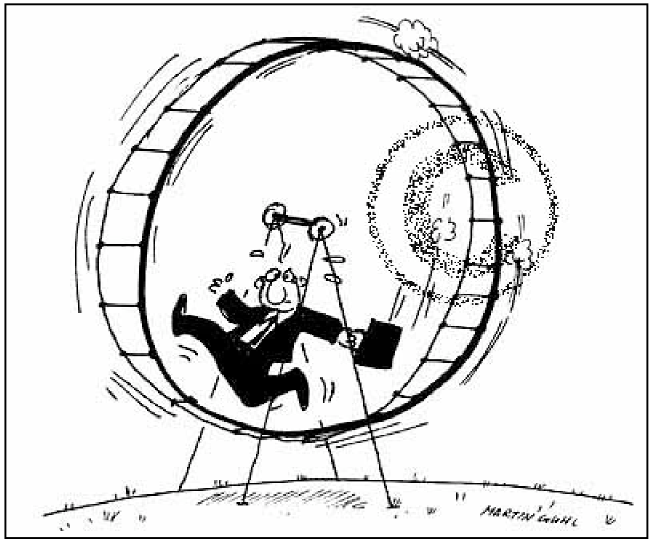
Didier Gonze

Circadian rhythms

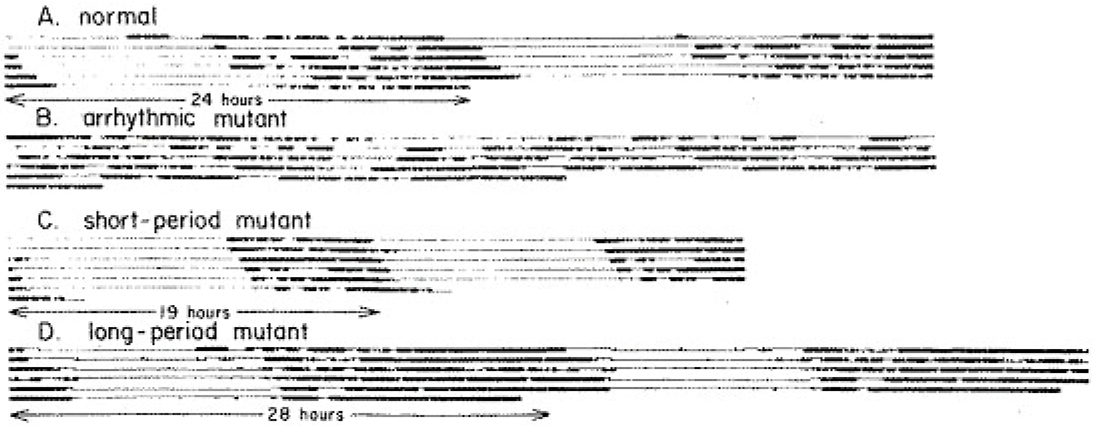
Circadian rhythms allow living organisms to live in phase with the alternance of day and night...



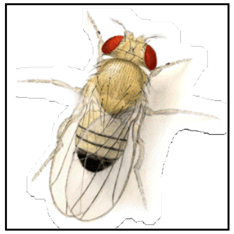




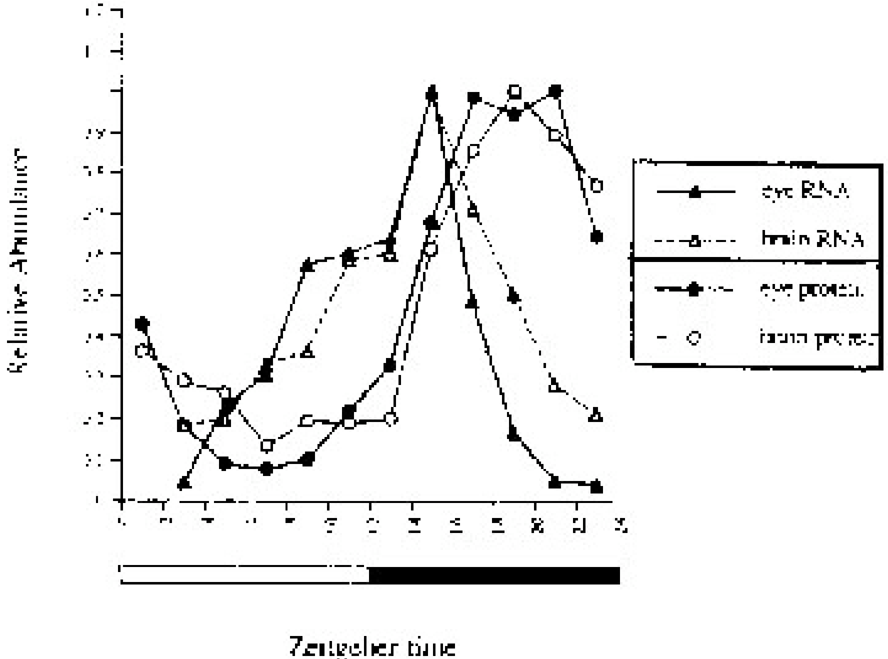
Circadian rhythms in *Drosophila*



Locomotor activity



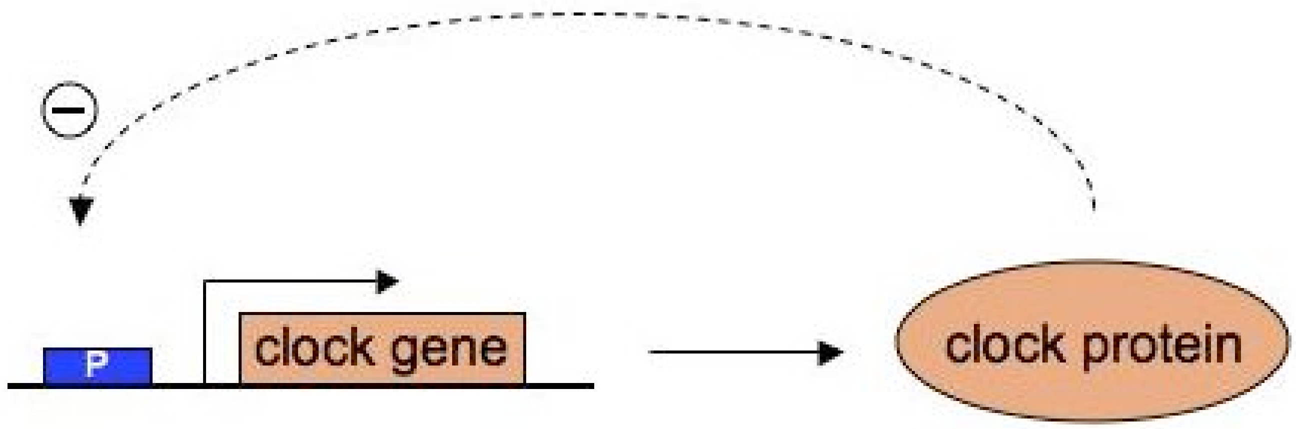
Expression of *per* gene



Konopka RJ & Benzer S (1971) Clock mutants of *Drosophila melanogaster*. *Proc Natl Acad Sci USA* **68,** 2112-6.

Molecular mechanism of circadian clocks

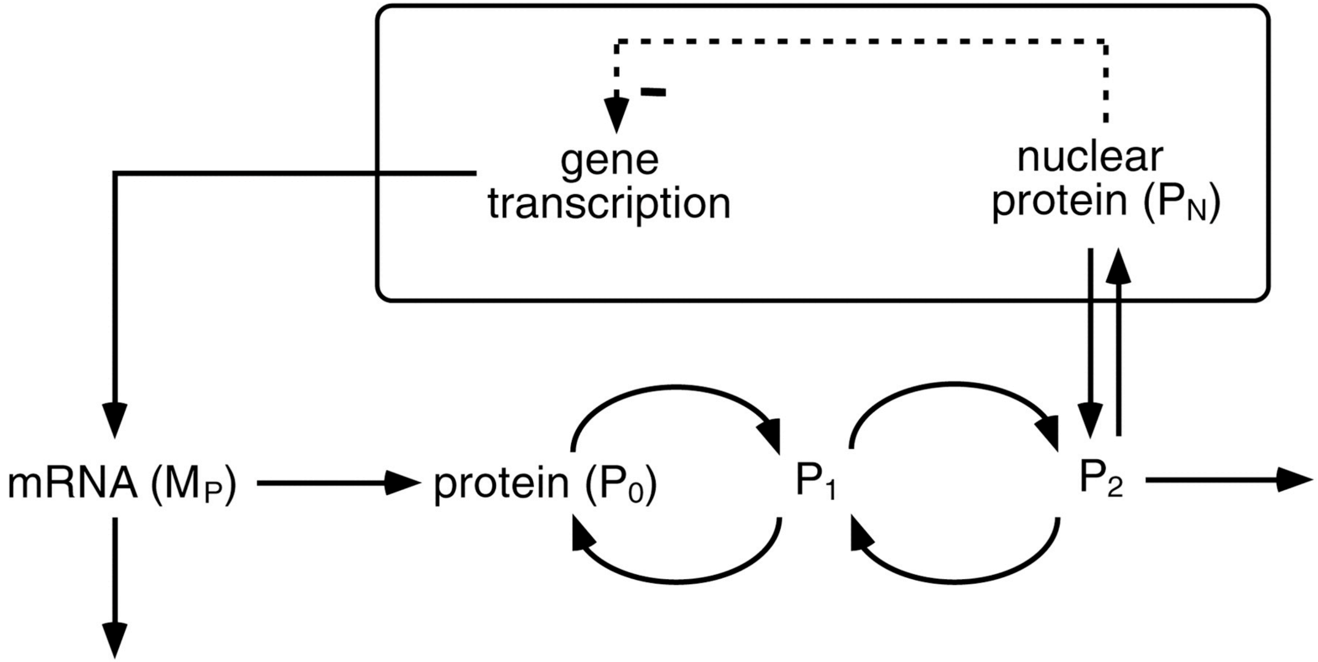
Core mechanism: negative feedback loop



	clock gene
Drosophila	***per*** *(period),* ***tim*** *(timeless)*
Mammals	***mper1-3*** *(period homologs)*
Neurospora	***frq*** *(frequency)*

Deterministic models for circadian rhythms

Goldbeter's 5-variable model



Goldbeter A (1995) A model for circadian oscillations in the *Drosophila* period protein (PER). *Proc. R. Soc. Lond. B. Biol. Sci.* 261, 319-24.

Goldbeter's 5-variable model

per mRNA	$\frac{dM_P}{dt} = v_s \frac{K_I^n}{K_I^n + P_N^n} - v_m \frac{M_P}{K_m + M_P}$
PER protein (unphosph.)	$\frac{dP_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}$
PER protein (monophosph.)	$\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}$
PER protein (biphosph.)	$\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N$
nuclear PER protein	$\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N$

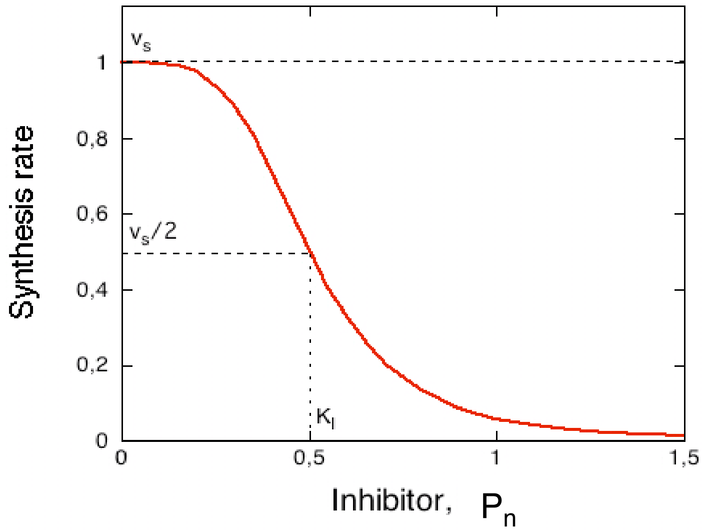
Goldbeter A (1995) A model for circadian oscillations in the *Drosophila* period protein (PER). *Proc. R. Soc. Lond. B. Biol. Sci.* 261, 319-24.

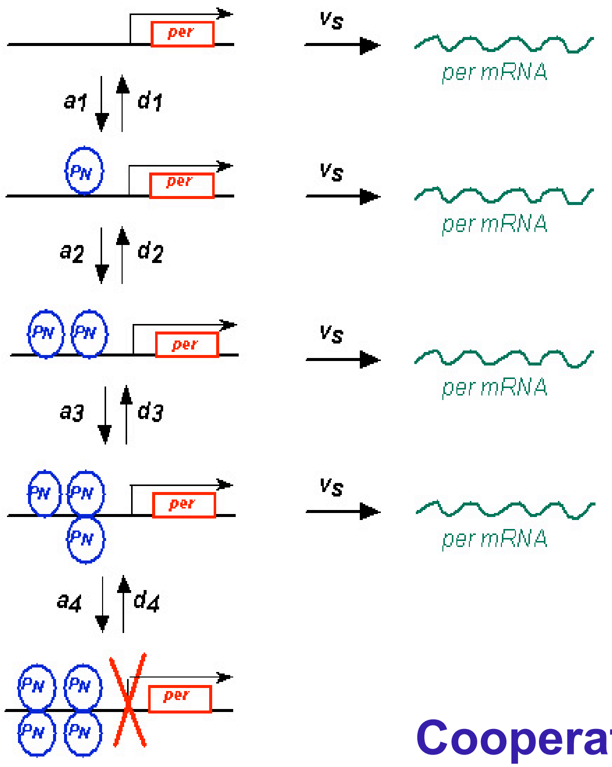
Goldbeter's 5-variable model

Dynamics of *per* mRNA (M_P): 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





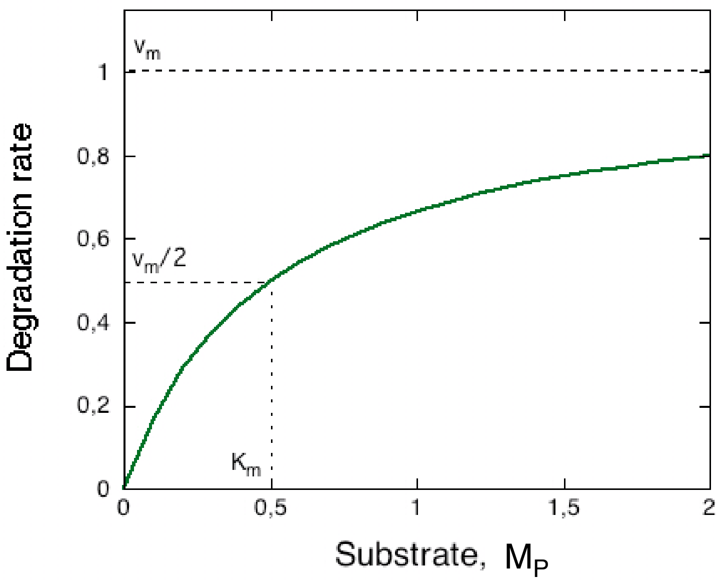
Cooperativity

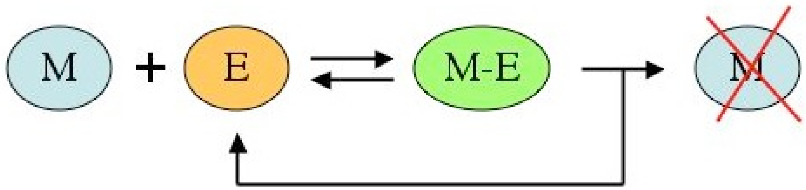
Goldbeter's 5-variable model

Dynamics of *per* mRNA (M_P): degradation

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

Degradation: Michaelis-Menten





$E << M$

$k_1, k_{-1} >> k_2$

$E_{tot} = E + ME$

$K_M = (k_{-1} + k_2) / k_1$ $v_m = k_2 E_{tot}$

Goldbeter's 5-variable model

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

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

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

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 phosphorylation/dephosphorylation:
Michaelis-Menten

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

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

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

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

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

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

PER degradation: Michaelis-Menten

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

Goldbeter's 5-variable model

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

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

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

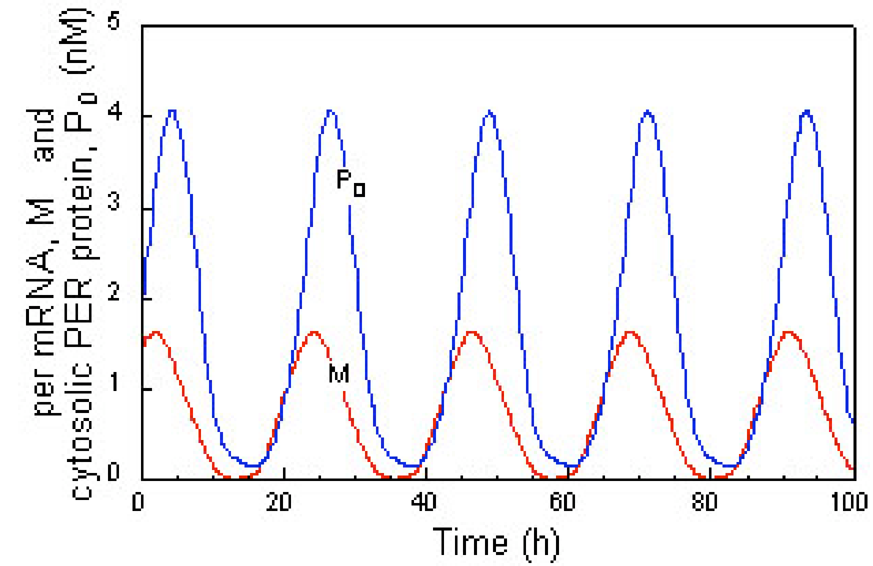
$$\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - \boxed{k_1 P_2 + k_2 P_N}$$

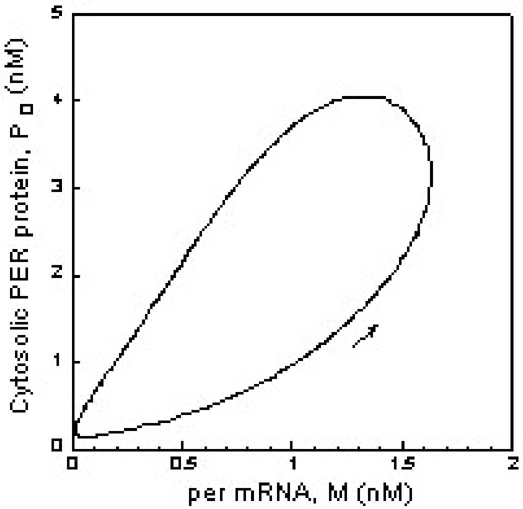
PER nuclear transport: linear

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

Goldbeter's 5-variable model

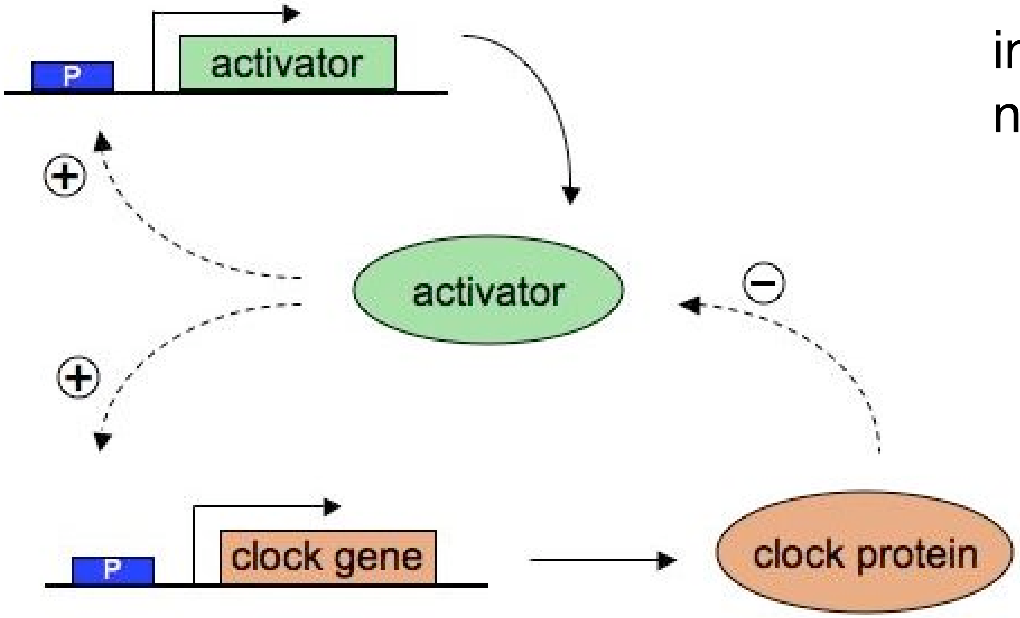
Limit-cycle oscillations





- Mutants (long-period, short-period, arrythmic)
- Entrainment by light-dark cycles
- Phase shift induced by light pulses
- Suppression of oscillations by a light pulse
- Temperature compensation
- ...

Molecular mechanism of circadian clocks



interlocked positive and negative feedback loops

	Clock gene	Activator	Effect of light
Drosophila	*per, tim*	*clk, cyc*	TIM degradation
Mammals	*mper1-3, cry1,2*	*clock, bmal1*	*per* transcription
Neurospora	*frq*	*wc-1, wc-2*	*frq* transcription

Dunlap JC (1999) Molecular bases for circadian clocks. *Cell* **96**: 271-290.
Young MW & Kay SA (2001) Time zones: a comparative genetics of circadian clocks. *Nat. Genet.* **2**: 702-715.

Molecular mechanism of circadian clocks

Example: circadian clock in mammals

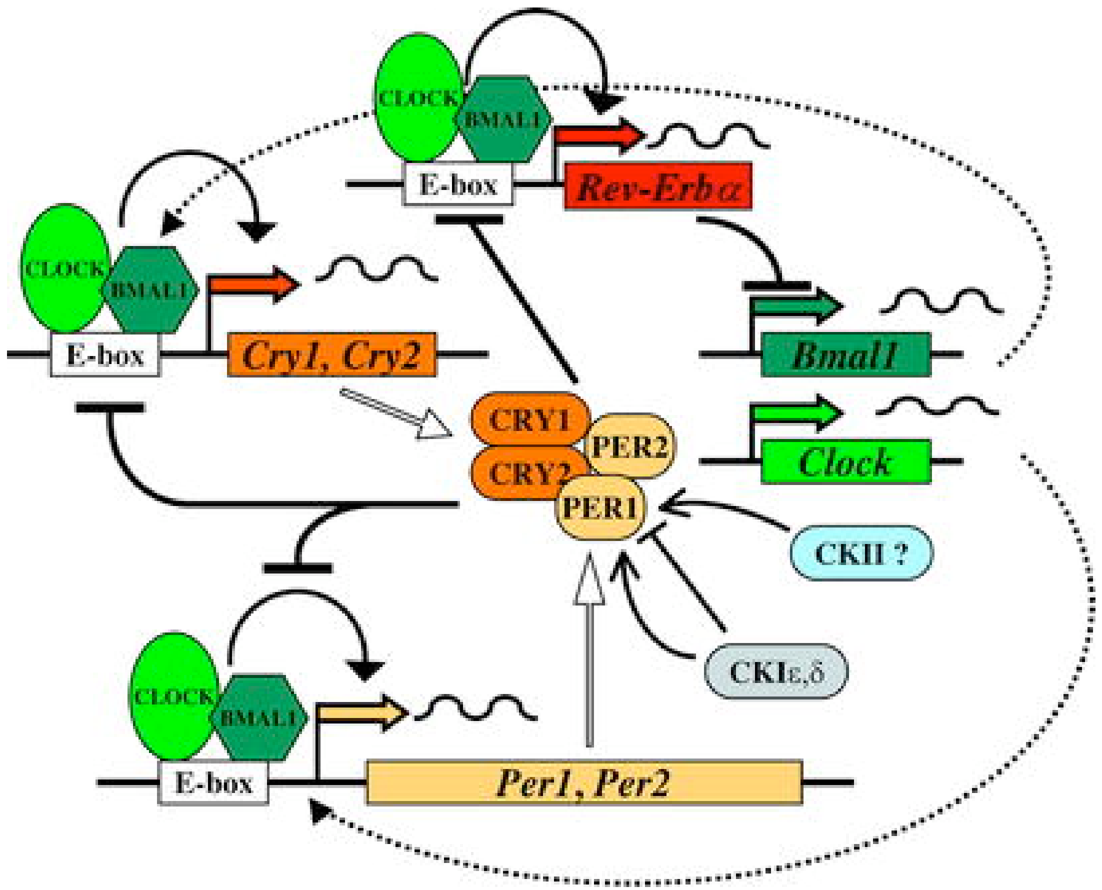
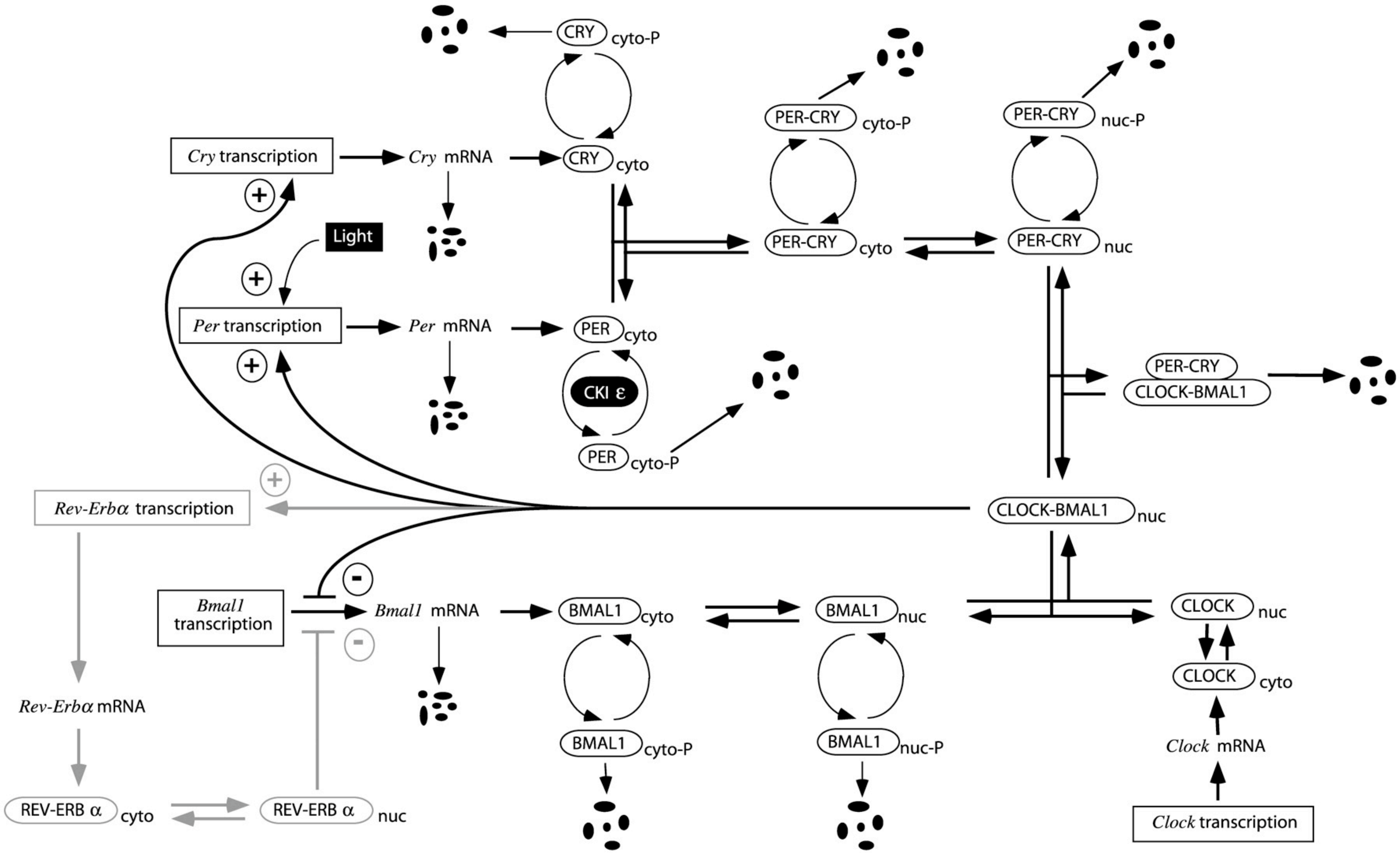


Figure from Gachon, Nagoshi, Brown, Ripperger, Schibler (2004) The mammalian circadian timing system: from gene expression to physiology. *Chromosomia* **113**: 103-112.

Model for the mammalian circadian clock



16-variable model including
per, cry, bmal1, rev-erbα

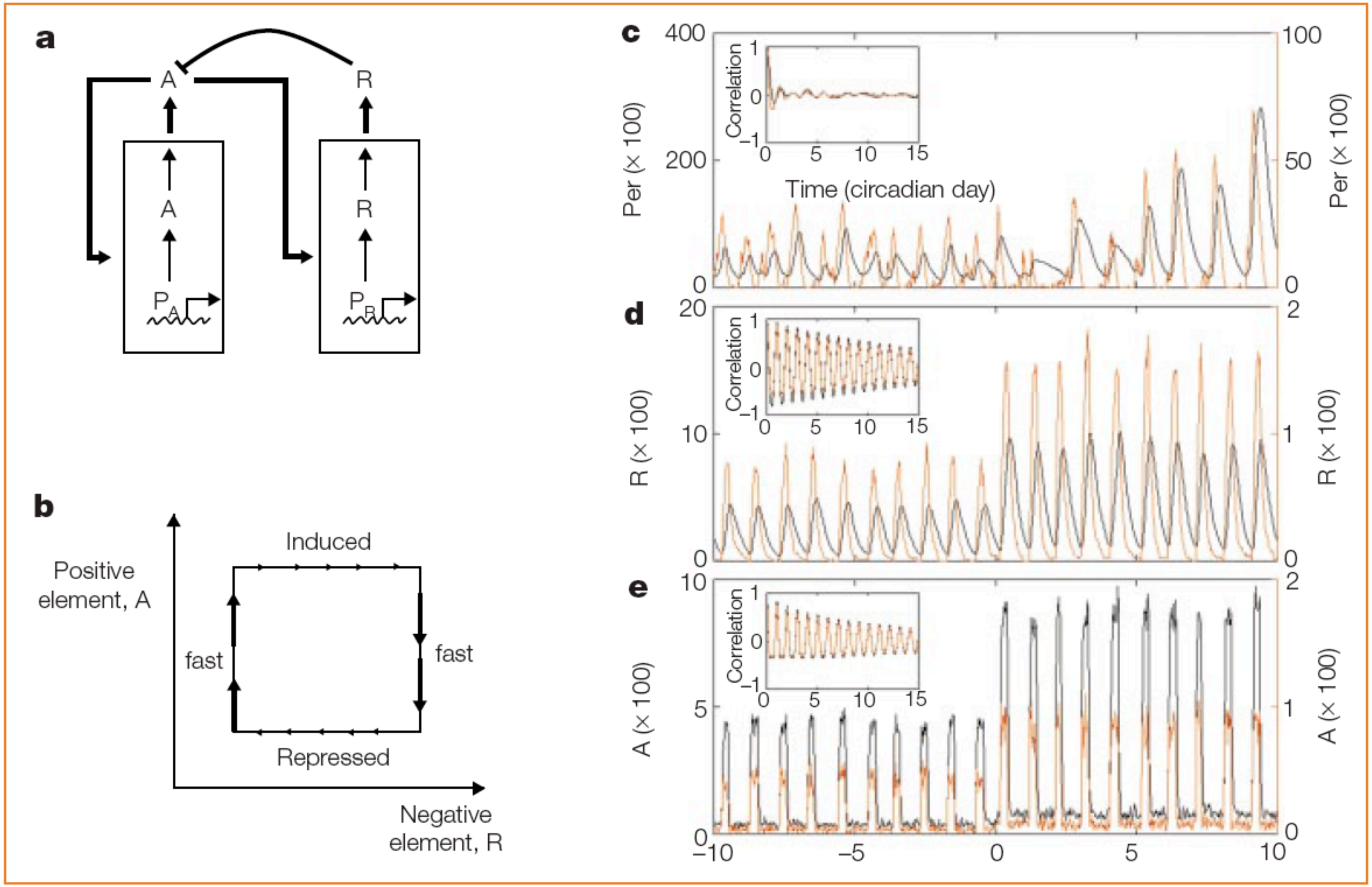
Leloup J-C & Goldbeter A (2003) Toward a detailed computational model for the mammalian circadian clock. *Proc Natl Acad Sci USA.* 100: 7051-7056.

Stochastic models for circadian rhythms

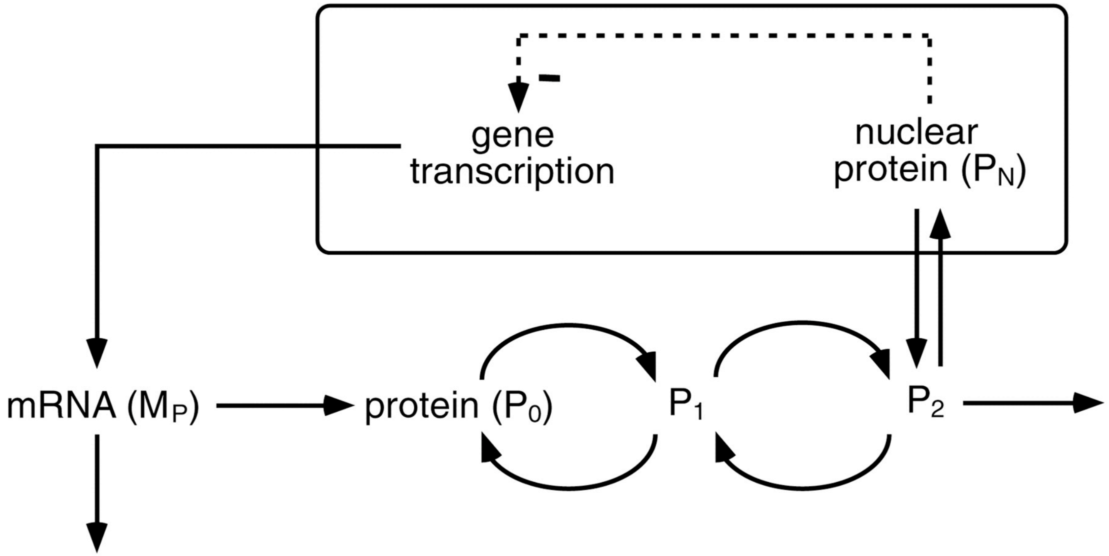
Circadian clocks limited by noise ?

Circadian clocks limited by noise

N. Barkai & S. Leibler, *Nature* (2000) 403: 267-268



Goldbeter's 5-variable model



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

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

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

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

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

Goldbeter A (1995) A model for circadian oscillations in the *Drosophila* period protein (PER). *Proc. R. Soc. Lond. B. Biol. Sci.* 261, 319-24.

Stochastic simulations

Fluctuations are due the limited number of molecules **(molecular noise)**. They can be assessed thanks to stochastic simulations.

Such an approach requires a description in term of the number of molecules (instead of concentrations).

Here, we will focus on several robustness factors:

- Number of molecules
- Degree of cooperativity
- Periodic forcing (LD cycle)
- Proximity of a bifurcation point
- Coupling between cells

Detailed reaction scheme

$$G + P_N \rightleftharpoons GP_N$$
$$GP_N + P_N \rightleftharpoons GP_{N2}$$
$$GP_{N2} + P_N \rightleftharpoons GP_{N3}$$
$$GP_{N3} + P_N \rightleftharpoons GP_{N4}$$

Successive binding of 4 P_N molecules to the gene G

$$[G, GP_{N1}, GP_{N2}, GP_{N3}] \rightarrow M + [G, GP_{N1}, GP_{N2}, GP_{N3}]$$

Transcription

$$M + E_m \rightleftharpoons C_m \rightarrow E_m$$

Degradation of mRNA

$$M \rightarrow M + P_0$$

Translation

$$P_0 + E_1 \rightleftharpoons C_1 \rightarrow P_1 + E_1$$
$$P_1 + E_2 \rightleftharpoons C_2 \rightarrow P_0 + E_2$$
$$P_1 + E_3 \rightleftharpoons C_3 \rightarrow P_2 + E_3$$
$$P_2 + E_4 \rightleftharpoons C_4 \rightarrow P_1 + E_4$$

Two reversible phosphorylation steps

$$P_2 + E_d \rightleftharpoons C_d \rightarrow E_d$$

Degradation of protein

$$P_2 \rightleftharpoons P_n$$

Translocation of protein

Gillespie algorithm

A **reaction rate w_i** is associated to each reaction step. These probabilites are related to the kinetics constants.

Initial number of molecules of each species are specified.

The **time interval** is computed stochastically according the reation rates.

At each time interval, the **reaction** that occurs is chosen randomly according to the probabilities w_i and both the number of molecules and the reaction rates are updated.

$$A \xrightarrow{w_1} B$$

$$B + C \xrightarrow{w_2} D$$

$$D \xrightarrow{w_3} E + F$$

...

Gillespie D.T. (1977) Exact stochastic simulation of coupled chemical reactions. *J. Phys. Chem.* 81: 2340-2361.
Gillespie D.T., (1976) A General Method for Numerically Simulating the Stochastic Time Evolution of Coupled Chemical Reactions. *J. Comp. Phys.*, 22: 403-434.

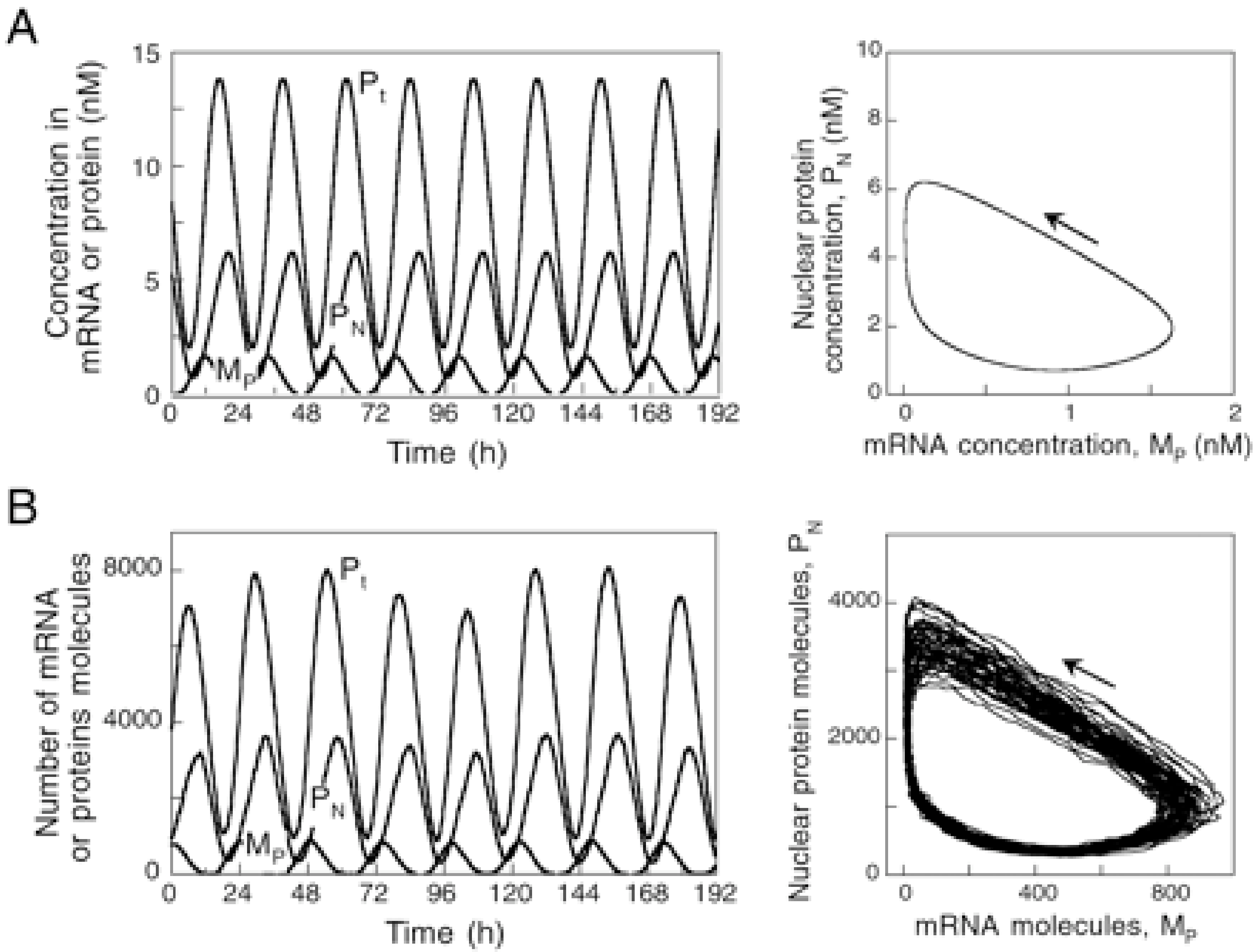
Stochastic description of the model

Reaction number	Reaction step	Probability of reaction
1	$G + P_N \xrightarrow{a_1} GP_N$	$w_1 = a_1 \times G \times P_N / \Omega$
2	$GP_N \xrightarrow{d_1} G + P_N$	$w_2 = d_1 \times GP_N$
3	$GP_N + P_N \xrightarrow{a_2} GP_{N2}$	$w_3 = a_2 \times GP_N \times P_N / \Omega$
4	$GP_{N2} \xrightarrow{d_2} GP_N + P_N$	$w_4 = d_2 \times GP_{N2}$
5	$GP_{N2} + P_N \xrightarrow{a_3} GP_{N3}$	$w_5 = a_3 \times GP_{N2} \times P_N / \Omega$
6	$GP_{N3} \xrightarrow{d_3} GP_{N2} + P_{N3}$	$w_6 = d_3 \times GP_{N3}$
7	$GP_{N3} + P_N \xrightarrow{a_4} GP_{N4}$	$w_7 = a_4 \times GP_{N3} \times P_N / \Omega$
8	$GP_{N4} \xrightarrow{d_4} GP_{N3} + P_N$	$w_8 = d_4 \times GP_{N4}$
9	$[G, GP_N, GP_{N2}, GP_{N3}] \xrightarrow{v_s} M_P$	$w_9 = v_s \times (G + GP_N + GP_{N2} + GP_{N3})$
10	$M_P + E_m \xrightarrow{k_{m1}} C_m$	$w_{10} = k_{m1} \times M_p \times E_m / \Omega$
11	$C_m \xrightarrow{k_{m2}} M_P + E_m$	$w_{11} = k_{m2} \times C_m$
12	$C_m \xrightarrow{k_{m3}} E_m$	$w_{12} = k_{m3} \times C_m$
13	$M_P \xrightarrow{k_s} M_P + P_0$	$w_{13} = k_s \times M_p$
14	$P_0 + E_1 \xrightarrow{k_{11}} C_1$	$w_{14} = k_{11} \times P_0 \times E_1 / \Omega$
15	$C_1 \xrightarrow{k_{12}} P_0 + E_1$	$w_{15} = k_{12} \times C_1$
16	$C_1 \xrightarrow{k_{13}} P_1 + E_1$	$w_{16} = k_{13} \times C_1$
17	$P_1 + E_2 \xrightarrow{k_{21}} C_2$	$w_{17} = k_{21} \times P_1 \times E_2 / \Omega$
18	$C_2 \xrightarrow{k_{22}} P_1 + E_2$	$w_{18} = k_{22} \times C_2$
19	$C_2 \xrightarrow{k_{23}} P_0 + E_2$	$w_{19} = k_{23} \times C_2$
20	$P_1 + E_3 \xrightarrow{k_{31}} C_3$	$w_{20} = k_{31} \times P_1 \times E_3 / \Omega$
21	$C_3 \xrightarrow{k_{32}} P_1 + E_3$	$w_{21} = k_{32} \times C_3$
22	$C_3 \xrightarrow{k_{33}} P_2 + E_3$	$w_{22} = k_{33} \times C_3$
23	$P_2 + E_4 \xrightarrow{k_{41}} C_4$	$w_{23} = k_{41} \times P_2 \times E_4 / \Omega$
24	$C_4 \xrightarrow{k_{42}} P_2 + E_4$	$w_{24} = k_{42} \times C_4$
25	$C_4 \xrightarrow{k_{43}} P_1 + E_4$	$w_{25} = k_{43} \times C_4$
26	$P_2 + E_d \xrightarrow{k_{d1}} C_d$	$w_{26} = k_{d1} \times P_2 \times E_d / \Omega$
27	$C_d \xrightarrow{k_{d2}} P_2 + E_d$	$w_{27} = k_{d2} \times C_d$
28	$C_d \xrightarrow{k_{d3}} E_d$	$w_{28} = k_{d3} \times C_d$
29	$P_2 \xrightarrow{k_1} P_N$	$w_{29} = k_1 \times P_2$
30	$P_N \xrightarrow{k_2} P_2$	$w_{30} = k_2 \times P_N$

Stochastic oscillations and limit cycle

Deterministic

Stochastic



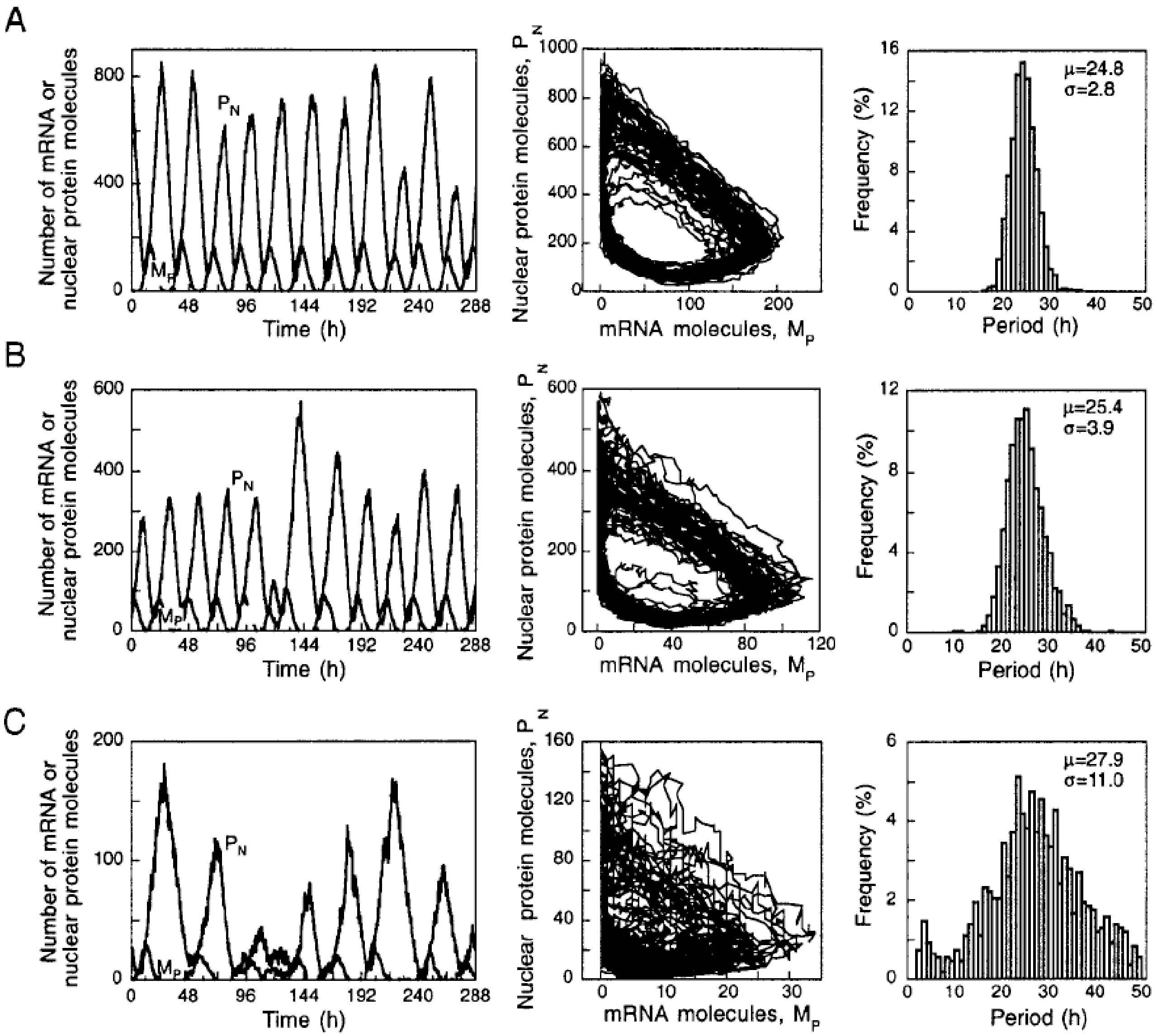
Gonze D, Halloy J, Goldbeter A (2002) Robustness of circadian rhythms with respect to molecular noise. *Proc. Natl. Acad. Sci. USA* 99: 673-678.

Effect of the number of molecules, Ω

Ω=1000

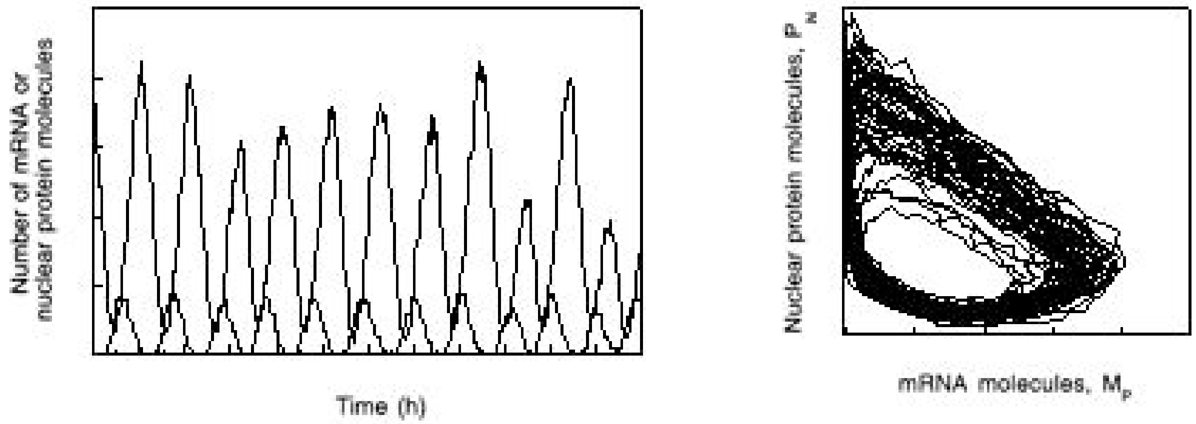
Ω=100

Ω=10

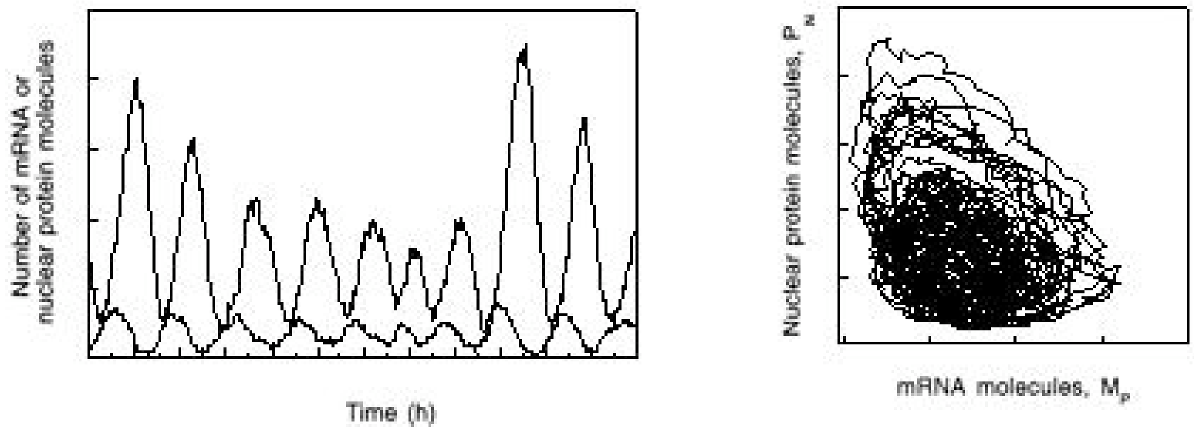


Effect of the degree of cooperativity, *n*

n = 4



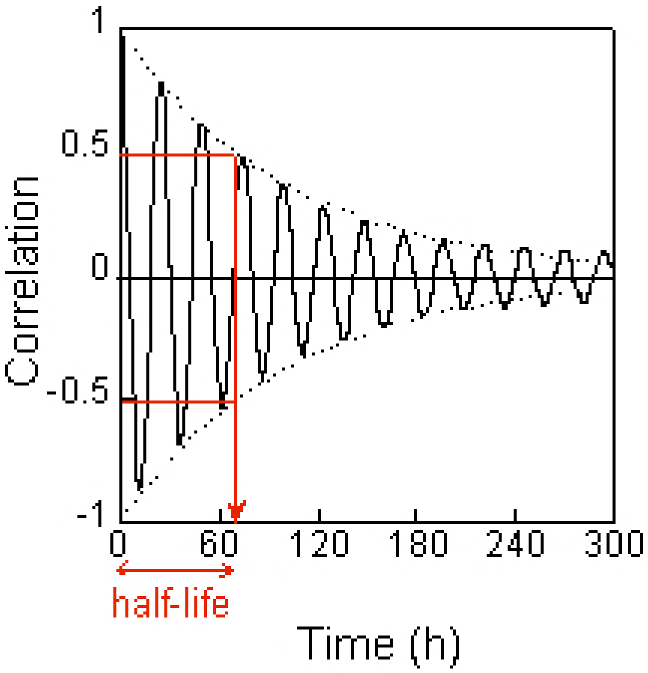
n = 1



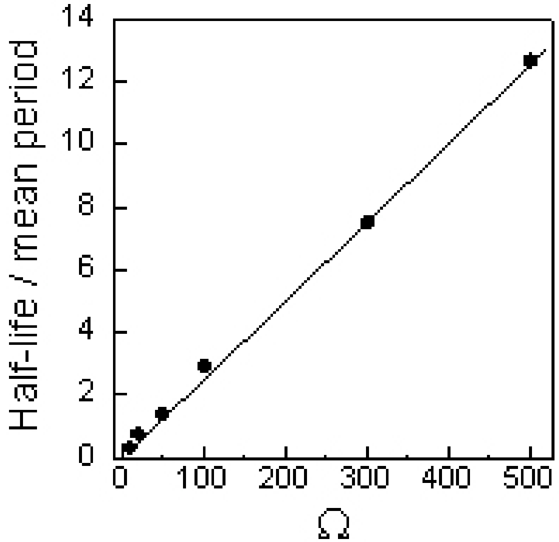
Gonze D, Halloy J, Goldbeter A (2002) Robustness of circadian rhythms with respect to molecular noise. *Proc. Natl. Acad. Sci. USA* 99: 673-678.

Quantification of the effect of noise

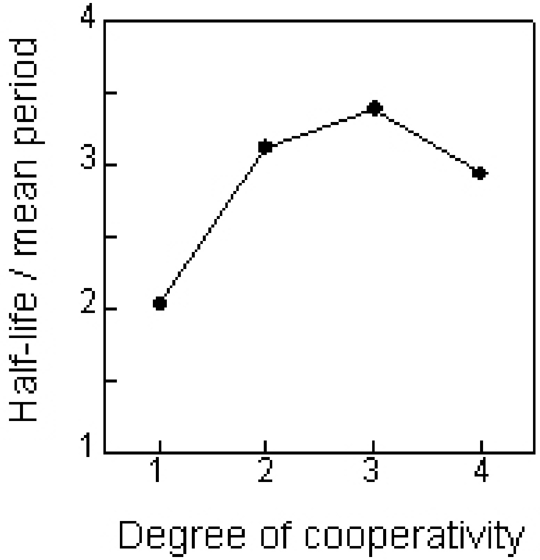
Auto-correlation function



Effect of the number of molecules, Ω



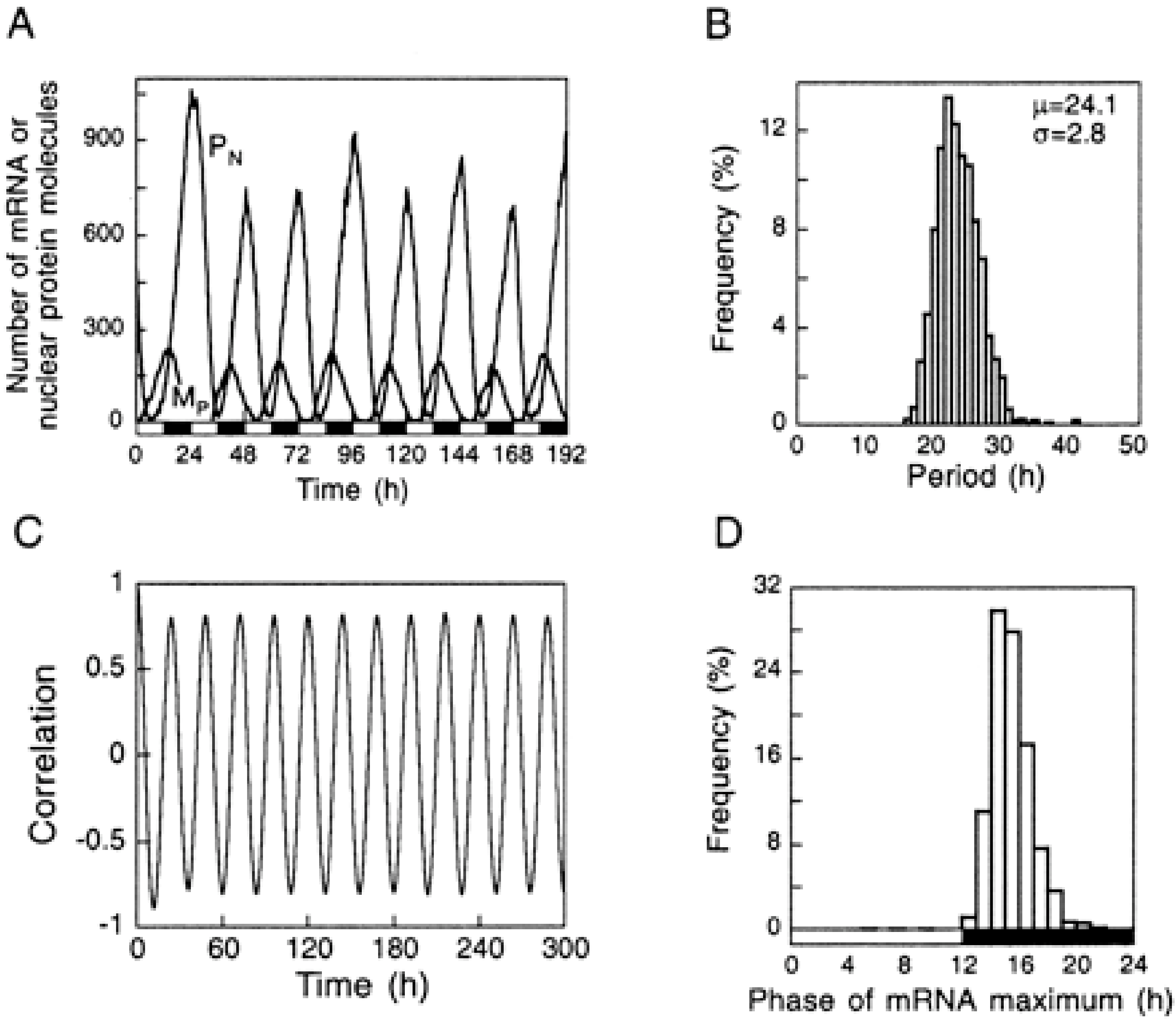
Effect of the degree of cooperativity, *n*



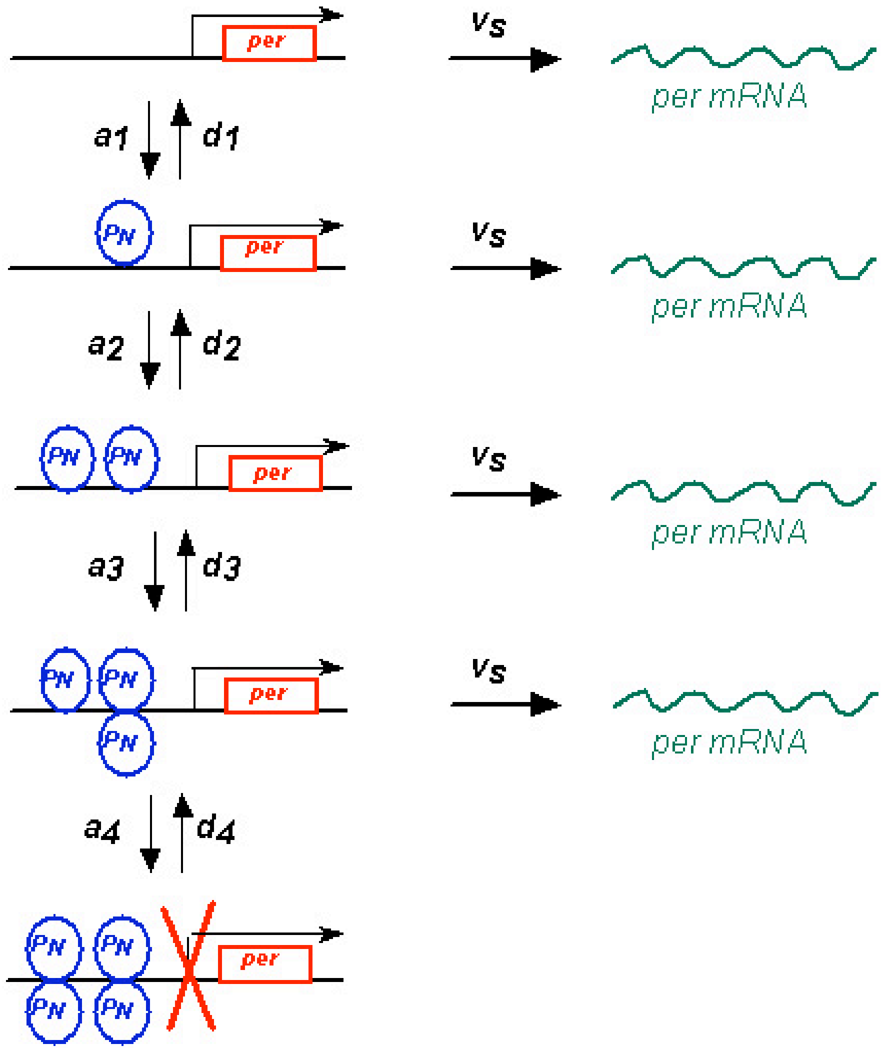
Effect of a periodic forcing (LD cycle)

Light-dark cycle
LD 12:12

light induces
PER protein
degradation, v_d



Cooperative protein-DNA binding



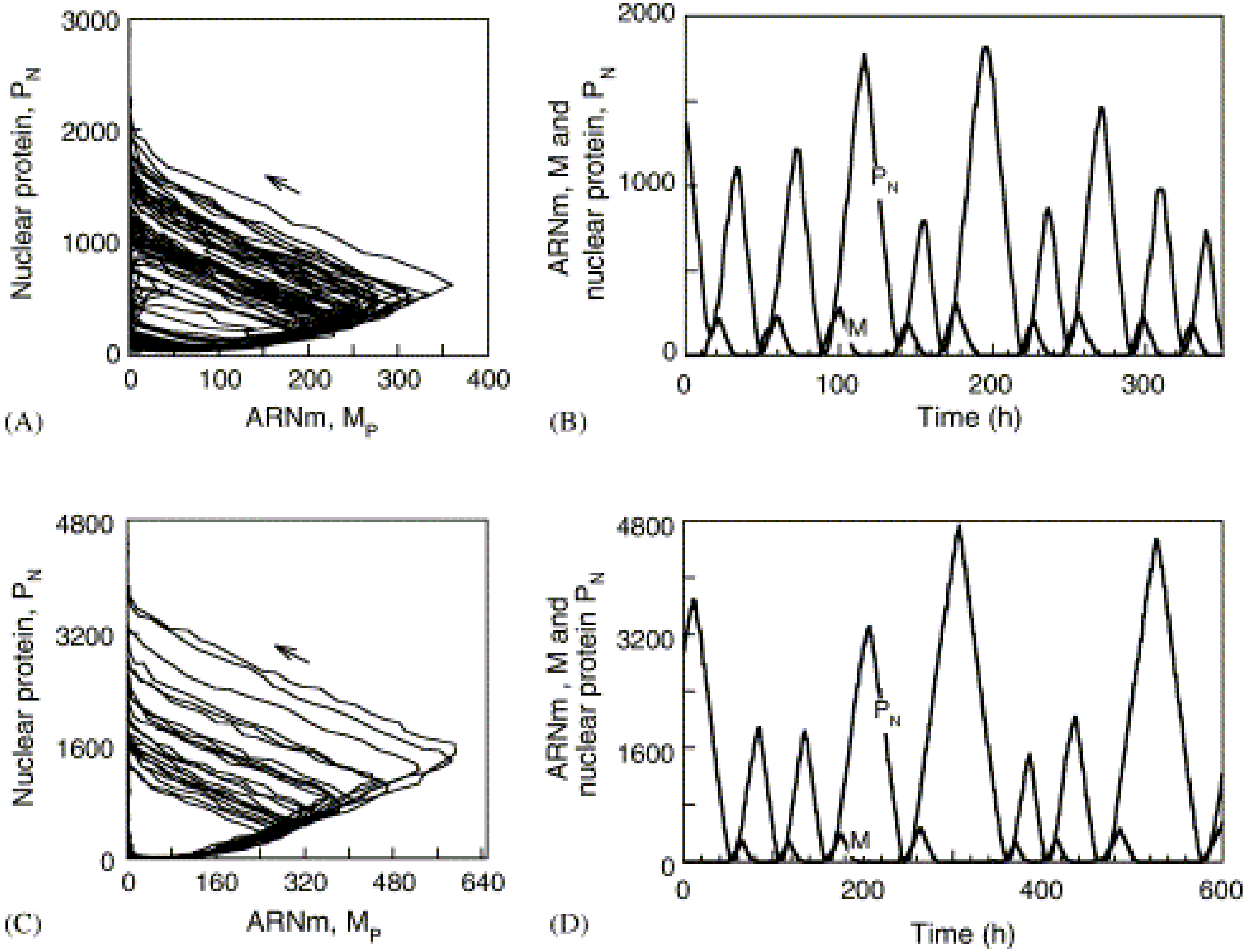
We define γ:

$$a_i \rightarrow a_i / \gamma \quad (i = 1,\ldots 4)$$
$$d_i \rightarrow d_i / \gamma \quad (i = 1,\ldots 4)$$

Influence of the protein-DNA binding rate

$\gamma = 100$

$\gamma = 1000$



Gonze D, Halloy J, Goldbeter A (2004) Emergence of coherent oscillations in stochastic models for circadian rhythms. *Physica A* 342: 221-233.

Developed deterministic model

$$\frac{dG}{dt} = -a_1 GP_N + d_1[GP_N] ,$$

$$\frac{d[GP_N]}{dt} = a_1 GP_N - d_1[GP_N] - a_2[GP_N]P_N + d_2[GP_{N2}] ,$$

$$\frac{d[GP_{N2}]}{dt} = a_2[GP_{N1}]P_N - d_2[GP_{N2}] - a_3[GP_{N2}]P_N + d_3[GP_{N3}] ,$$

$$\frac{d[GP_{N3}]}{dt} = a_3[GP_{N2}]P_N - d_3[GP_{N3}] - a_4[GP_{N3}]P_N + d_4[GP_{N4}] ,$$

$$\frac{d[GP_{N4}]}{dt} = a_4[GP_{N3}]P_N - d_4[GP_{N4}] ,$$

$$\frac{dM}{dt} = v_s(G + [GP_N] + [GP_{N2}] + [GP_{N3}]) - k_{11}ME_m + k_{12}C_m ,$$

$$\frac{dE_m}{dt} = -k_{m1}ME_m + k_{m2}C_m + k_{m3}C_m ,$$

$$\frac{dC_m}{dt} = k_{m1}ME_m - k_{m2}C_m - k_{m3}C_m ,$$

$$\frac{dP_0}{dt} = k_sM - k_{11}P_0E_1 + k_{12}C_1 + k_{23}C_2 ,$$

$$\frac{dE_1}{dt} = -k_{11}P_0E_1 + k_{12}C_1 + k_{13}C_1 ,$$

$$\frac{dC_1}{dt} = k_{11}P_0E_1 - k_{12}C_1 - k_{13}C_1 ,$$

$$\frac{dP_1}{dt} = -k_{21}P_1E_2 + k_{22}C_2 + k_{13}C_1 - k_{31}P_1E_3 + k_{32}C_3 + k_{43}C_4 ,$$

$$\frac{dE_2}{dt} = -k_{21}P_1E_2 + k_{22}C_2 + k_{23}C_2 ,$$

$$\frac{dC_2}{dt} = k_{21}P_1E_2 - k_{22}C_2 - k_{23}C_2 ,$$

$$\frac{dP_2}{dt} = k_{33}C_3 - k_{41}P_2E_4 + k_{42}C_4 - k_{d1}P_2E_d + k_{d2}C_d - k_1P_2 + k_2P_N ,$$

$$\frac{dE_3}{dt} = -k_{31}P_1E_3 + k_{32}C_3 + k_{33}C_3 ,$$

$$\frac{dC_3}{dt} = k_{31}P_1E_3 - k_{32}C_3 - k_{33}C_3 ,$$

$$\frac{dE_4}{dt} = -k_{41}P_2E_4 + k_{42}C_4 + k_{43}C_4 ,$$

$$\frac{dC_4}{dt} = k_{41}P_2E_4 - k_{42}C_4 - k_{43}C_4 ,$$

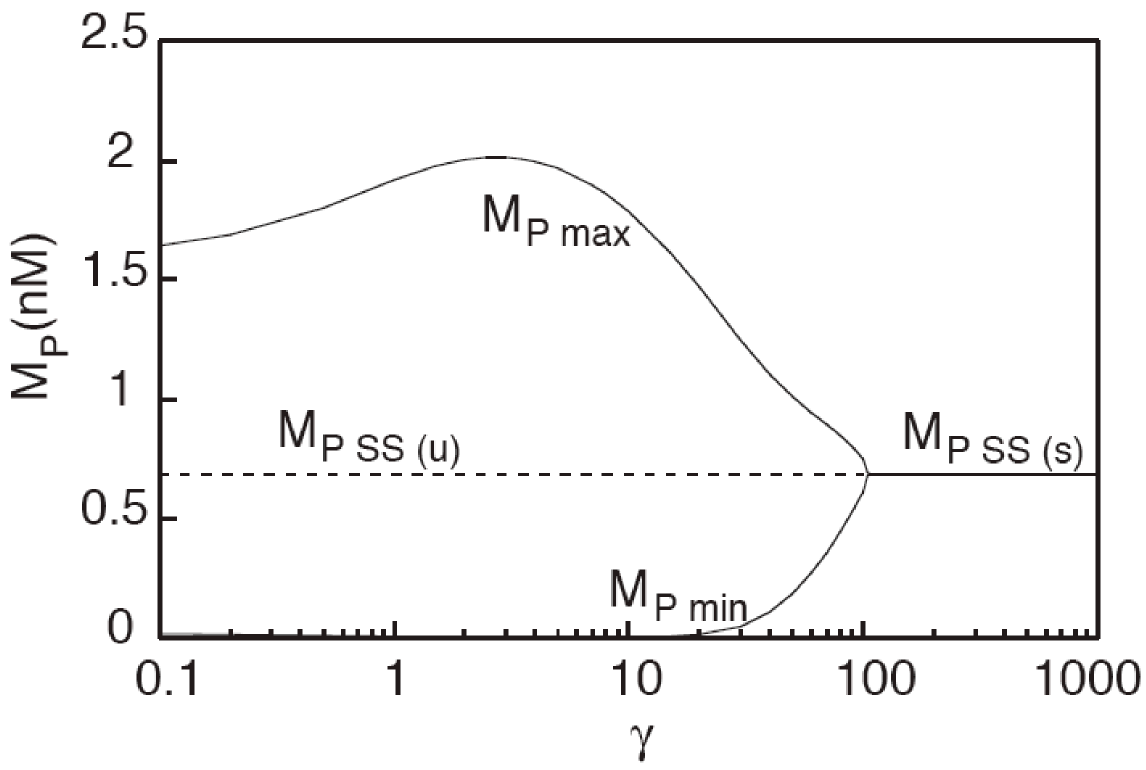
$$\frac{dE_d}{dt} = -k_{d1}P_2E_d + k_{d2}C_d + k_{d3}C_d ,$$

$$\frac{dC_d}{dt} = k_{d1}P_2E_d - k_{d2}C_d - k_{d3}C_d ,$$

$$\frac{dP_N}{dt} = -a_1GP_N + d_1[GP_N] - a_2[GP_{N1}]P_N + d_2[GP_{N2}] - a_3[GP_{N2}]P_N + d_3[GP_{N3}] - a_4[GP_{N3}]P_N + d_4[GP_{N4}] + k_1P_2 - k_2P_N$$

with $G_{tot} = G + GP_N + GP_{N2} + GP_{N3} + GP_{N4} = 1.$

Deterministic model: bifurcation diagram



fast binding rate

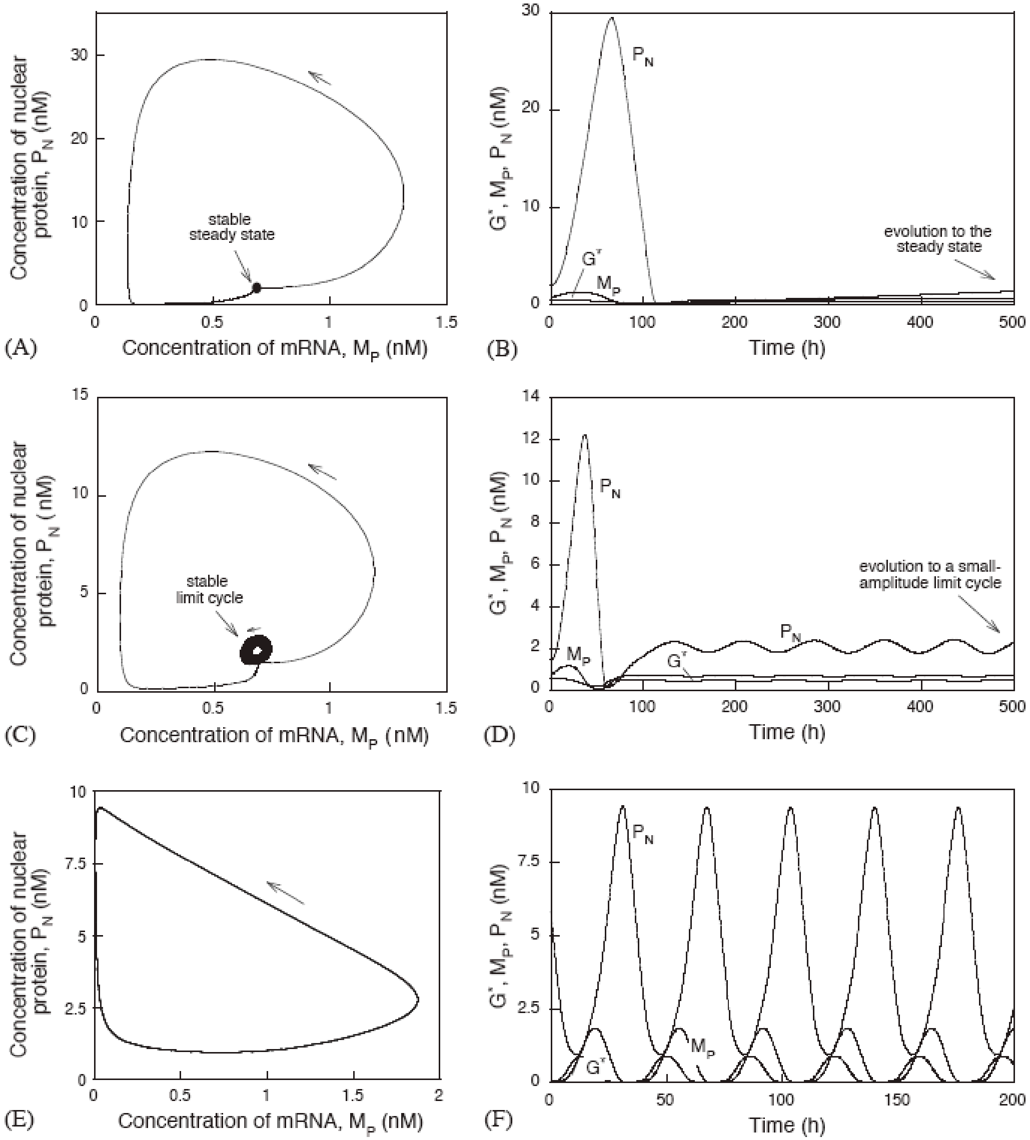
slow binding rate

Developed deterministic model: excitability

γ = 1000

γ = 100

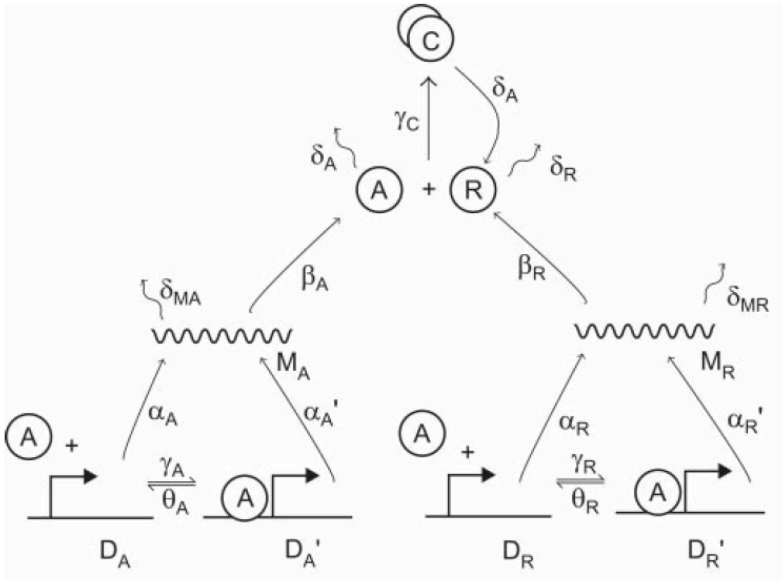
γ = 1

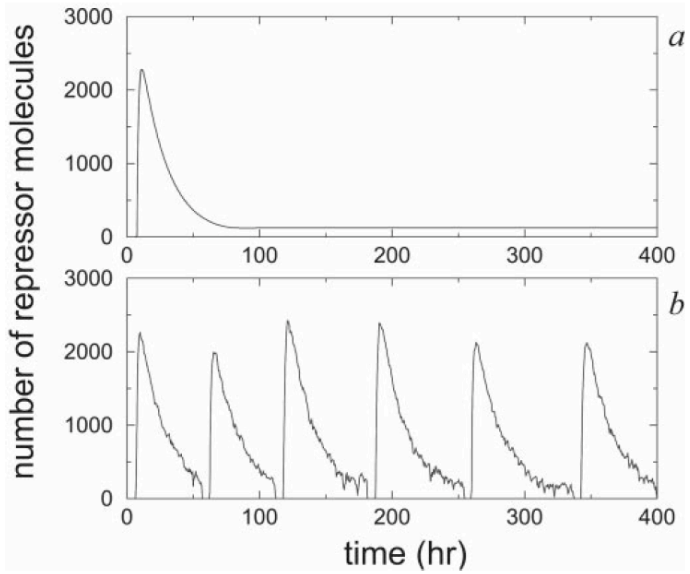


Mechanisms of noise-resistance

Mechanisms of noise-resistance in genetic oscillators

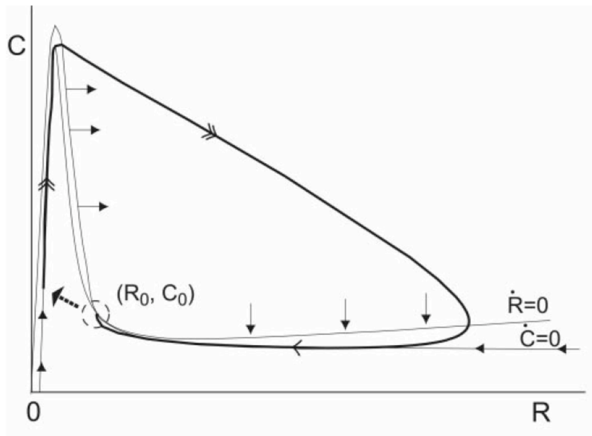
Vilar, Kueh, Barkai, Leibler, *PNAS* (2002) 99: 5988-5992





$$\frac{dR}{dt} = \frac{\beta_R}{\delta_{M_R}} \frac{\alpha_R\theta_R + \alpha'_R\gamma_R\tilde{A}(R)}{\theta_R + \gamma_R\tilde{A}(R)} - \gamma_C\tilde{A}(R)R + \delta_A C - \delta_R R$$

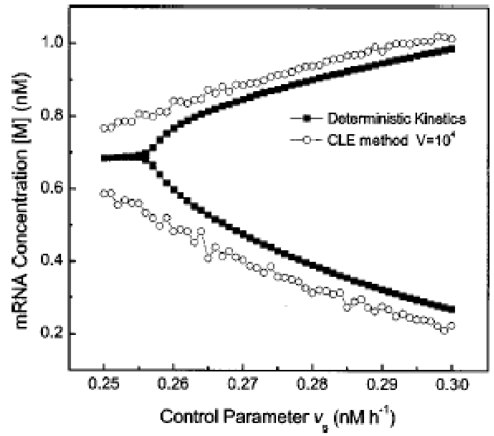
$$\frac{dC}{dt} = \gamma_C\tilde{A}(R)R - \delta_A C$$

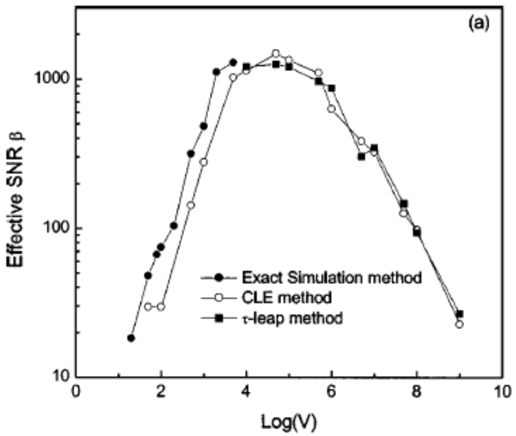


Stochastic resonance in circadian clock?

Internal noise stochastic resonance in a circadian clock system

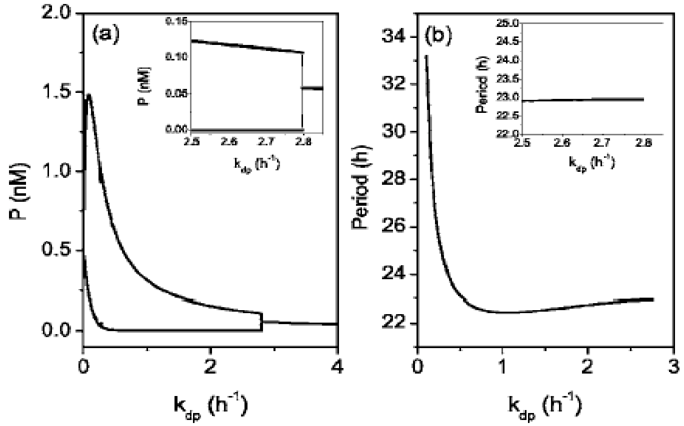
Hou & Xin, *J Chem Phys* (2003) 119: 11508

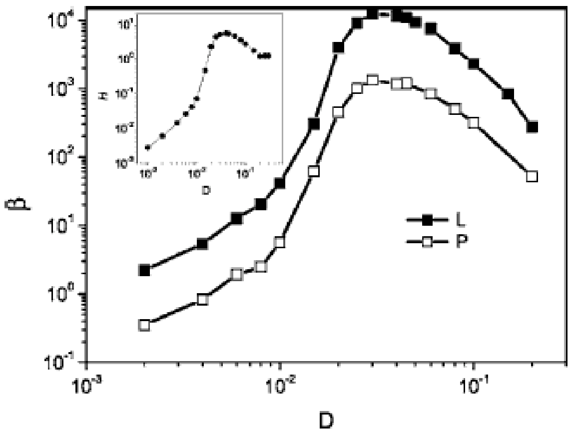




Light-noise induced supra-threshold circadian oscillations and coherent resonance in *Drosophila*

Yi & Jia, *Phys Rev E* (2005) 72: 012902





Conclusions

- **Robust circadian oscillations** are observed for a limited number of molecules, i.e. some tens mRNA molecules and hundreds proteins molecules.
- **Cooperativity** increases the robustness of the oscillations.
- The **periodic forcing** of the oscillations (LD cycle) increases the robustness by stabilizing the phase of the oscillations.
- The proximity of a **bifurcation point** decreases the robustness of the oscillations. In particular, near an excitable steady state, highly irregular oscillations are observed.
- **Coupling** between cells increases the robustness of the oscillations.

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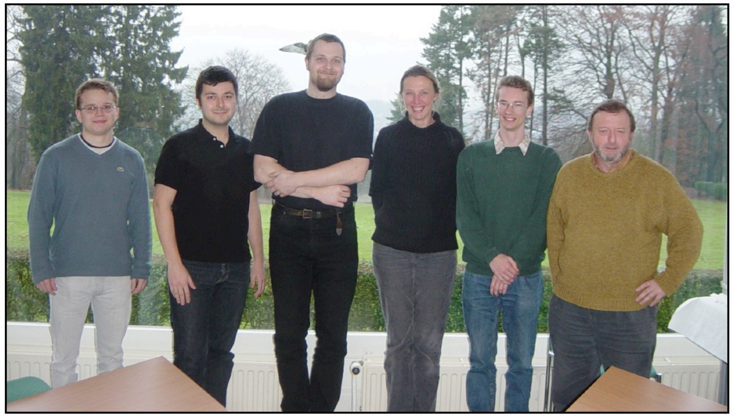

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