

Design principles of biological oscillators

Didier Gonze

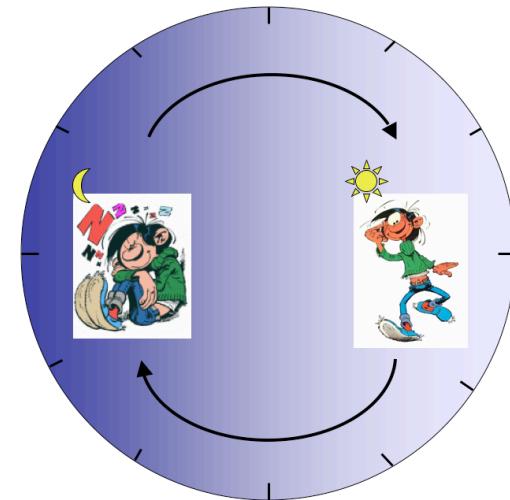
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Belgium*

Overview

■ Biological clocks

- Harmonic vs limit-cycle oscillations
- BZ reaction & the *Brusselator*
- The Goodwin model
- The van der Pol oscillator
- Sine-like vs relaxation oscillators
- A metabolic clock: glycolysis
- A genetic clock: the circadian clock
- A synthetic oscillator: the *Repressilator*

switch or clock?



■ Perturbing oscillators

- Perturbations and phase shifting
- Periodic forcing and entrainment
- Coupling and synchronization

} *will be discussed
in a subsequent
chapter*

Overview

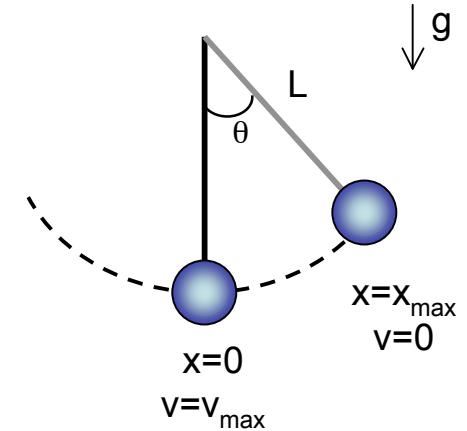
Questions:

- What is the difference between a pendulum and biological oscillators?
- How to build a limit-cycle oscillator?
- What is the role of positive circuits in oscillators?
- How to relate the structure of the oscillator with the shape of the oscillations and its dynamical properties?
- Why are natural biological oscillators complex?

We will answer (or at least provide some hints) to these questions by examining the design and dynamical properties of a few selected oscillators.

Biological clocks vs pendulum

An **harmonic oscillator** is a system that executes a periodic behavior. A swinging pendulum returns the same point in space (x) at regular intervals; furthermore its velocity (v) also rises and falls with regularity. The amplitude of the oscillations depends on the initial perturbation; i.e. the height from which it is released (θ).



Simple pendulum

$$\frac{d^2\theta}{dt^2} + \frac{g}{L}\theta = 0 \quad \longleftrightarrow \quad \begin{cases} \frac{d\theta}{dt} = \mu \\ \frac{d\mu}{dt} = -\frac{g}{L}\theta \end{cases} \quad \xrightarrow{\text{solution}} \quad \theta(t) = \theta_0 \cos\left(\sqrt{\frac{g}{L}}t\right)$$

- The amplitude of the oscillations depends on the initial condition $\theta_0=\theta(0)$.
- The frequency of the oscillations is given by $\omega = \sqrt{\frac{g}{L}}$

Biological clocks vs pendulum



Lynx vs hare

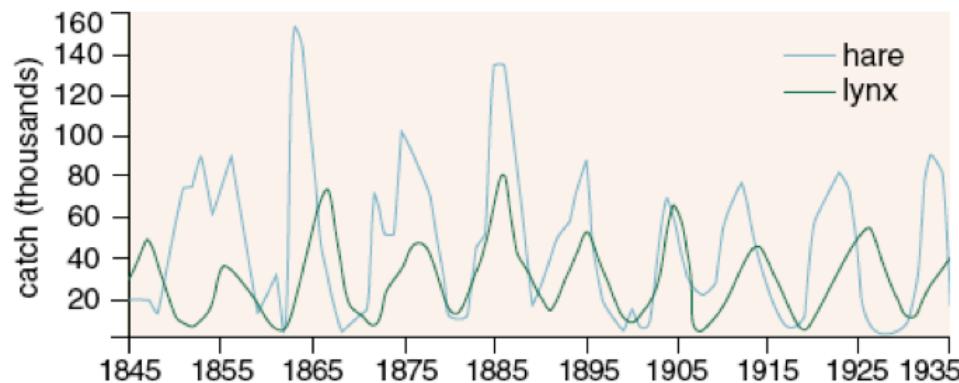
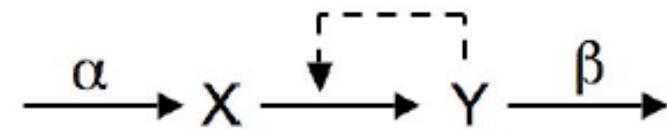


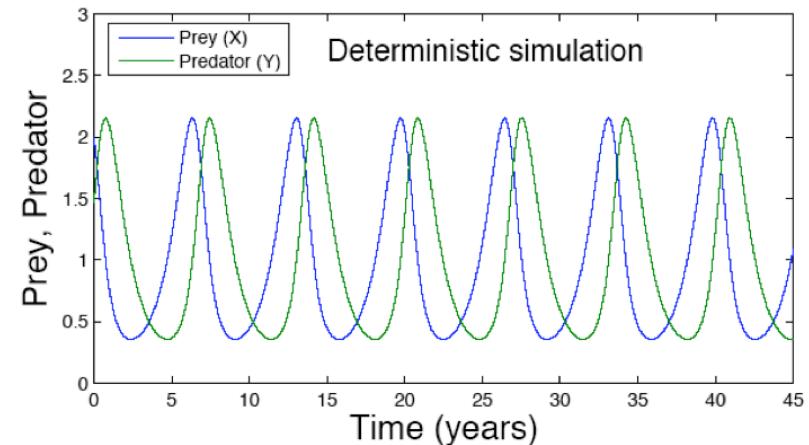
Figure 2. Early in the 20th century, Alfred Lotka and Vito Volterra developed equations now used by ecologists to understand predator-prey interactions. An example of predator-prey population dynamics is the record of annual Hudson Bay Company catches of Canadian lynx and snowshoe hares, which appears to show fluctuations on 9- to 11-year cycles. Through a combination of modeling and experiment, ecologists have found that these cycles can be predicted by a model including both predation (left) and changes in the hares' food supply. (Graph adapted from Eugene Odum's *Fundamentals of Ecology*, 1953.)

Strogatz, Sci. Am. dec 1993, pp.68-74

Lotka-Volterra model



$$\begin{cases} \frac{dX}{dt} = \alpha X - XY & \text{prey} \\ \frac{dY}{dt} = XY - \beta Y & \text{predator} \end{cases}$$



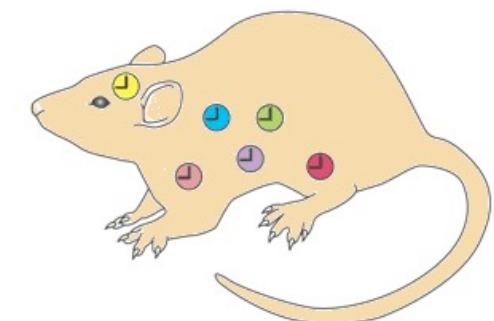
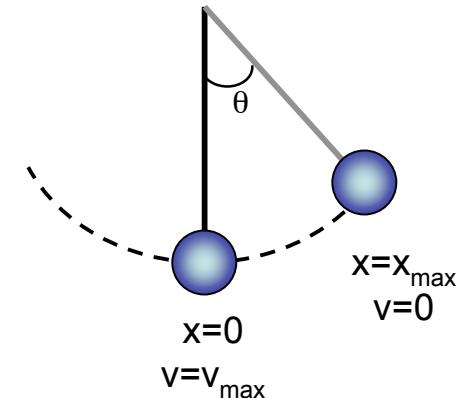
Biological clocks vs pendulum

An **harmonic oscillator** is a system that executes a periodic behavior. A swinging pendulum returns the same point in space (x) at regular intervals; furthermore its velocity (v) also rises and falls with regularity. The amplitude of the oscillations depends on the initial perturbation; i.e. the height from which it is released (θ).

The oscillations predicted by the Lotka-Volterra model are of such type: changing the initial number of preys/predators changes the amplitude of the oscillations.

Biological oscillators, in contrast, tend to have not only a characteristic period, but also a characteristic amplitude. In the phase space their trajectories correspond to a **limit cycle**. If a perturbation is exerted on such a system, they will automatically come back to their normal behavior, i.e. to their limit cycle.

They indeed incorporate a dissipative mechanism to damp oscillators that grow too large and a source of energy to pump up those that become too small.



Biological clocks

Table 1 | Survey of biochemical oscillators

Function	Components	Period	Class*	References
Metabolism	Glucose, ATP, phospho-fructokinase	2 min	3	52–54
Signalling	Cyclic AMP, receptor, adenylate cyclase	5 min	3	55,64
Signalling	Ca ²⁺ , Ins(1,4,5)P ₃	> 1 s	3	65
Signalling	NF-κB, IκB, IKK	~2 h	1	41,43
Signalling	p53, MDM2	5 h	1	39,40
			3	58,59
Signalling	Msn2, adenylate cyclase, cAMP, PKA	~10 min	1	66,67
Somitogenesis	Her1, Her7, Notch	30–90 min	1	40,68
Yeast endoreplication cycles	Cig2, Cdc10, Rum1	1–2 h	2	49
Frog egg cycles	CycB, Wee1, Cdc25, Cdc20	30 min	2	47,48
Circadian rhythm	PER, TIM, CLOCK, CYC	24 h	1	26
			2	30

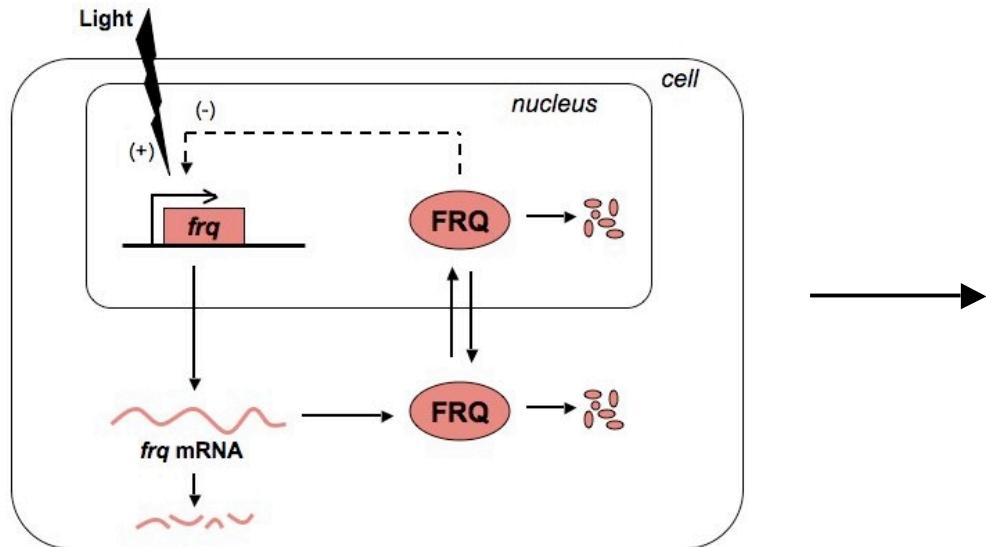
*See FIG. 5. Class 1 represents delayed negative-feedback loops; class 2 represents amplified negative-feedback loops; class 3 represents incoherently amplified negative-feedback loops. IκB, inhibitor of NF-κB; IKK, IκB kinase; Ins(1,4,5)P₃, inositol-1,4,5-triphosphate; NF-κB, nuclear factor κB.

Novak, Tyson (2008) Design principles of biochemical oscillators. *Nature review* 9:981-991.

Goldbeter (1996) *Biochemical Oscillations and Cellular Rhythms: The molecular bases of periodic and chaotic behaviour*, Cambridge University Press, Cambridge.

Goldbeter (2001) Computational approaches to cellular rhythms. *Nature* 420:238-45.

Circadian clocks



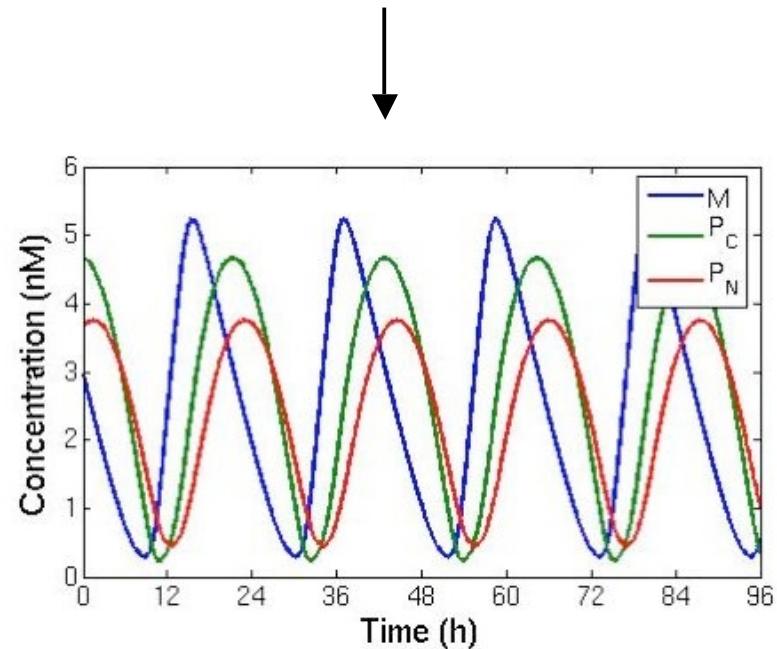
Circadian rhythms are biological rhythms characterized by a period of about 24h. Present in nearly all organisms, these endogenous rhythms allow the adaptation of the organisms to the alternance of day and night.

These oscillations are generated at the molecular level by a regulatory mechanism based on a negative feedback loop: a clock protein (*PER* in *Drosophila*, *FRQ* in *Neurospora*) inhibits the transcription of its own gene. This leads to oscillations in the level of mRNA and protein.

$$\frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + F_N^n} - v_m \frac{M}{K_m + M}$$

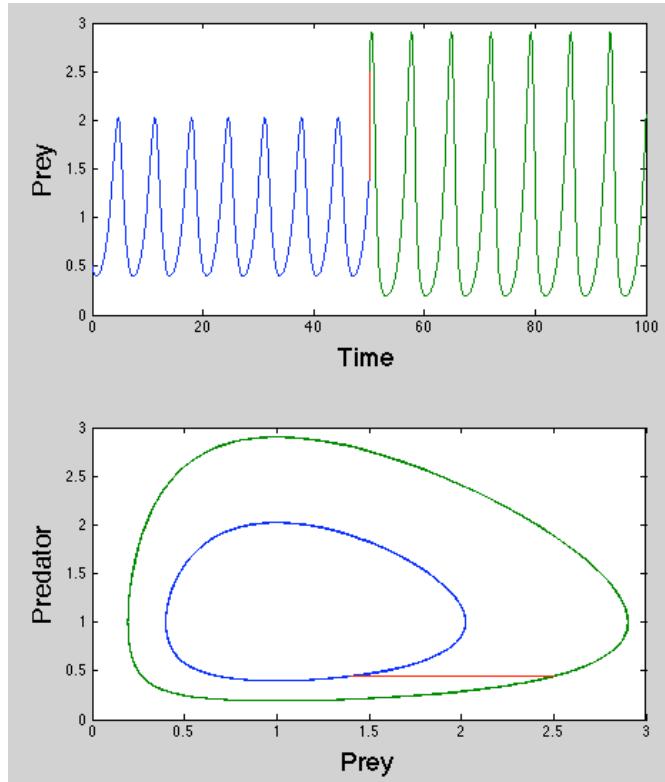
$$\frac{dF_C}{dt} = k_s M - k_1 F_C + k_2 F_N - v_d \frac{F_C}{K_d + F_C}$$

$$\frac{dF_N}{dt} = k_1 F_C - k_2 F_N - v_n \frac{F_N}{K_n + F_N}$$



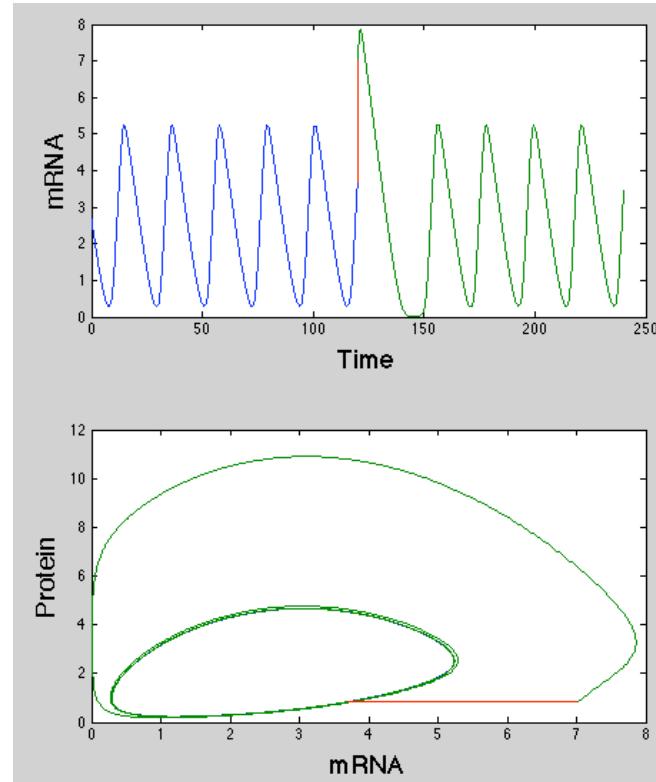
Harmonic vs limit-cycle oscillations

Lotka-Volterra system



The Lotka-Volterra model behaves like an harmonic oscillator: its period and amplitude depend on the initial conditions.

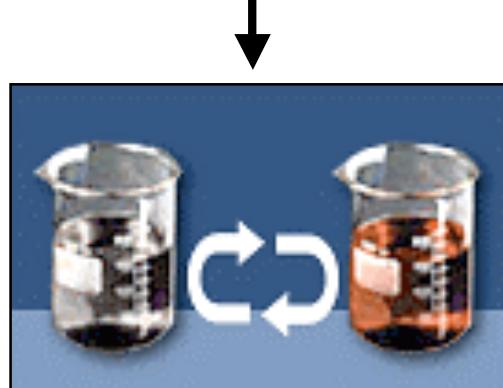
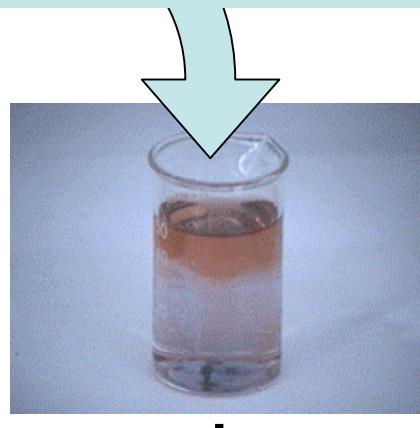
Circadian clock model



This model for circadian clock generates limit-cycle oscillations, characterized by a fixed period and amplitude. If a perturbation is applied, the system returns to its limit cycle.

Chemical oscillations: BZ reaction

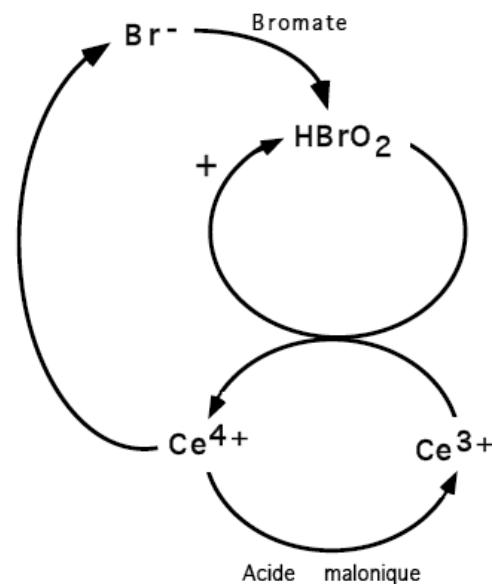
bromate de sodium + d'acide sulfurique + acide malonique + bromure de sodium + eau distillée



In a well stirring reactor (CSTR), oscillations occur spontaneously



When the same reaction is done in a Petri dish, spatial patterns (including concentric and spiral waves) occur spontaneously.



En présence du bromate, Br^- est transformé en HBrO_2 .

HBrO_2 active sa propre production pendant que Ce^{3+} est oxydé en Ce^{4+} .

L'acide malonique permet la réduction du Ce^{4+} en Ce^{3+} tout en régénérant du Br^- .

C'est cette "boucle de rétroaction" qui permet la génèse des oscillations entretenues.

Chemical oscillations: BZ reaction

Brusselator

The *Brusselator* is an hypothetical set of chemical reactions which can produce limit-cycle oscillations (Lefever & Prigogine, 1968)



Prigogine (1917-2003),
Nobel Chemistry (1977)

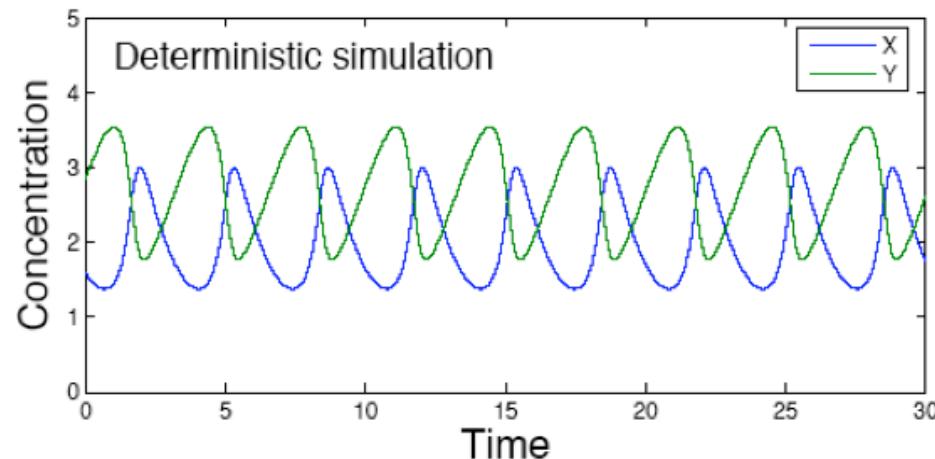
This model is sometimes referred to as the *trimolecular model* (indeed, the 3rd reaction is trimolecular - which is a quite unrealistic assumption). The name *Brusselator* was coined by Tyson in 1973.

r	reaction	rate
1	$A \xrightarrow{k_1} X$	$v_1 = k_1 A$
2	$B + X \xrightarrow{k_2} Y + C$	$v_2 = k_2 BX$
3	$2X + Y \xrightarrow{k_3} 3X$	$v_3 = k_3 X^2 Y$
4	$X \xrightarrow{k_4} D$	$v_4 = k_4 X$

reactional scheme

$$\begin{cases} \frac{dX}{dt} = k_1 a - k_2 b X + k_3 X^2 Y - k_4 X \\ \frac{dY}{dt} = k_2 b X - k_3 X^2 Y \end{cases}$$

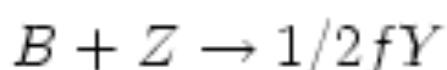
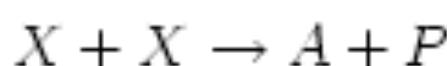
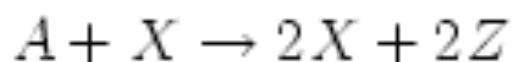
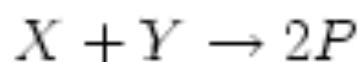
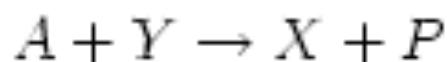
kinetic equations



limit-cycle oscillations

Chemical oscillations: BZ reaction

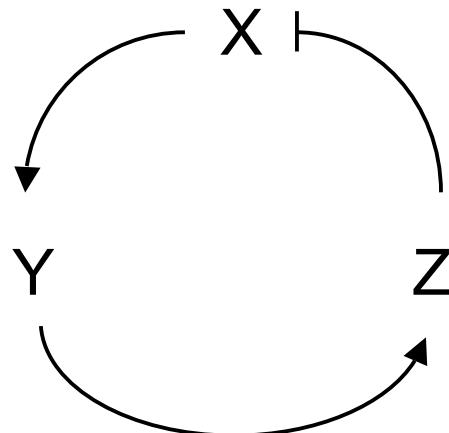
A 3-variable variant of the *Brusselator* was developed by Field and Noyes (1974) at the University of Oregon. This model, named the *Oregonator*, is the simplest realistic model of the chemical dynamics of the oscillatory BZ reaction. It is obtained by reduction of the complex chemical mechanism of the BZ reaction suggested by Field, Korös and Noyes (1974) and referred to as the FKN mechanism. The model contains both an autocatalytic step and a delayed negative feedback loop. It is composed of 5 coupled reactions:



Exercise

- Write the kinetic equations for X, Y and Z (A and B are supposed constant).
- Write the jacobian matrix and identify the negative feedback loop.
- Simulate the system for the following parameter values: A=0.06, B=0.02, $k_1=1.28$, $k_2=2.4 \cdot 10^6$, $k_3=33.6$, $k_4=2400$, $k_5=1$, f=1, and the following initial condition: $x_0=y_0=z_0=1$. Use a method of integration for "stiff" systems and a time span of at least 1000.

Biochemical oscillations: Goodwin model



The Goodwin model was originally proposed to model oscillatory processes in enzymatic control processes. Due to its generic nature it was subsequently used and reinterpreted in different contexts (e.g. gene regulatory processes).

This is a minimal model, based on a delayed negative feedback loop.

Goodwin model (1965)

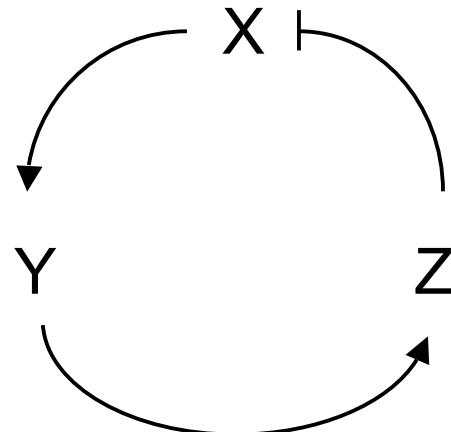
$$\begin{aligned}\frac{dX}{dt} &= k_1 \frac{1}{K_I + Z} - k_2 X \\ \frac{dY}{dt} &= k_3 X - k_4 Y \\ \frac{dZ}{dt} &= k_5 Y - k_6 Z\end{aligned}$$

No limit-cycle oscillations can occur in this model.

The oscillations reported in the original paper by Goodwin were due to numerical artefacts...

Goodwin (1965) Oscillatory behavior in enzymatic control processes, in *Advances in Enzyme Regulation*, vol. 3, p. 425, Oxford.

Biochemical oscillations: Goodwin model



Correction of the Goodwin model (Griffith, 1968)

$$\begin{aligned}\frac{dX}{dt} &= k_1 \frac{1}{K_I^n + Z^n} - k_2 X \\ \frac{dY}{dt} &= k_3 X - k_4 Y \\ \frac{dZ}{dt} &= k_5 Y - k_6 Z\end{aligned}$$

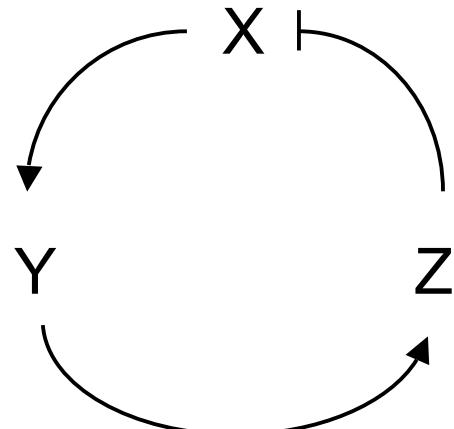
Limit-cycle oscillations occurs in this model when $n > 8$.

A strong non-linear repression function is thus needed (as demonstrated mathematically).

Griffith (1968) Mathematics of Cellular Control Processes. I. Negative feedback to one gene, *J Theor Biol* 20: 202-208.

Biochemical oscillations: Goodwin model

A variant of the Goodwin model



$$\frac{dX}{dt} = v_1 \frac{K_1^n}{K_1^n + Z^n} - v_2 \frac{X}{K_2 + X},$$

$$\frac{dY}{dt} = k_3 X - v_4 \frac{Y}{K_4 + Y},$$

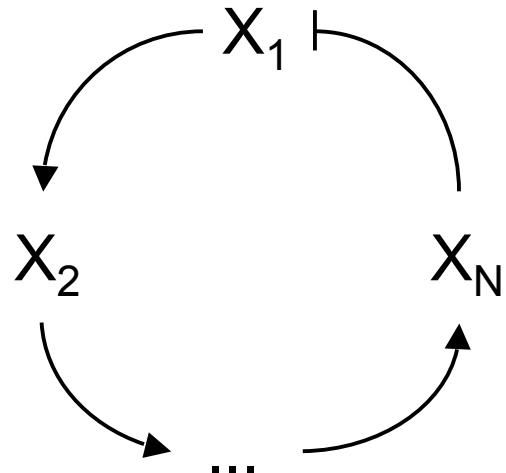
$$\frac{dZ}{dt} = k_5 Y - v_6 \frac{Z}{K_6 + Z}.$$

Limit-cycle oscillations can occur in this model with low values of n (even for $n=1$); the non-linearity is distributed in different degradation terms.

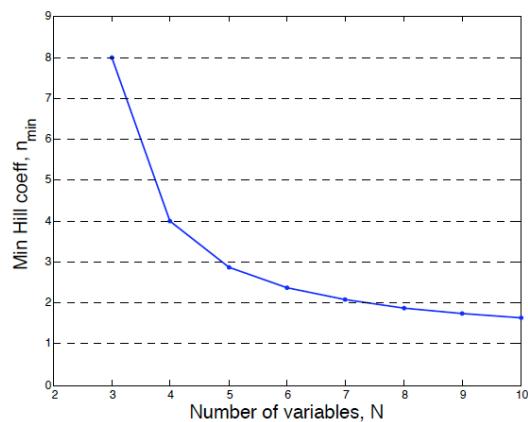
Gonze, Bernard, Waltermann, Kramer, Herzel (2005) Spontaneous synchronization of coupled circadian oscillators. *Biophys. J.* 89: 120-129.

Biochemical oscillations: Goodwin model

Generalisation of the Goodwin model (Tyson & Othmer, 1978)



$$\begin{aligned}\frac{dX_1}{dt} &= k_1 \frac{1}{K_I^n + X_N^n} - k_2 X_1 \\ \frac{dX_2}{dt} &= a_1 X_1 - b_2 X_2 \\ &\dots \\ \frac{dX_N}{dt} &= a_{N-1} X_{N-1} - b_N X_N\end{aligned}$$



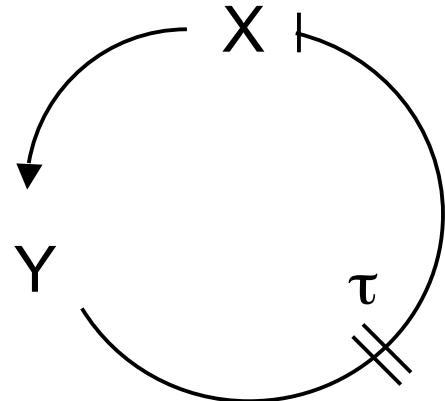
Limit-cycle oscillations occurs in this model when $n > \sec^N(\pi/N)$ where N is the number of elements involved in the feedback loop.

In other words, we need either a strong non-linear feedback or a large delay in the feedback loop for the oscillations to occur.

Tyson & Othmer (1978) The dynamics of feedback control circuits in Biochemical Pathways, *Prog Theor Biol* 5: 1-62.

Biochemical oscillations: Goodwin model

Delay version of the Goodwin model



$$\begin{aligned}\frac{dX}{dt} &= k_1 \frac{1}{K_I^n + Y_{(t-\tau)}^n} - k_2 X \\ \frac{dY}{dt} &= k_3 X - k_4 Y\end{aligned}$$

A succession of linear steps can be replaced by an explicit delay. The system obtained is thus referred to as a delay differential model (DDE).

Exercise

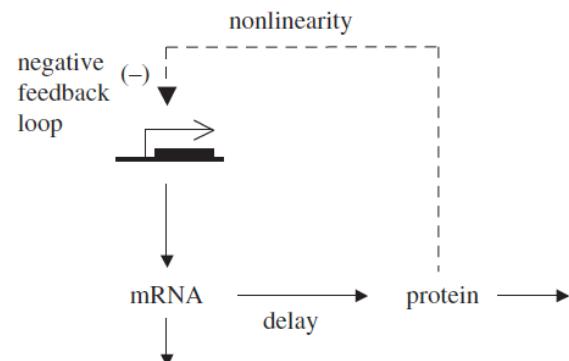
Simulate the system for various parameter values; find parameter values to obtain oscillations and find the condition of the delay τ and coefficient n to obtain oscillations. NB: this exercise requires a specific solver for DDE (ex: dde23 function in Matlab).

Biochemical oscillations: Goodwin model

The Goodwin model: summary

The Goodwin model produces self-sustained (limit cycle) oscillations provided that the **non-linearity** is sufficient and that the **delay** (explicit or implicit) is large enough.

Use of the Goodwin model today



Ruoff *et al* - model for the *Neurospora* circadian clock

Ruoff P, Vinsjevik M, Monnerjahn C, Rensing L (1999) The Goodwin oscillator: on the importance of degradation reactions in the circadian clock. *J Biol Rhythms* 14:469-79.

Ruoff P, Vinsjevik M, Mohsenzadeh S, Rensing L (1999) The Goodwin model: simulating the effect of cycloheximide and heat shock on the sporulation rhythm of *Neurospora crassa*. *J Theor Biol* 196:483-94.

Ruoff P, Vinsjevik M, Monnerjahn C, Rensing L (2001) The Goodwin model: simulating the effect of light pulses on the circadian sporulation rhythm of *Neurospora crassa*. *J Theor Biol* 209:29-42.

Ruoff P, Loros JJ, Dunlap JC (2005) The relationship between FRQ-protein stability and temperature compensation in the *Neurospora* circadian clock. *Proc Natl Acad Sci USA* 102:17681-6

Gonze *et al* - model for the synchronization of circadian oscillators in the SCN

Gonze D, Bernard S, Waltermann C, Kramer A, Herzog H (2005) Spontaneous synchronization of coupled circadian oscillators. *Biophys J.* 89:120-9

Zeiser *et al* - model for Hes1 oscillations

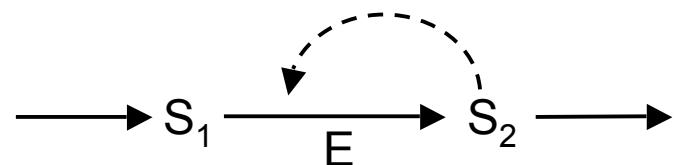
Zeiser S, Müller J, Liebscher V (2007) Modeling the Hes1 oscillator. *J Comput Biol* 14:984-1000.

Biochemical oscillations: Selkov model

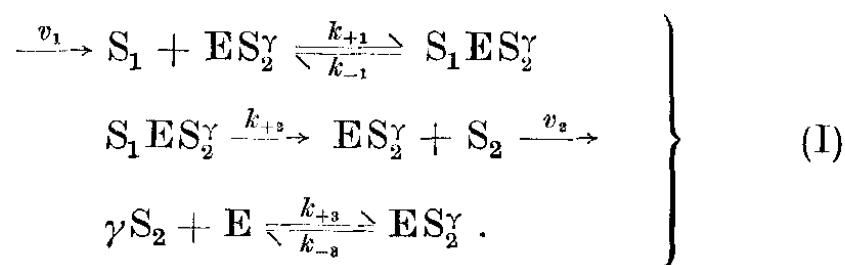
Self-Oscillations in Glycolysis

1. A Simple Kinetic Model

E. E. SEL'KOV



Consider a simple kinetic model of enzyme catalysis with product activation of the enzyme:



According to the law of mass action and the law of mass conservation reaction (I) is described by the equation system:

$$\left. \begin{array}{l} \frac{ds_1}{dt} = v_1 - k_{+1} s_1 x_1 + k_{-1} x_2 \\ \frac{ds_2}{dt} = k_{+2} x_2 - k_{+3} s_2^\gamma e + k_{-3} x_1 - k_2 s_2 \\ \frac{dx_1}{dt} = -k_{+1} s_1 x_1 + (k_{-1} + k_{+2}) x_2 + \\ \qquad \qquad \qquad k_{+3} s_2^\gamma e - k_{-3} x_1 \\ \frac{dx_2}{dt} = k_{+1} s_1 x_1 - (k_{-1} + k_{+2}) x_2 \\ \frac{de}{dt} = -k_{+3} s_2^\gamma e + k_{-3} x_1 \end{array} \right\} \text{(4)}$$

Sel'kov (1968) Self-oscillations in Glycolysis, *Eur J Biochem* 4: 79-86.

Biochemical oscillations: Selkov model

Self-Oscillations in Glycolysis

1. A Simple Kinetic Model

E. E. SEL'KOV

Under some assumptions the model can be reduced as:

$$\frac{dx}{d\tau} = 1 - xy^\gamma$$

$$\frac{dy}{d\tau} = \alpha y(x y^{\gamma-1} - 1)$$

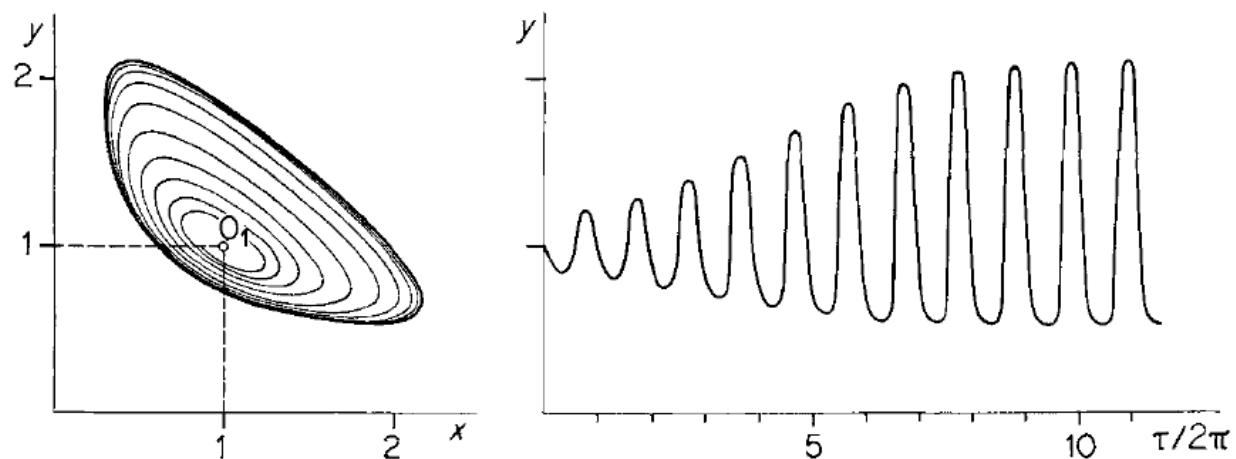


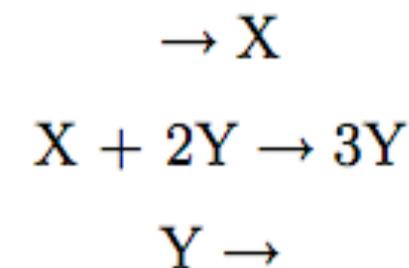
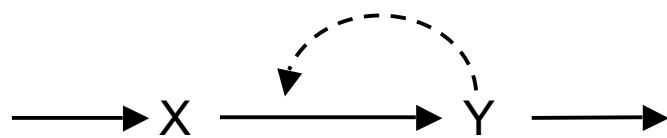
Fig. 3. On the left: the transition of system (II) to the limit cycle from unstable focus O_1 ; on the right: time display of the process. The curves have been obtained by the digital computer solution of system (II) at a fixed integration step $h = 0.1$; $\gamma = 2$, $\alpha = 1.1$. x and y as in Fig. 2. τ is dimensionless time

Sel'kov (1968) Self-oscillations in Glycolysis, *Eur J Biochem* 4: 79-86.

Exercise : Write the Jacobian matrix and show that there is a negative circuit involving x and y . This circuit is not obvious on the scheme. How would you interpret it?

Biochemical oscillations: Selkov model

A variant of the Selkov model: the Schnakenberg model



$$\begin{aligned}\frac{dx}{dt} &= v - k_1 xy^2 \\ \frac{dy}{dt} &= k_1 xy^2 - k_2 y\end{aligned}$$

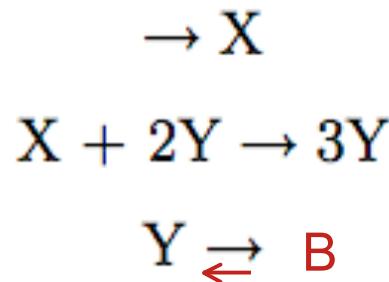
It can be shown that under some conditions, the steady state can be unstable. However, using the Poincaré-Bendixson theorem, it is possible to show that the system does not display limit-cycle oscillations.

Schnakenberg (1979) Simple chemical reaction systems with limit cycle behaviour,
J. Theor Biol 81:389-400

Biochemical oscillations: Selkov model

A variant of the Selkov model: the Schnakenberg model

In order to allow limit-cycle oscillations, Schnakenberg introduces the following modification:



Exercise

- *How should the kinetic equations be changed?*
- *Calculate the steady state and determine its stability.*
- *Draw the nullclines in the phase space and use the Poincaré-Bendixson theorem to show that the system can display limit cycle oscillations.*
- *Simulate the system and show the oscillations.*

Biochemical oscillations: allosteric model

Glycolytic oscillations originate from positive feedback regulation of the allosteric enzyme phosphofructokinase (PFK)

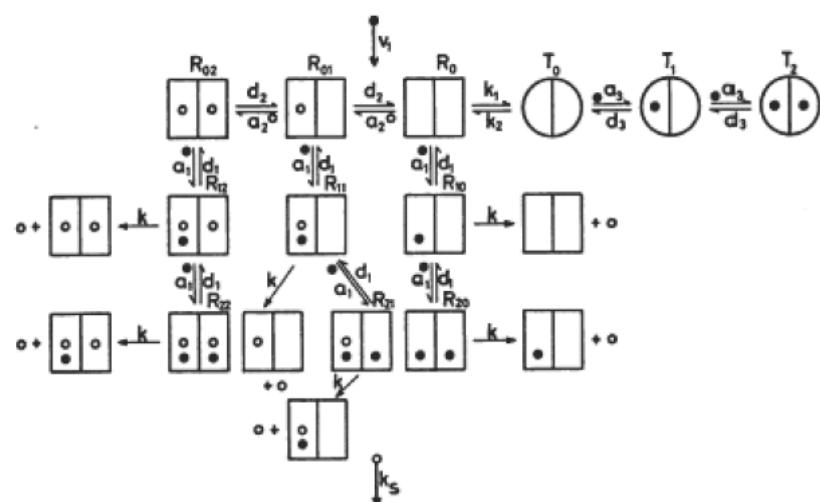
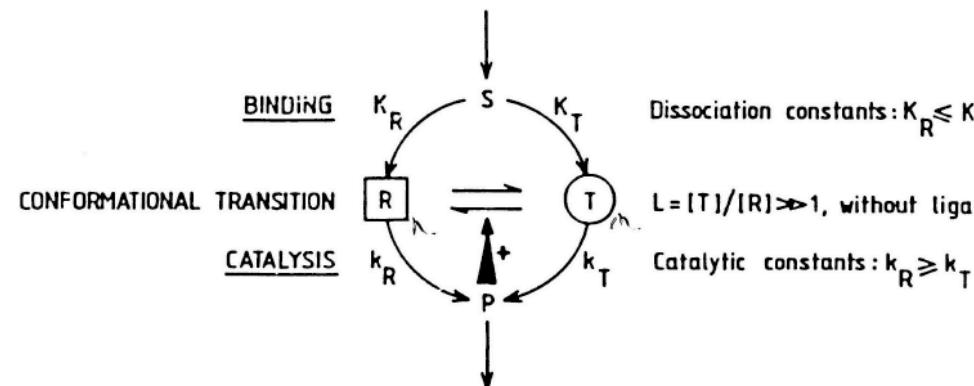
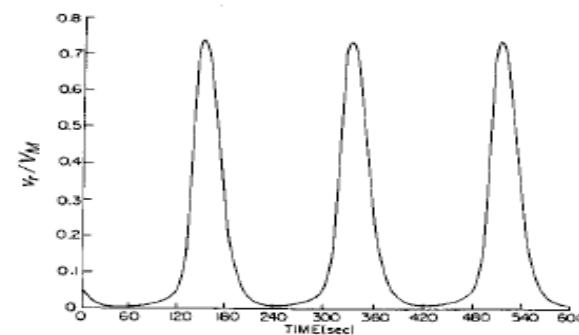
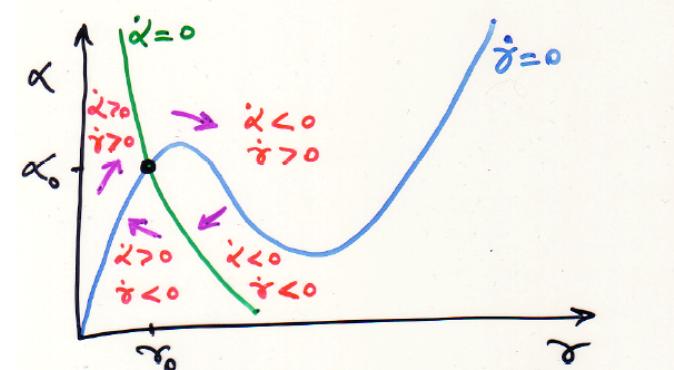


FIGURE 1 Model I (see text). ●, substrate; ○, product.

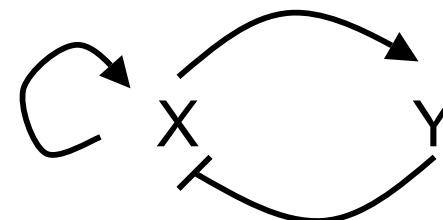
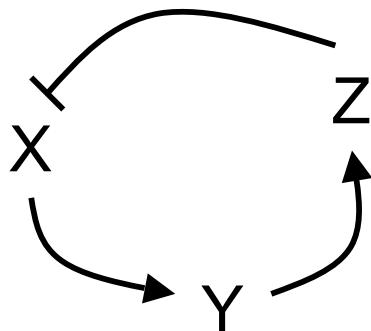
$$\left\{ \begin{array}{l} \frac{d\alpha}{dt} = \dot{\alpha} = v - \tau \phi \\ \frac{d\gamma}{dt} = \dot{\gamma} = q \tau \phi - k \gamma \end{array} \right.$$

$$\text{with } \phi = \frac{\alpha(1+\alpha)(1+\gamma)^2}{L + (1+\alpha)^2(1+\gamma)^2}$$



How to build an limit-cycle oscillator?

How to build a limit-cycle oscillator?

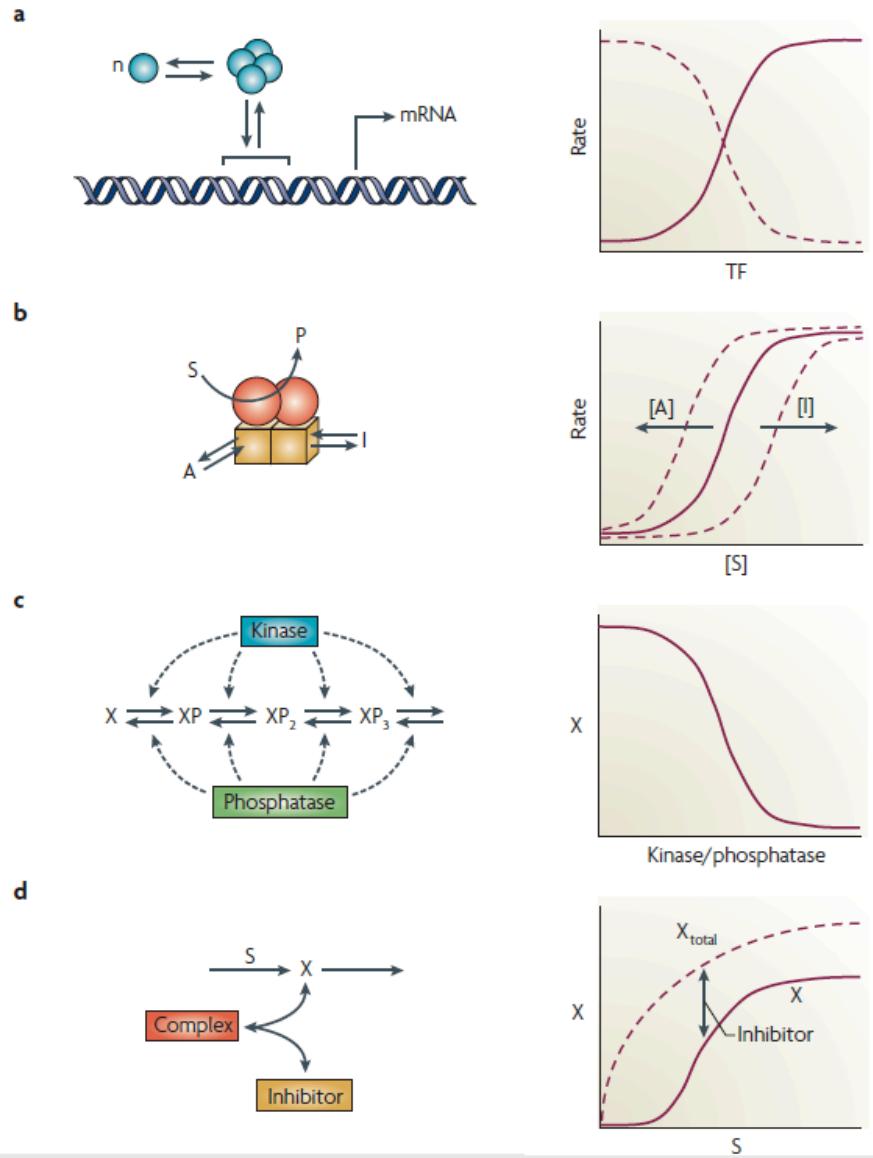


Necessary conditions:

- Negative feedback loop (or, at least, a negative circuit)
- Delay in the feedback, either explicit (DDE) or implicit (intermediary steps), or auto-catalysis (positive circuit).
- One or several sources of non-linearities

Novak, Tyson (2008) Design principles of biochemical oscillators. *Nature review* 9:981-991.

How to build an limit-cycle oscillator?



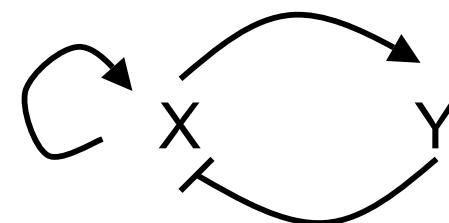
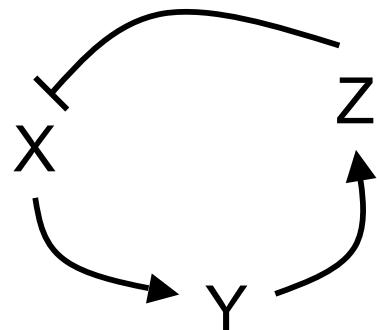
Source of non-linearity in biological systems

Figure 4 | Sources of nonlinearity. **a** | Oligomer binding. Left: a transcription factor (blue ball) forms an n -component homo-oligomer, which then binds upstream of a structural gene and either activates or represses mRNA synthesis. Right: the rate of mRNA synthesis as a function of transcription factor (TF) concentration, for an activator (solid line) or a repressor (dashed line). **b** | Cooperativity and allostery. Left: an enzyme, consisting of two catalytic subunits (spheres) and two regulatory subunits (cubes), catalyses the conversion of substrate (S) into product (P). Activators (A) and inhibitors (I) bind to specific sites on the regulatory subunits. Right: if the binding of substrate to the catalytic subunits is cooperative, then the rate of reaction as a function of substrate concentration is sigmoidal (solid line). The rate curve can be shifted to the left or to the right by increasing the concentration of the activator or inhibitor, respectively. **c** | Multisite phosphorylation. Left: a regulatory protein, X , is phosphorylated on multiple sites by a protein kinase and is dephosphorylated by a protein phosphatase. Right: the concentration of the unphosphorylated form of X as a function of the ratio of activities of kinase and phosphatase. **d** | Stoichiometric inhibition. Left: a regulatory protein, X , is synthesized in response to a signal, S . X binds strongly to an inhibitor to form an inactive complex. Right: the concentration of total X increases hyperbolically with S (dashed line), but the concentration of 'free' X is a sigmoidal function of S (solid line).

Novak, Tyson (2008) Design principles of biochemical oscillators. *Nature review* 9:981-991.

How to build an limit-cycle oscillator?

Do the two types of oscillators generate the same kind of oscillations?

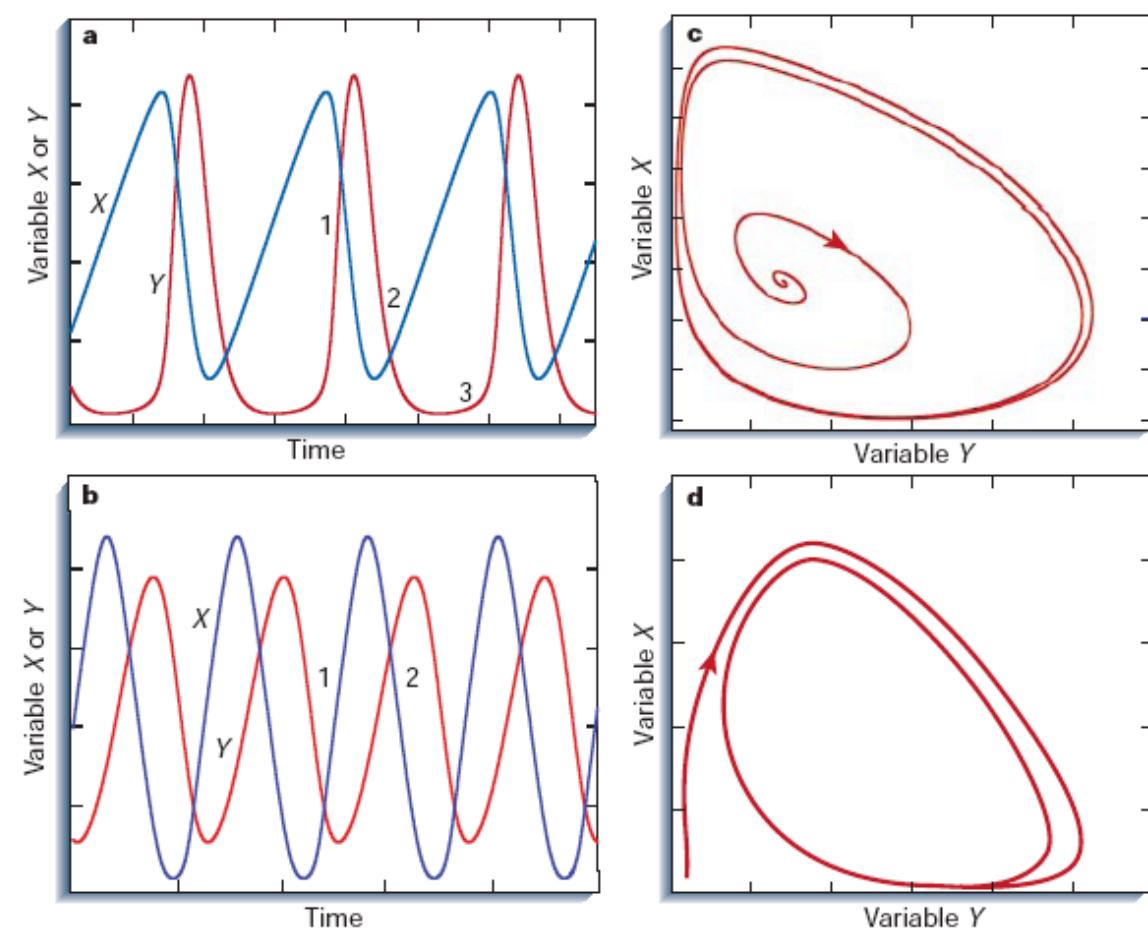


Smooth vs relaxation oscillations

Depending on the "architecture" of the model (wiring + kinetics + parameter values), different types of oscillations can be obtained (smooth vs relaxation)

Figure 1 Sustained oscillations can occur in models based on positive or negative feedback. **a**, Typical oscillations obtained in models based on positive feedback. The particular oscillations shown are obtained in a two-variable model for the product-activated phosphofructokinase reaction responsible for glycolytic oscillations^{3,81}.

Y represents the reaction product, and X represents the substrate of the enzyme. Similar oscillations are obtained in models for Ca^{2+} oscillations based on Ca^{2+} -induced Ca^{2+} release^{3,17} or cAMP oscillations in *Dictyostelium amoebae*^{3,28}. In the case of Ca^{2+} oscillations, Y denotes cytosolic Ca^{2+} , whereas X represents the Ca^{2+} content of intracellular stores. For cAMP oscillations in *Dictyostelium*, which rely on a mixture of positive and negative feedback (see text), X represents the fraction of active (non-desensitized) cAMP receptor, and Y represents the level of extracellular cAMP. **b**, Oscillations obtained in a five-variable model based on negative feedback for the circadian rhythmic variation of the PER protein (Y) and its mRNA (X) in *Drosophila*^{3,50}. **c**, Limit cycle in the phase plane (X, Y), corresponding to the oscillations shown in **a**. Initial conditions are such that the limit cycle here is reached from a point located in the vicinity of the unstable steady state. **d**, Limit cycle corresponding to the oscillations shown in **b**.



Smooth vs relaxation oscillations

van der Pol oscillator

The van der Pol oscillator was originally proposed by the physicist Balthasar van der Pol. Van der Pol found stable oscillations, now known as limit cycle, in electrical circuits.

$$\ddot{x} - \epsilon(1 - x^2)\dot{x} + x = 0$$



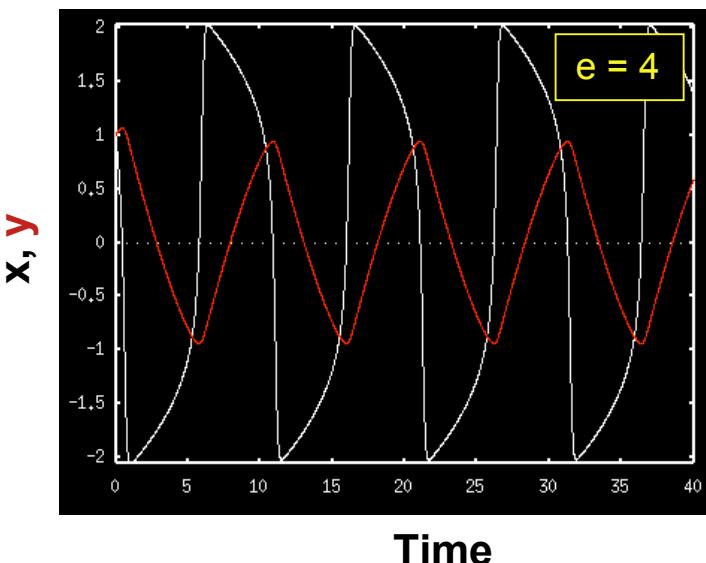
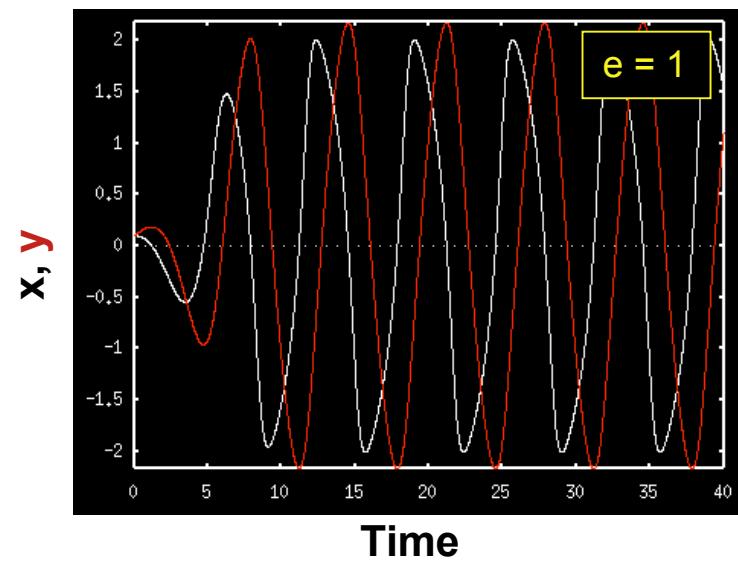
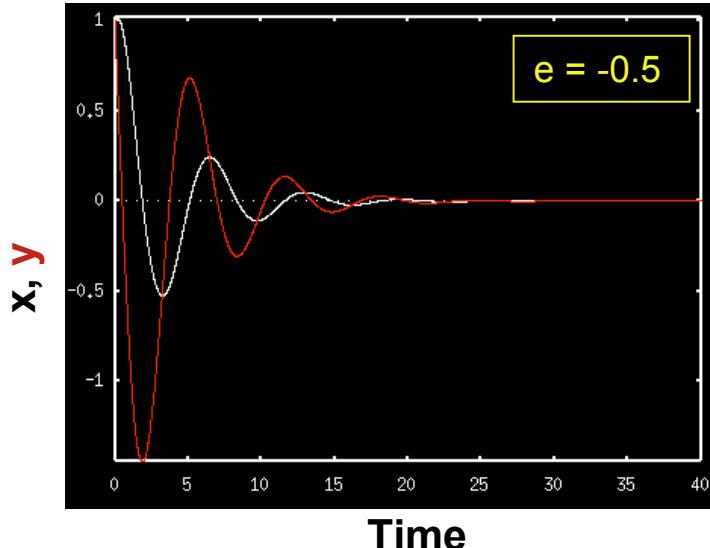
Depending on the value of ϵ , different behaviors are observed:

- When $\epsilon < 0$, the system will be damped.
- When $\epsilon = 0$, i.e. there is no damping; the system behaves as a simple harmonic oscillator. The amplitude and period of the oscillations depends on the initial condition; the energy is conserved.
- When $\epsilon > 0$, the system will enter a limit cycle; energy can be gained/lost.
- When $\epsilon \gg 0$, the system converges very quickly to the limit cycle (it loses energy extremely quickly).

van der Pol & van der Mark (1927) Frequency demultiplication, *Nature* 120:363-364

Smooth vs relaxation oscillations

```
# Van der Pol model  
  
dx/dt=e*(x-x^3/3-y)  
dy/dt=x/e  
  
param e=1  
  
ini x=0.1, y=0.1  
  
@ total=40, dt=0.01  
@ nplot=2, ypl1=x, ypl2=y  
  
done
```



Smooth vs relaxation oscillations

van der Pol oscillator

The second order van der Pol equation:

$$\ddot{x} - \epsilon(1 - x^2)\dot{x} + x = 0$$

can be rewritten as a system of 2 first-order equations:

$$\begin{aligned}\dot{x} &= \epsilon \left(x - \frac{1}{3}x^3 - y \right) \\ \dot{y} &= \frac{x}{\epsilon}\end{aligned}$$

Periodic Forcing

Van der Pol had examined the response of the van der Pol oscillator to a periodic forcing, which can be formulated as

$$\ddot{x} - \epsilon(1 - x^2)\dot{x} + x = F \cos\left(\frac{2\pi t}{T_{in}}\right)$$

van der Pol & van der Mark (1927) Frequency demultiplication, *Nature* 120:363-364

van der Pol (1920) A theory of the amplitude of free and forced triode vibrations, *Radio Review* 1:701-710; 1:754-762.

Exercise

- Show that the 2nd-order equation is equivalent to the system of two 1st-order equations.
- Write the Jacobian matrix and identify the negative circuit.
- Simulate the van der Pol model for various values of ϵ and compare the shapes of the limit cycles

Smooth vs relaxation oscillations

Use of the van der Pol oscillator today

Forger, Kronauer *et al* - mammalian circadian pacemaker

Forger DB, Jewett ME, Kronauer RE (1999) A simpler model of the human circadian pacemaker. *J Biol Rhythms*. 14:532-7.

Jewett ME, Forger DB, Kronauer RE (1999) Revised limit cycle oscillator model of human circadian pacemaker. *J Biol Rhythms*. 14:493-9.

Gonze *et al* - cyanobacteria circadian pacemaker

Gonze D, Roussel MR, Goldbeter A (2002) A model for the enhancement of fitness in cyanobacteria based on resonance of a circadian oscillator with the external light-dark cycle. *J Theor Biol*. 214:577-97.

Neuronal models

Example: Kawahara T (1980) Coupled Van der Pol oscillators---a model of excitatory and inhibitory neural interactions. *Biol Cybern*. 39:37-4.

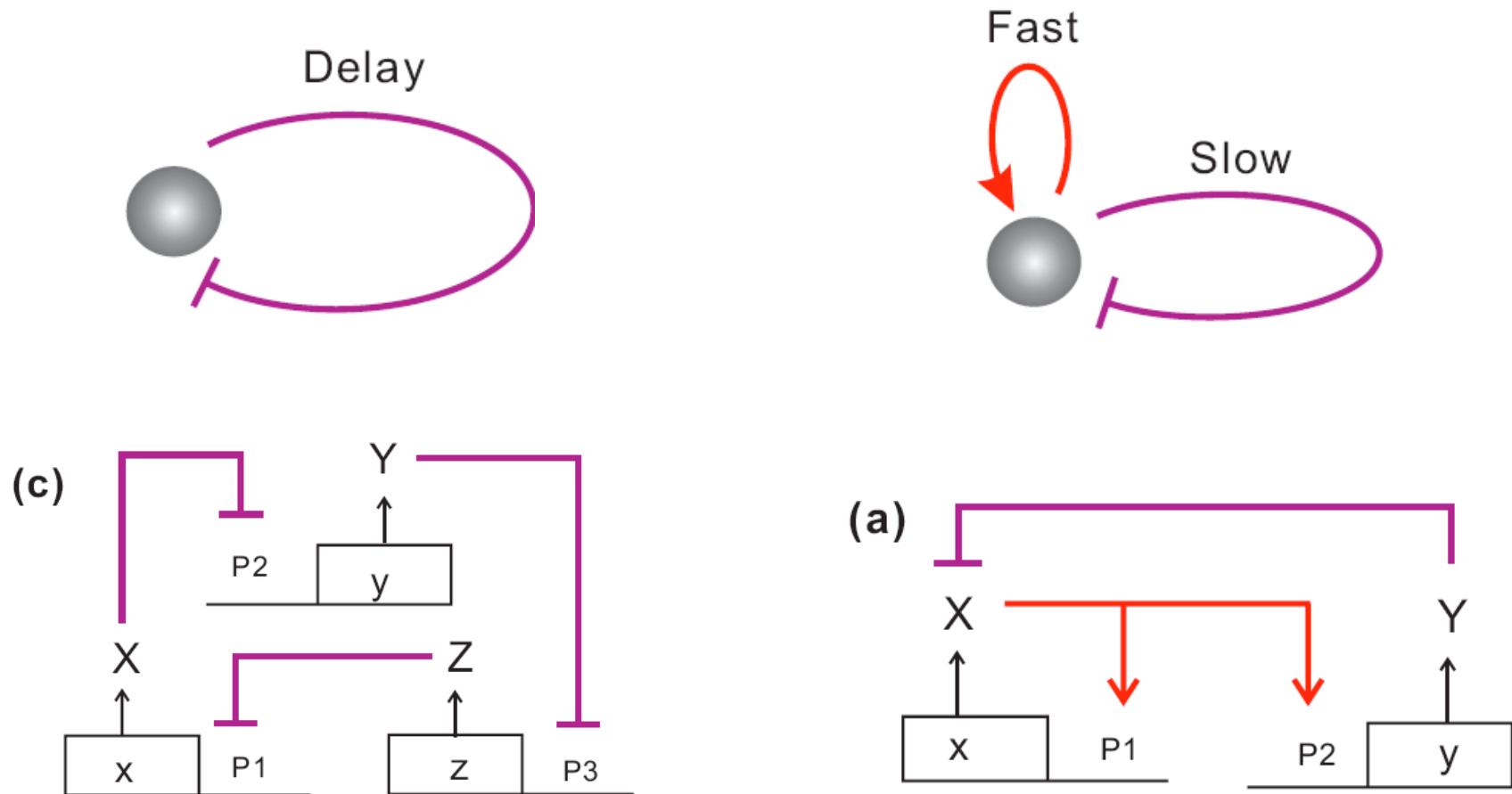
Theoretical studies

Many current researches still deal with coupled van der Pol oscillators and entrainment of van der Pol oscillators by a periodic forcing.

Example: Nana, Woaf (2006) Synchronization in a ring of four mutually coupled van der Pol oscillators: theory and experiment. *Phys Rev E* 74:046213.

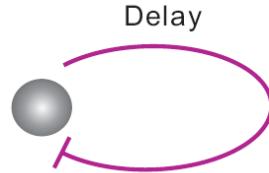
Smooth vs relaxation oscillations

Smooth (sine-like) vs relaxation oscillations



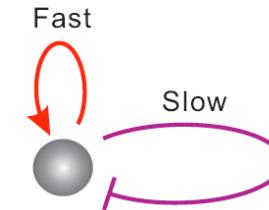
Smooth vs relaxation oscillations

Smooth (sine-like) vs relaxation oscillations



$$\begin{aligned}\frac{dx}{dt} &= \frac{\alpha_1}{1+z^n} - \beta_1 x + \gamma_1 \\ \frac{dy}{dt} &= \frac{\alpha_2}{1+x^n} - \beta_2 y + \gamma_2 \\ \frac{dz}{dt} &= \frac{\alpha_3}{1+y^n} - \beta_3 z + \gamma_3\end{aligned}$$

$$\alpha_1=\alpha_2=\alpha_3=216, \beta_1=\beta_2=\beta_3=1, n=3, \gamma=0.5 \text{ or } 1.5$$



$$\begin{aligned}\frac{dx}{dt} &= \alpha_1 \frac{1+\rho x^n}{1+x^n} - \beta_1 x - \gamma xy \\ \frac{dy}{dt} &= \alpha_2 \frac{1+\rho x^n}{1+x^n} - \beta_2 y\end{aligned}$$

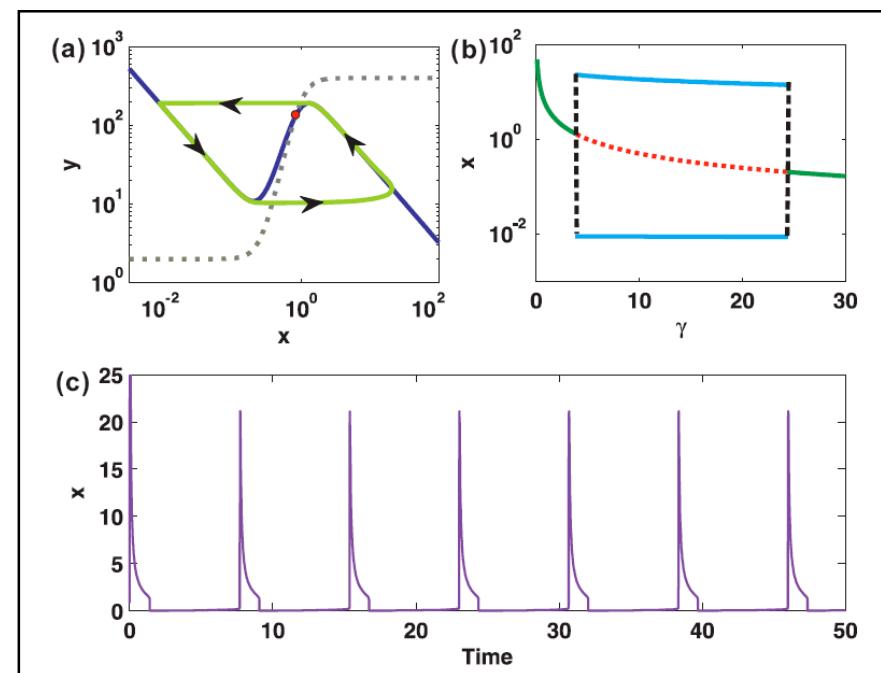
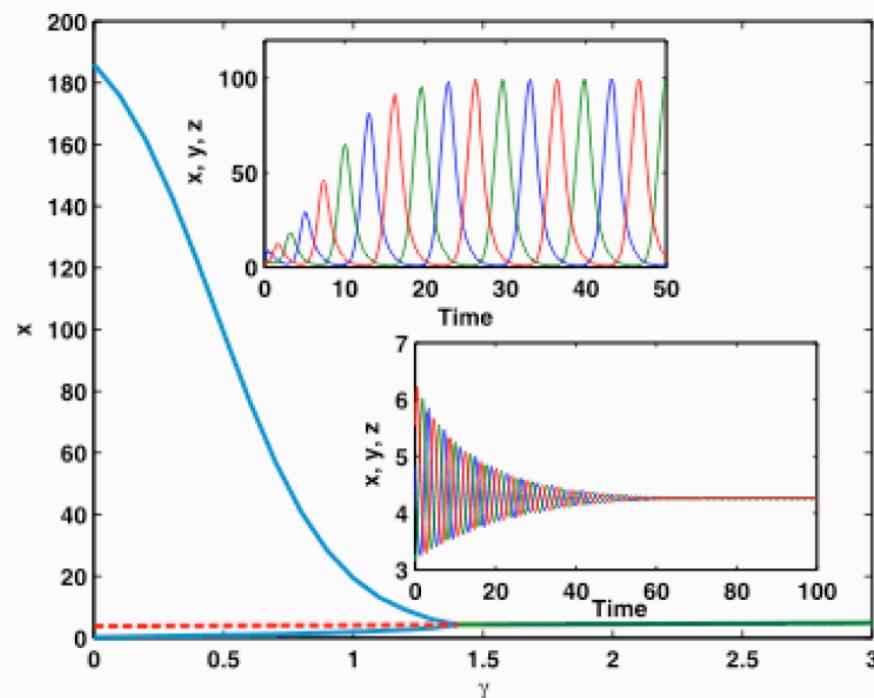
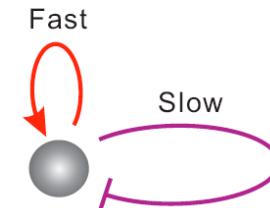
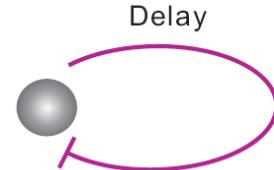
$$\alpha_1=10, \alpha_2=1, \beta_1=\beta_2=0.5, \rho=200, n=4, \gamma=6$$

Exercise

Simulate the two systems and show that the first system gives sine-like oscillations while the second model displays relaxation oscillations. Determine the influence of some parameters on the shape, the period and the amplitude of the oscillations.

Smooth vs relaxation oscillations

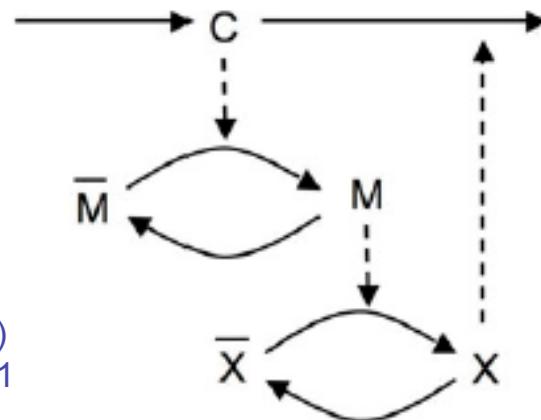
Smooth (sine-like) vs relaxation oscillations



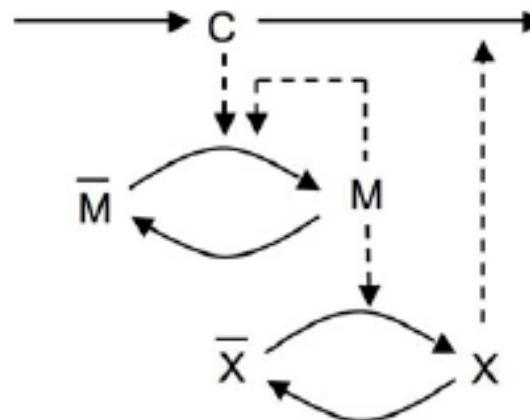
Smooth vs relaxation oscillations

Minimal mitotic oscillator

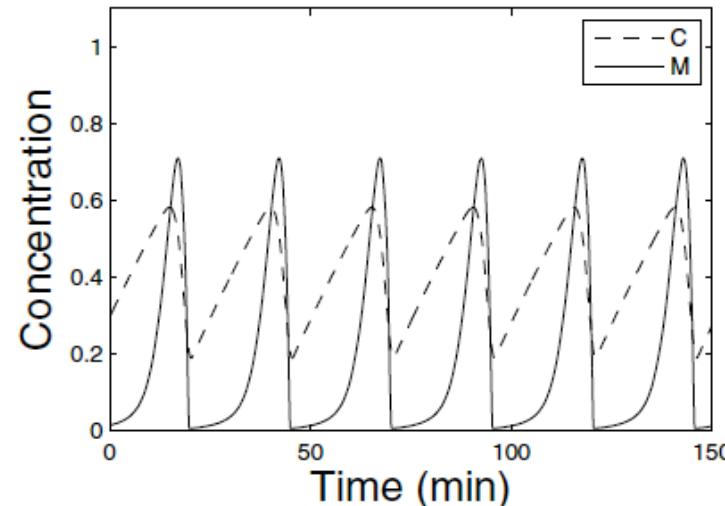
Goldbeter (1991)
PNAS 88: 9107-1



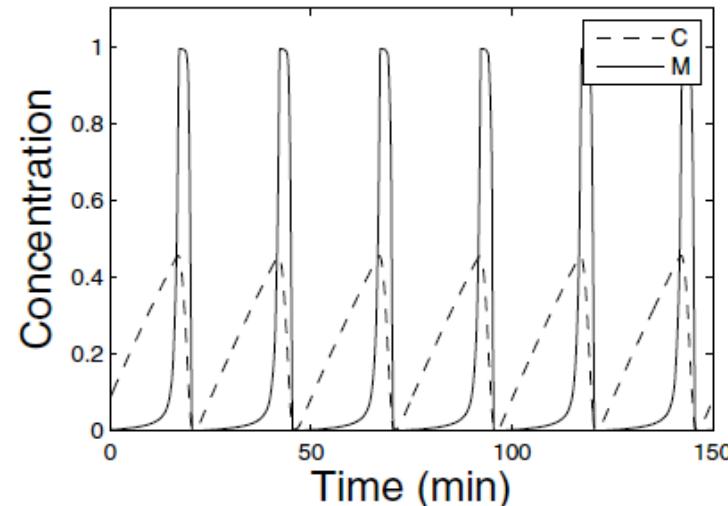
Version of the mitotic model with a positive feedback loop



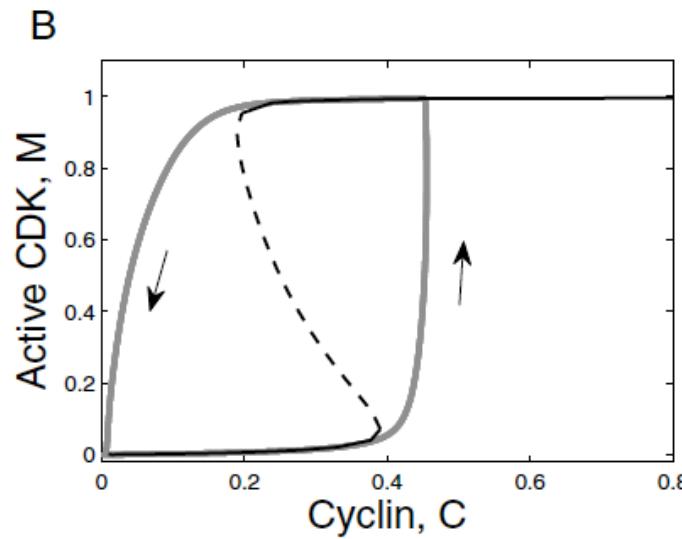
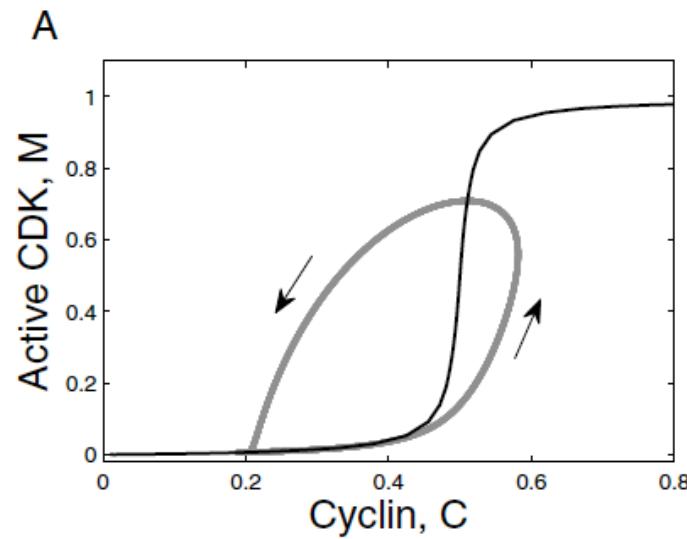
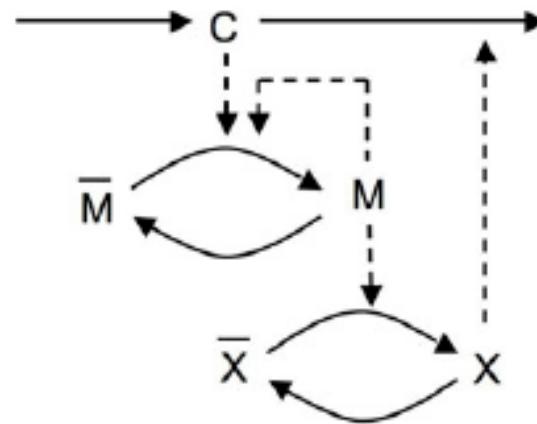
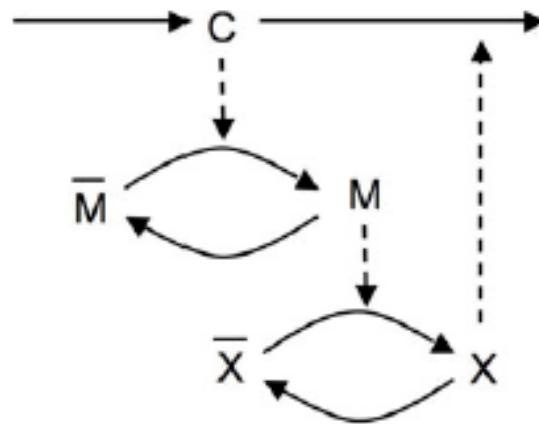
A



B



Smooth vs relaxation oscillations



Smooth vs relaxation oscillations

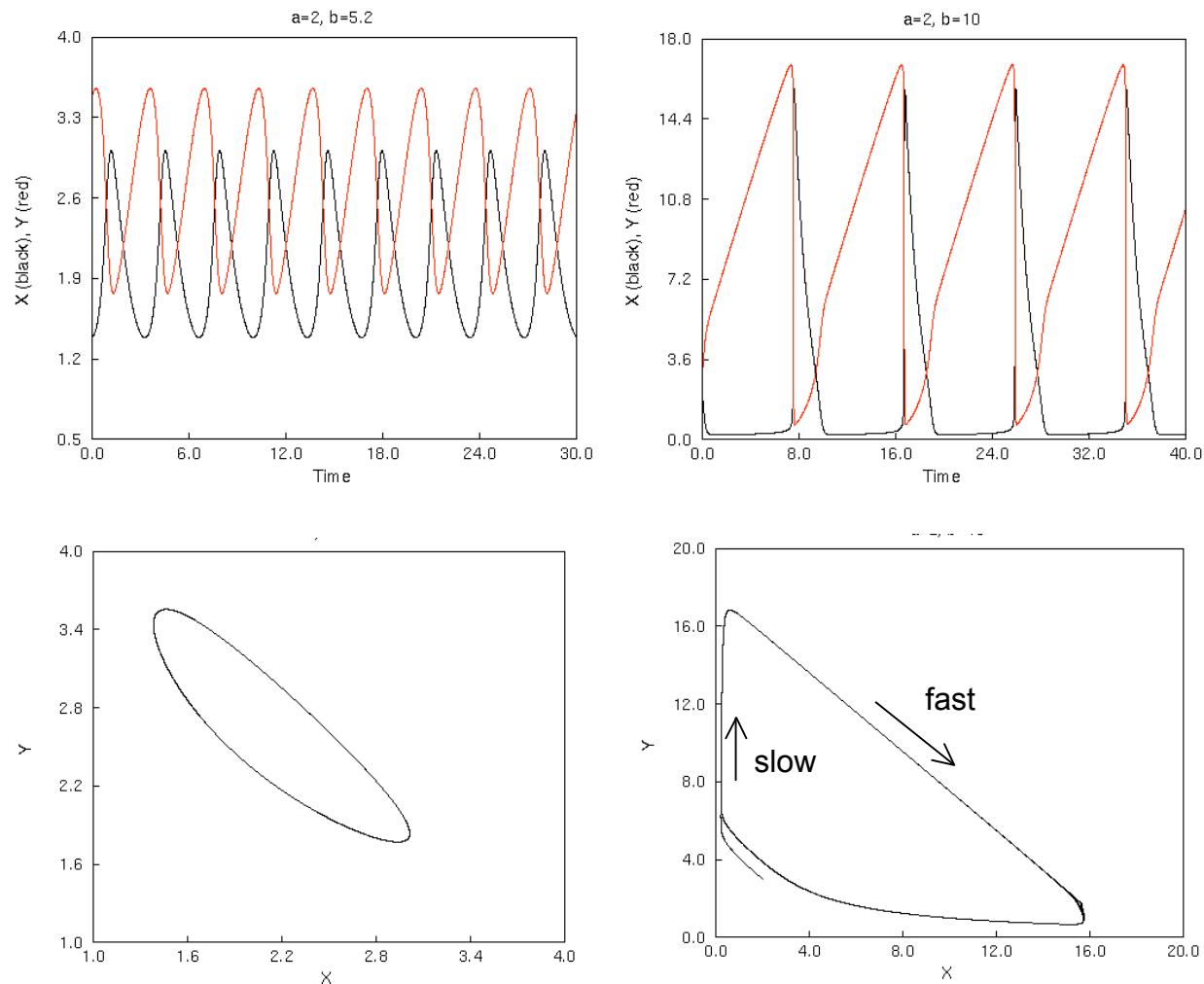
Rem: Relaxation oscillations require different time scales, typically (*always?*) a fast positive circuit and a slow negative circuit, *but not necessarily bistability/hysteresis (?)*.

Ex: Brusselator

r	reaction	rate
1	$A \xrightarrow{k_1} X$	$v_1 = k_1 A$
2	$B + X \xrightarrow{k_2} Y + C$	$v_2 = k_2 BX$
3	$2X + Y \xrightarrow{k_3} 3X$	$v_3 = k_3 X^2 Y$
4	$X \xrightarrow{k_4} D$	$v_4 = k_4 X$

$$\begin{cases} \frac{dX}{dt} = k_1 a - k_2 bX + k_3 X^2 Y - k_4 X \\ \frac{dY}{dt} = k_2 bX - k_3 X^2 Y \end{cases}$$

By drawing the nullclines in the phase space, it is easy to show that there is no bistability in the system. Nevertheless, for large value of b , relaxation oscillations are obtained (the speed on the different phases on the limit cycle is not constant)

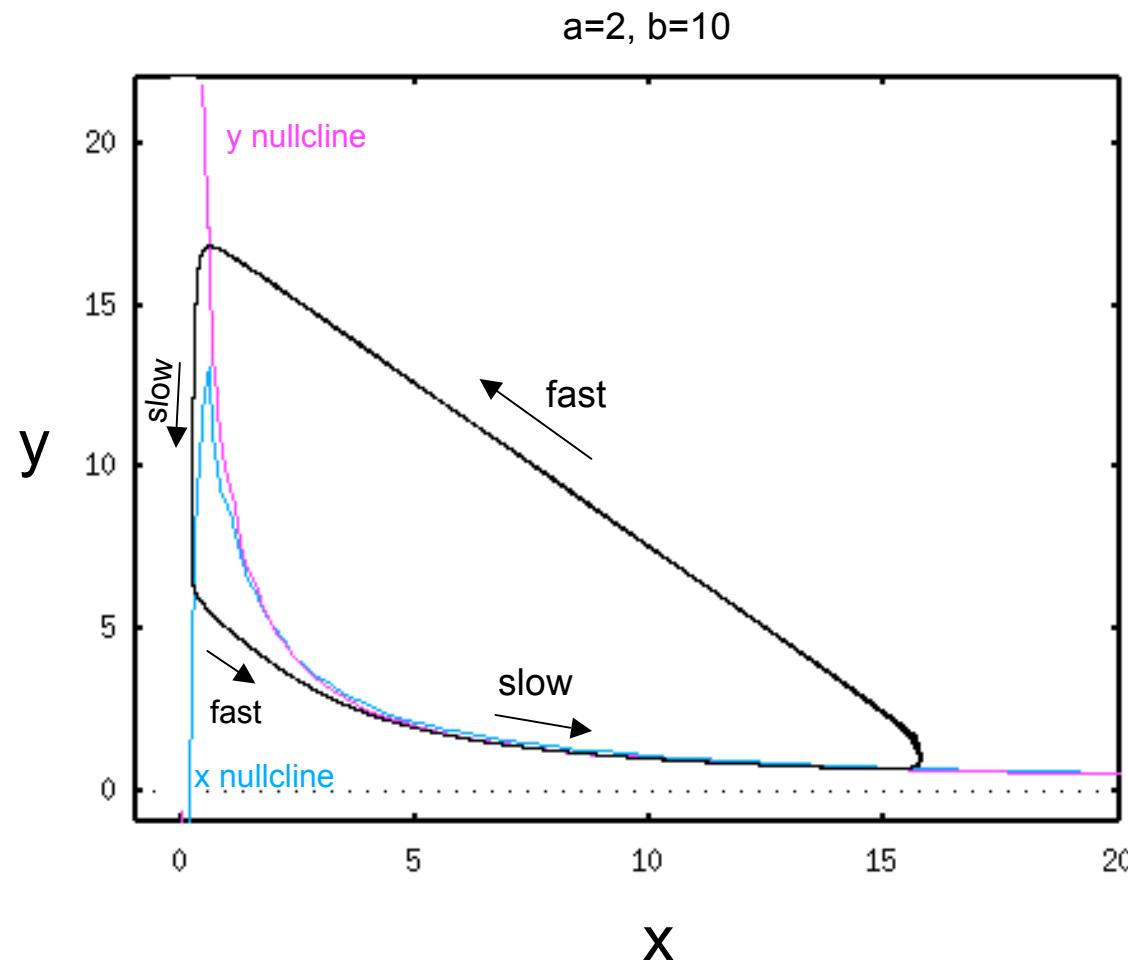


Smooth vs relaxation oscillations

Brusselator - original version

$$\begin{aligned} dx/dt &= a - (b+1)x + x^2y \\ dy/dt &= b*x - x^2*y \end{aligned}$$

Do relaxation oscillations require bistability / hysteresis?



Smooth vs relaxation oscillations

Brusselator - change of variables

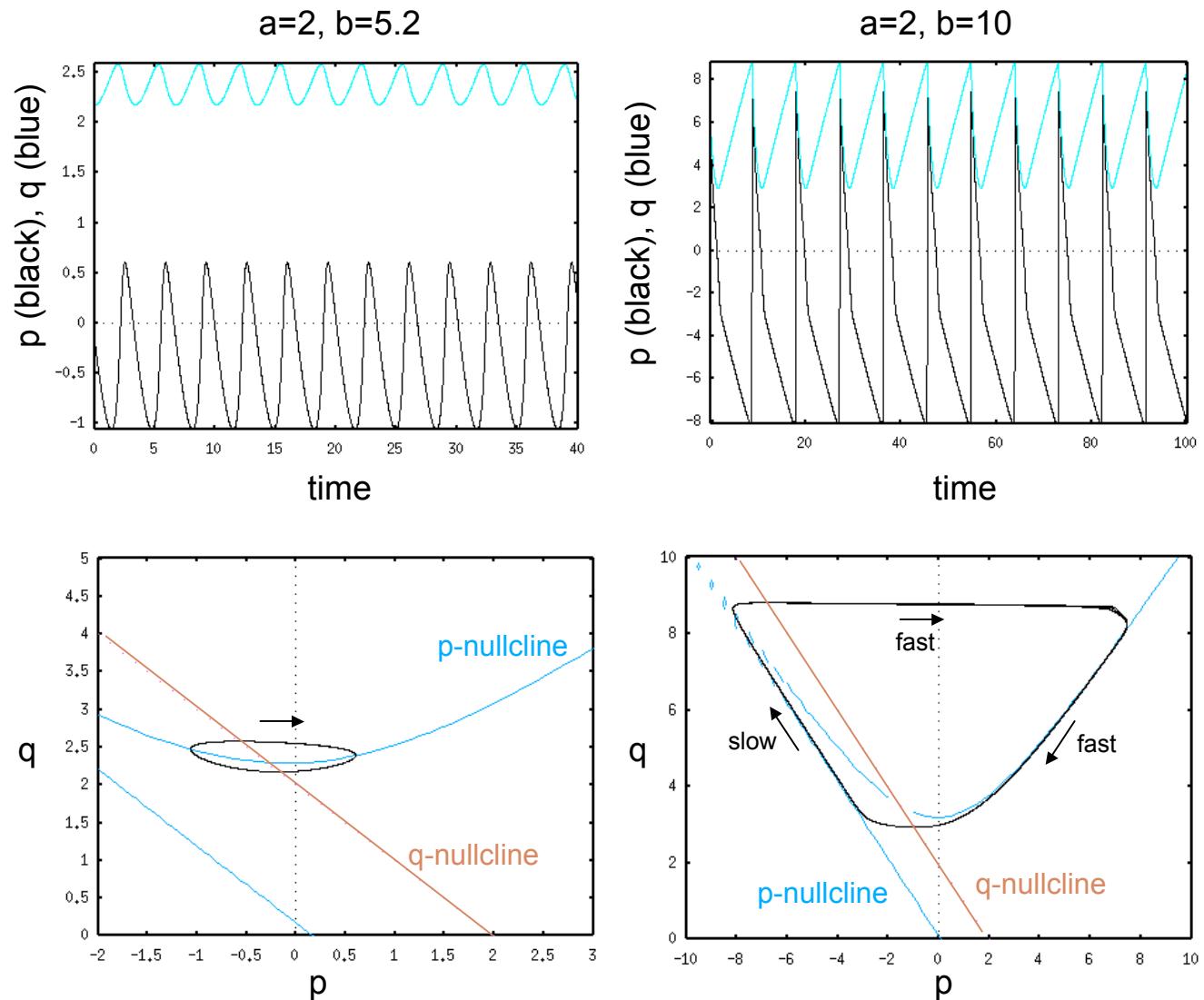
$$p = (x+y)/2$$

$$q = (x-y)/2$$

$$\begin{aligned} dp/dt &= a/2 - (p+q)(b - (p+q)(p-q) + 1/2) \\ dq/dt &= -1/2(p+q) + a/2 \end{aligned}$$

When this change of variable is applied, q is the slow variable (q can be treated as a parameter) and the p -nullcline takes a "N" shape, making an "hysteresis" appear...

M. Kaufman
H. Croisier
D. Gonze
Nov. 2009



Smooth vs relaxation oscillations



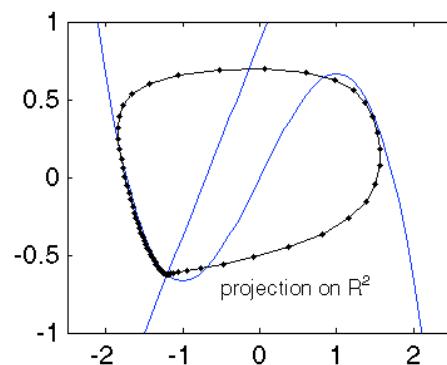
Neuronal models:

Fitzhugh-Nagumo

Hodgkin-Huxley



The Fitzhugh-Nagumo model is a example of a two-dimensional excitable system. It was proposed as a simplification of the famous model by Hodgkin and Huxley to describe the response of an excitable nerve membrane to external current stimuli.



$$\begin{cases} \frac{\epsilon dx}{dt} = f(x) - y \\ \frac{dy}{dt} = \gamma x - \beta y + b - s(t) \end{cases}$$

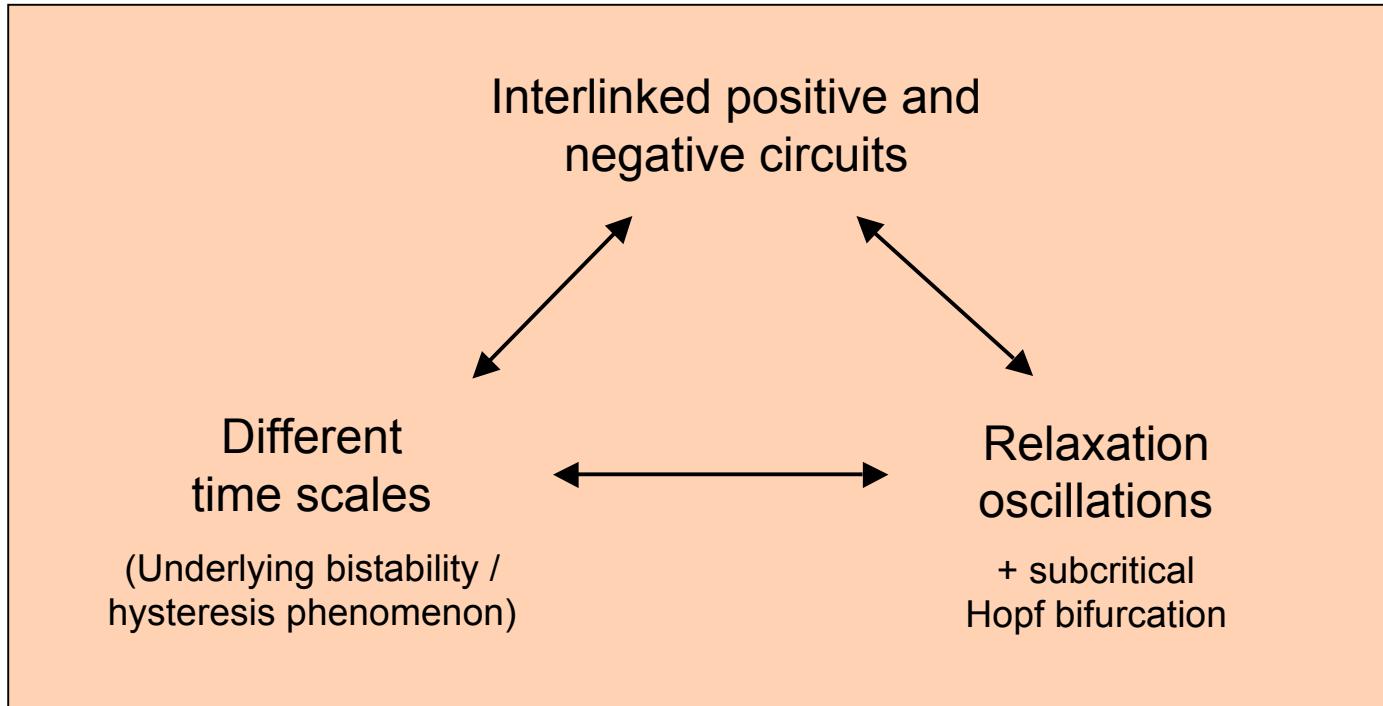
This model is conceptually close to the van der Pol model: it displays excitability and spike-like oscillations

FitzHugh (1955) Mathematical models of threshold phenomena in the nerve membrane. *Bull. Math. Biophysics* 17:257-278.

FitzHugh (1961) Impulses and physiological states in theoretical models of nerve membrane. *Biophysical J.* 1:445-466.

Nagumo *et al* (1962) An active pulse transmission line simulating nerve axon. *Proc IRE.* 50:2061-2070.

Smooth vs relaxation oscillations



- If you want to generate relaxation oscillations, you will need to build an oscillator using different time scale (fast + slow processes).
- If you want to explain (experimentally) observed relaxation oscillations, you will have to search for the origin of different time scales.
- If you build an oscillator based on different time scales, you may expect to generate relaxation oscillations.

Interlinked positive and negative feedbacks

Robust, Tunable Biological Oscillations from Interlinked Positive and Negative Feedback Loops

Tony Yu-Chen Tsai,^{1,*} Yoon Sup Choi,^{1,2,*} Wenzhe Ma,^{3,4} Joseph R. Pomerening,⁵ Chao Tang,^{3,4} James E. Ferrell Jr.^{1,†}

A simple negative feedback loop of interacting genes or proteins has the potential to generate sustained oscillations. However, many biological oscillators also have a positive feedback loop, raising the question of what advantages the extra loop imparts. Through computational studies, we show that it is generally difficult to adjust a negative feedback oscillator's frequency without compromising its amplitude, whereas with positive-plus-negative feedback, one can achieve a widely tunable frequency and near-constant amplitude. This tunability makes the latter design suitable for biological rhythms like heartbeats and cell cycles that need to provide a constant output over a range of frequencies. Positive-plus-negative oscillators also appear to be more robust and easier to evolve, rationalizing why they are found in contexts where an adjustable frequency is unimportant.

Possible roles of positive feedback loops:

- Provide oscillations with the ability to tune its frequency without changing its amplitude.
- Provide a greater robustness and reliability.

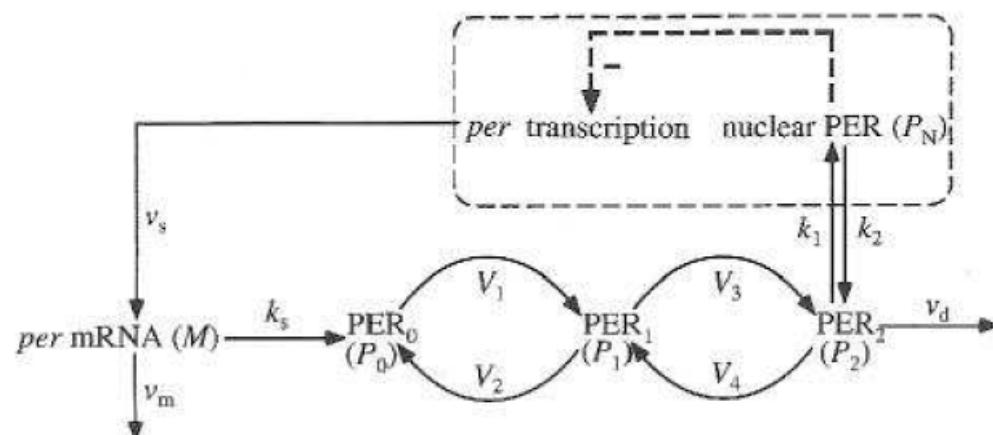
Molecular oscillator models

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

ALBERT GOLDBETER

Faculté des Sciences, Université Libre de Bruxelles, Campus Plaine, C. P. 231, B-1050 Brussels, Belgium

The informations obtained by theoretical studies can be used to model biological oscillators: look for negative feedback loops, sources of delay, sources of non-linearity, etc



5-variable model for circadian oscillations
(1 gene / 1 feedback loop)

Goldbeter (1995) *Proc R Soc Lond B* 261:319-324

$$\frac{dM}{dt} = v_s \frac{K_1^n}{K_1 + P_N^n} - v_m \frac{M}{K_m + M} \quad (1a)$$

$$\frac{dP_0}{dt} = k_s M - V_1 \frac{P_0}{K_1 + P_0} + V_2 \frac{P_1}{K_2 + P_1} \quad (1b)$$

$$\frac{dP_1}{dt} = V_1 \frac{P_0}{K_1 + P_0} - V_2 \frac{P_1}{K_2 + P_1} - V_3 \frac{P_1}{K_3 + P_1} + V_4 \frac{P_2}{K_4 + P_2} \quad (1c)$$

$$\frac{dP_2}{dt} = V_3 \frac{P_1}{K_3 + P_1} - V_4 \frac{P_2}{K_4 + P_2} - k_1 P_2 + k_2 P_N - v_d \frac{P_2}{K_d + P_2} \quad (1d)$$

$$\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N \quad (1e)$$

The total (nonconserved) quantity of PER protein, P_t , is given by:

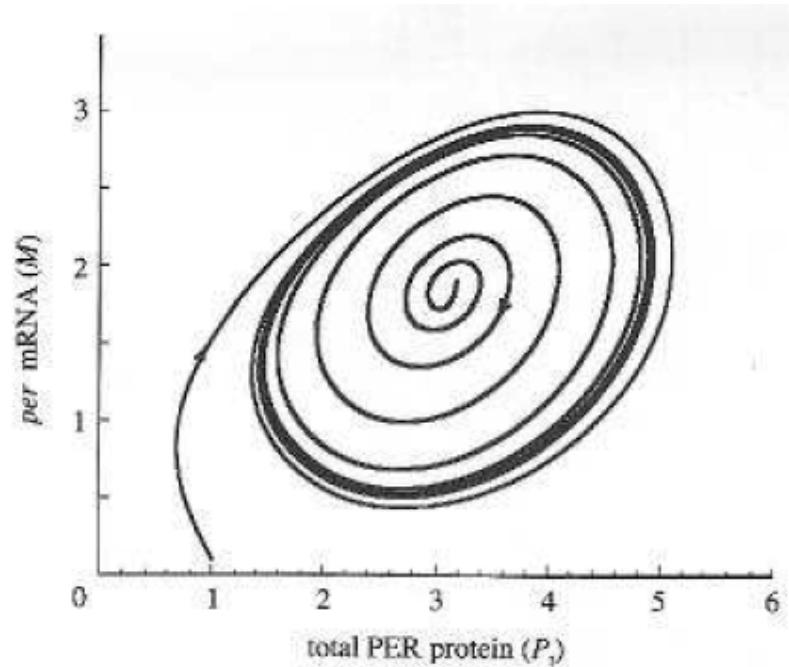
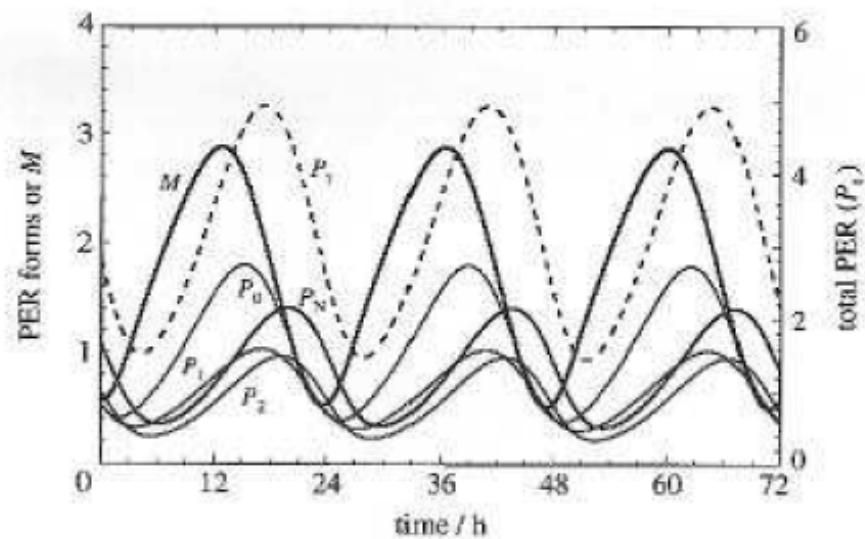
$$P_t = P_0 + P_1 + P_2 + P_N \quad (2)$$

Molecular oscillator models

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

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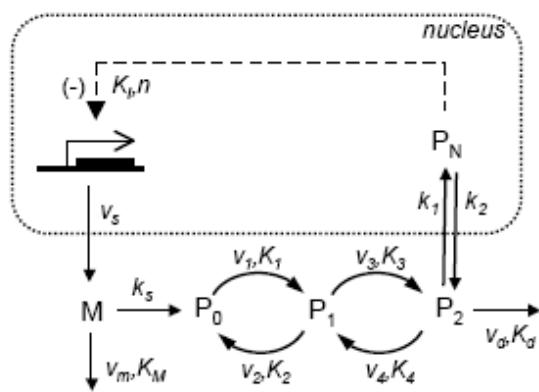


Goldbeter (1995) Proc R Soc Lond B 261:319-324

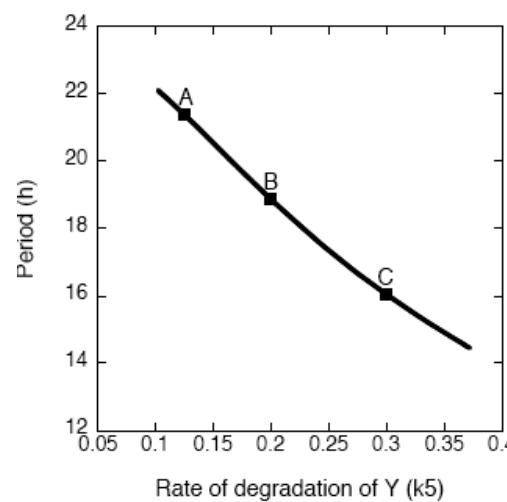
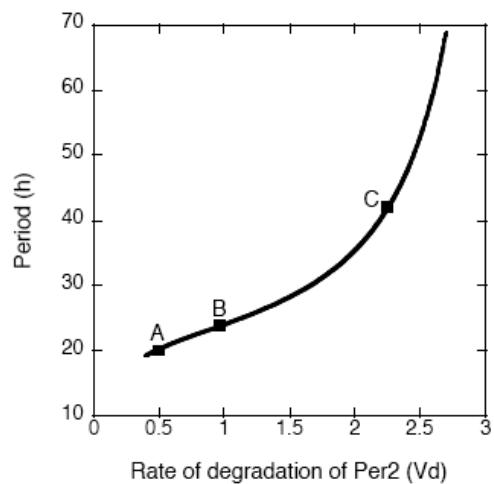
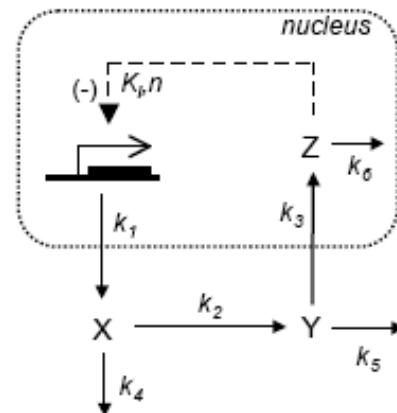
Molecular oscillator models

But some surprises remains...

Goldbeter's model



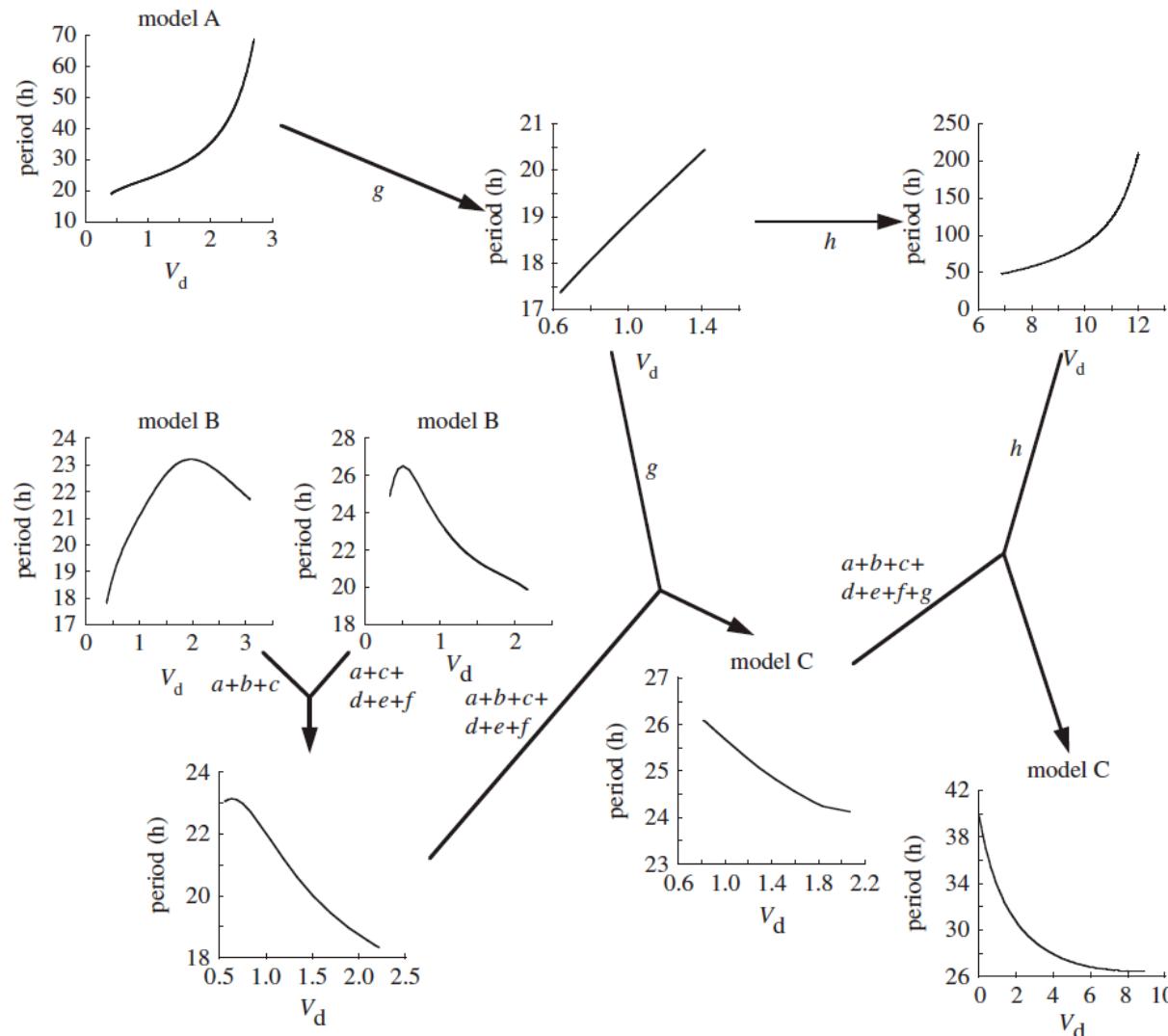
Ruoff's (Goodwin) model



Gérard, Gonze,
Goldbeter (2009)
Phil Trans Roy Soc A
357:4665-83

Molecular oscillator models

But some surprises remains...



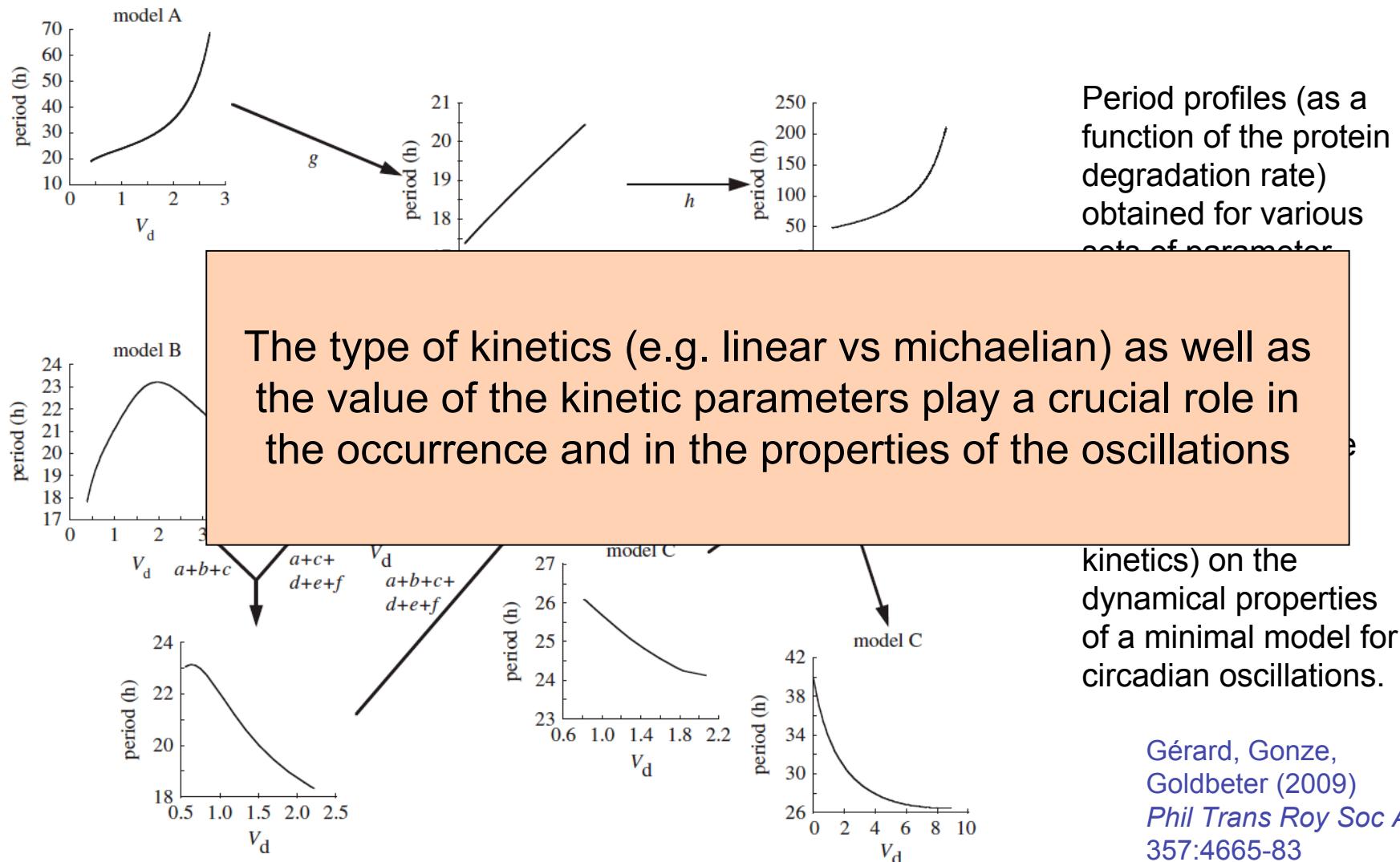
Period profiles (as a function of the protein degradation rate) obtained for various sets of parameter values.

This comparative study highlights the role of non-linear terms (saturating kinetics) on the dynamical properties of a minimal model for circadian oscillations.

Gérard, Gonze,
Goldbeter (2009)
Phil Trans Roy Soc A
357:4665-83

Molecular oscillator models

But some surprises remains...



Detailed molecular oscillator models

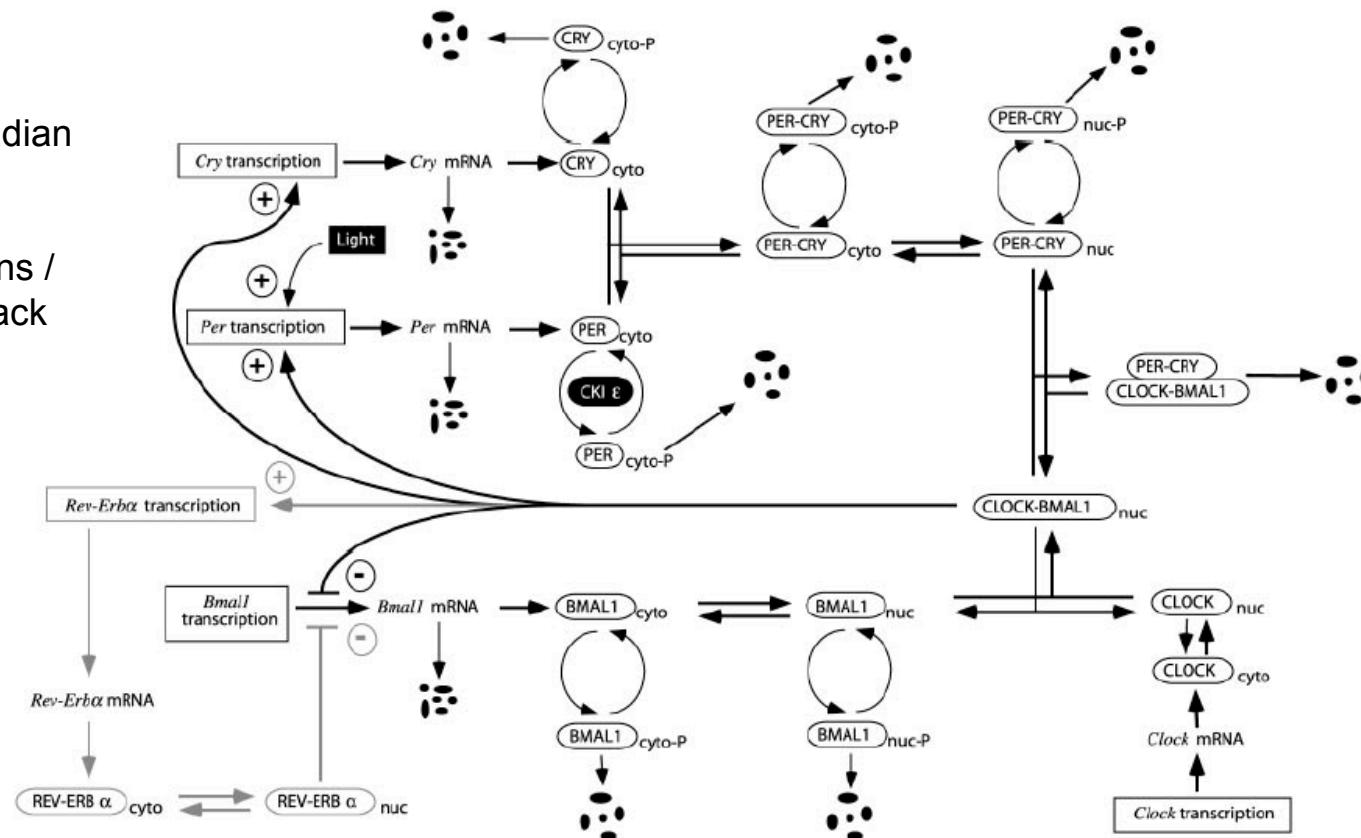
Toward a detailed computational model for the mammalian circadian clock

Jean-Christophe Leloup* and Albert Goldbeter†

Unité de Chronobiologie Théorique, Faculté des Sciences, Université Libre de Bruxelles, Campus Plaine, C. P. 231, B-1050 Brussels, Belgium

PNAS | June 10, 2003 | vol. 100 | no. 12 | 7051–7056

16/19-variable
model for circadian
oscillations in
mammals (4/5
genes + proteins /
multiple feedback
loops).



Detailed molecular oscillator models

Toward a detailed computational model for the mammalian circadian clock

Kinetic Equations. The time evolution of the model of Fig. 5 is governed by the system of kinetic equations 1–16. For the sake of clarity, we have grouped these equations for the various mRNAs, the phosphorylated and nonphosphorylated proteins PER and CRY in the cytosol, the phosphorylated and nonphosphorylated PER–CRY complex in cytosol and nucleus, the phosphorylated and nonphosphorylated protein BMAL1 in the cytosol and nucleus, and the complex between PER–CRY and CLOCK–BMAL1 in the nucleus:

(i) mRNAs of *Per*, *Cry*, and *Bmal1*:

$$\frac{dM_p}{dt} = v_{sp} \frac{B_N^n}{K_{AP}^n + B_N^n} - v_{mp} \frac{M_p}{K_{mP} + M_p} - k_{dmp} M_p$$

$$\frac{dM_c}{dt} = v_{sc} \frac{B_N^n}{K_{AC}^n + B_N^n} - v_{mc} \frac{M_c}{K_{mC} + M_c} - k_{dmc} M_c$$

$$\frac{dM_b}{dt} = v_{sb} \frac{K_{lb}^m}{K_{mb}^m + B_N^m} - v_{mb} \frac{M_b}{K_{mb} + M_b} - k_{dmb} M_b$$

(ii) Phosphorylated and nonphosphorylated proteins PER and CRY in the cytosol:

$$\frac{dP_C}{dt} = k_{sp} M_p - V_{1P} \frac{P_C}{K_p + P_C} + V_{2P} \frac{P_{CP}}{K_{dp} + P_{CP}} + k_4 P_C C_C - k_3 P_C C_C - k_{dn} P_C$$

$$\frac{dC_C}{dt} = k_{sc} M_c - V_{1C} \frac{C_C}{K_p + C_C} + V_{2C} \frac{C_{CP}}{K_{dp} + C_{CP}} + k_4 P_C C_C - k_3 P_C C_C - k_{dn} C_C$$

$$\frac{dP_{CP}}{dt} = V_{1P} \frac{P_C}{K_p + P_C} - V_{2P} \frac{P_{CP}}{K_{dp} + P_{CP}} - v_{dPC} \frac{P_{CP}}{K_d + P_{CP}} - k_{dn} P_{CP}$$

$$\frac{dC_{CP}}{dt} = V_{1C} \frac{C_C}{K_p + C_C} - V_{2C} \frac{C_{CP}}{K_{dp} + C_{CP}} - v_{dCC} \frac{C_{CP}}{K_d + C_{CP}} - k_{dn} C_{CP}$$

(iii) Phosphorylated and nonphosphorylated PER–CRY complex in cytosol and nucleus:

$$\frac{dP_{CC}}{dt} = -V_{1PC} \frac{P_C C_C}{K_p + P_C C_C} + V_{2PC} \frac{P_{CP} C_C}{K_{dp} + P_{CP} C_C} - k_4 P_C C_C + k_3 P_C C_C + k_2 P_C N - k_1 P_C C_C - k_{dn} P_C C_C$$
[8]

$$\frac{dP_{CN}}{dt} = -V_{3PC} \frac{P_C N}{K_p + P_C N} + V_{4PC} \frac{P_{NP} N}{K_{dp} + P_{NP} N} - k_2 P_C N + k_1 P_C C_C - k_7 B_N P_C N + k_8 J_N - k_{dn} P_C N$$
[9]

$$\frac{dP_{CP}}{dt} = V_{1PC} \frac{P_C C_C}{K_p + P_C C_C} - V_{2PC} \frac{P_{CP} C_C}{K_{dp} + P_{CP} C_C} - v_{dPCC} \frac{P_{CP} C_C}{K_d + P_{CP} C_C} - k_{dn} P_{CP} C_C$$
[10]

$$\frac{dP_{NP}}{dt} = V_{3PC} \frac{P_C N}{K_p + P_C N} - V_{4PC} \frac{P_{NP} N}{K_{dp} + P_{NP} N} - v_{dPNC} \frac{P_{NP} N}{K_d + P_{NP} N} - k_{dn} P_{NP} N$$
[11]

(iv) Phosphorylated and nonphosphorylated protein BMAL1 in the cytosol and nucleus:

$$\frac{dB_C}{dt} = k_{sB} M_b - V_{1B} \frac{B_C}{K_p + B_C} + V_{2B} \frac{B_{CP}}{K_{dp} + B_{CP}} - k_5 B_C + k_6 B_N - k_{dn} B_C$$
[12]

$$\frac{dB_{CP}}{dt} = V_{1B} \frac{B_C}{K_p + B_C} - V_{2B} \frac{B_{CP}}{K_{dp} + B_{CP}} - v_{dBC} \frac{B_{CP}}{K_d + B_{CP}} - k_{dn} B_{CP}$$
[13]

$$\frac{dB_N}{dt} = -V_{3B} \frac{B_N}{K_p + B_N} + V_{4B} \frac{B_{NP}}{K_{dp} + B_{NP}} + k_5 B_C - k_6 B_N - k_7 B_N P_C N + k_8 J_N - k_{dn} B_N$$
[14]

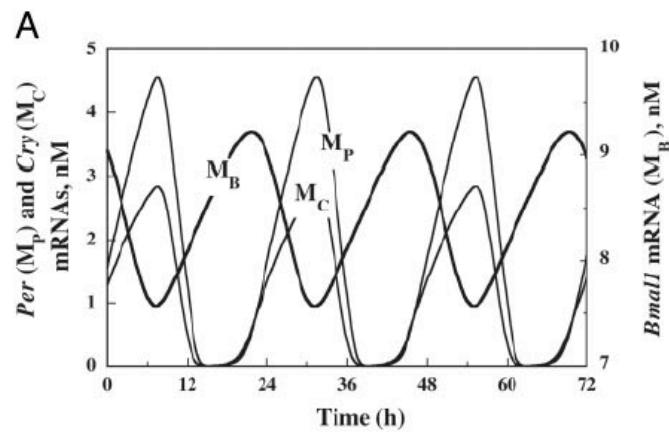
$$\frac{dB_{NP}}{dt} = V_{3B} \frac{B_N}{K_p + B_N} - V_{4B} \frac{B_{NP}}{K_{dp} + B_{NP}} - v_{dBN} \frac{B_{NP}}{K_d + B_{NP}} - k_{dn} B_{NP}$$
[15]

Detailed molecular oscillator models

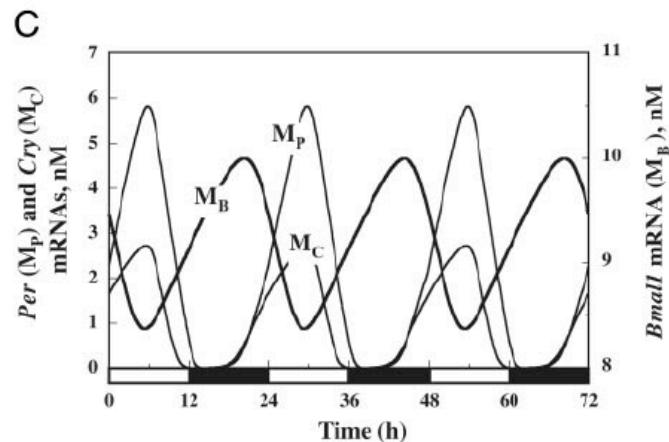
Toward a detailed computational model for the mammalian circadian clock

Jean-Christophe Leloup* and Albert Goldbeter†

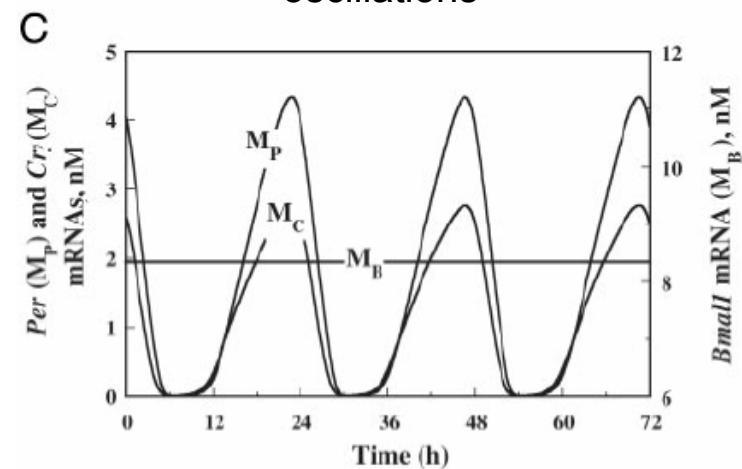
Unité de Chronobiologie Théorique, Faculté des Sciences, Université Libre de Bruxelles, Campus Plaine, C. P. 231, B-1050 Brussels, Belgium



limit-cycle
oscillations



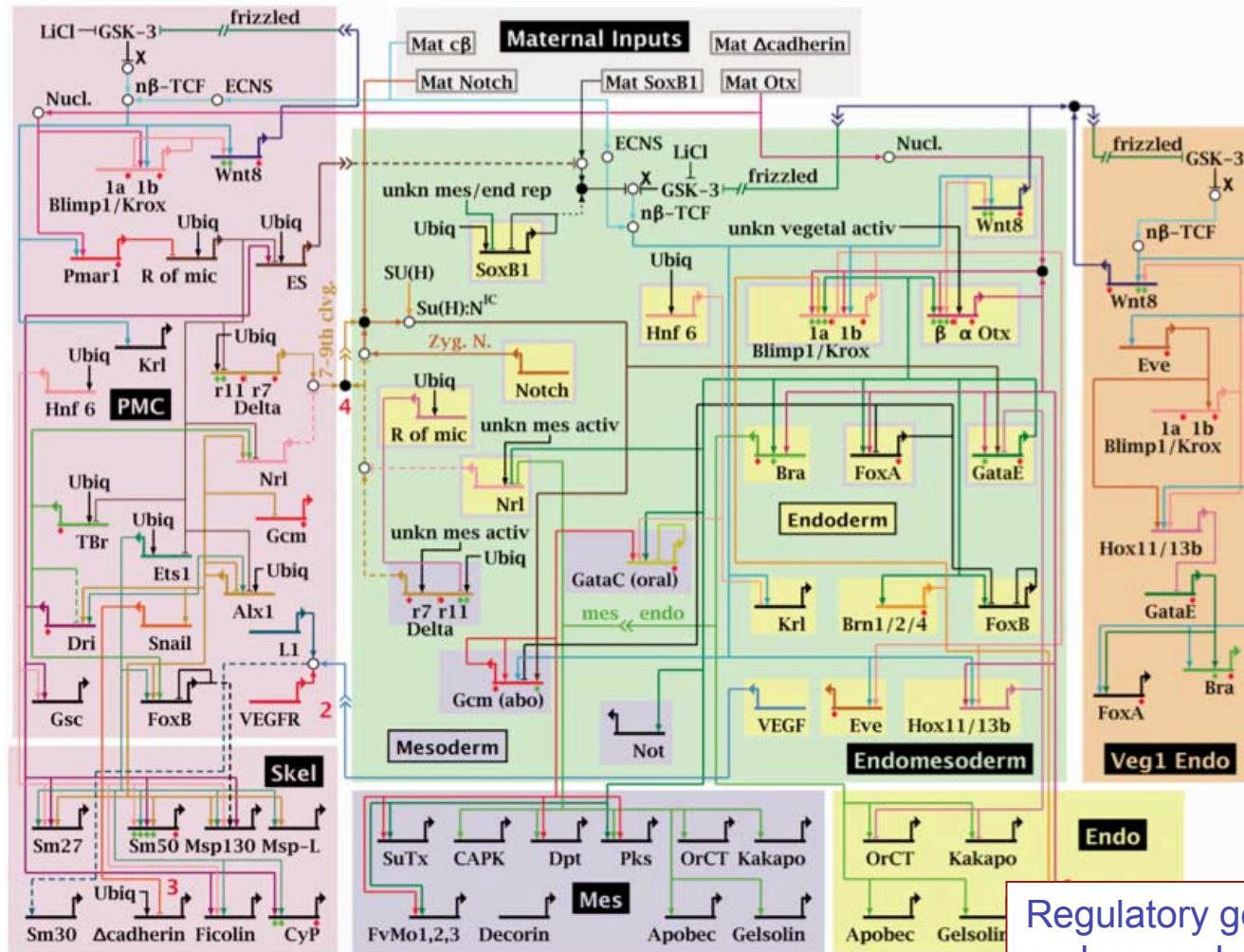
entrainment
by a LD cycle



multiple sources of
oscillations

Complex biological oscillators

Most biological systems combine negative and positive feedback loops.



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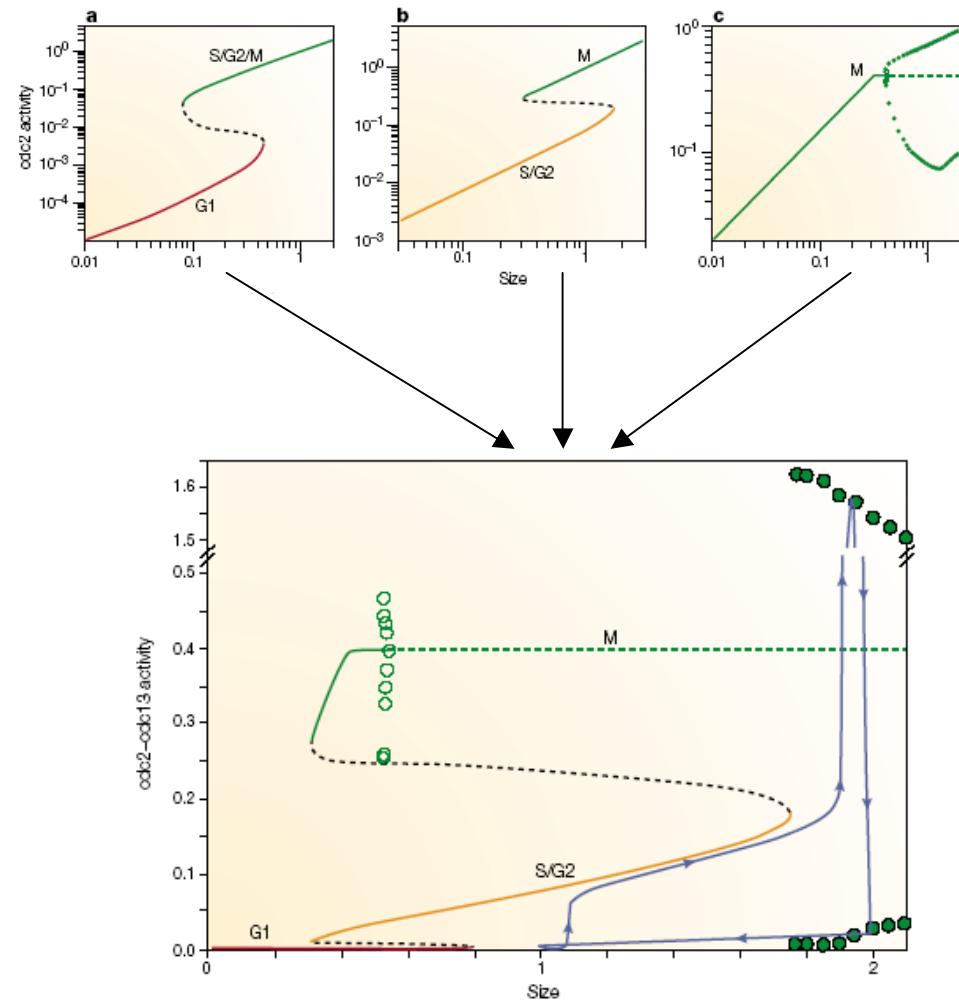
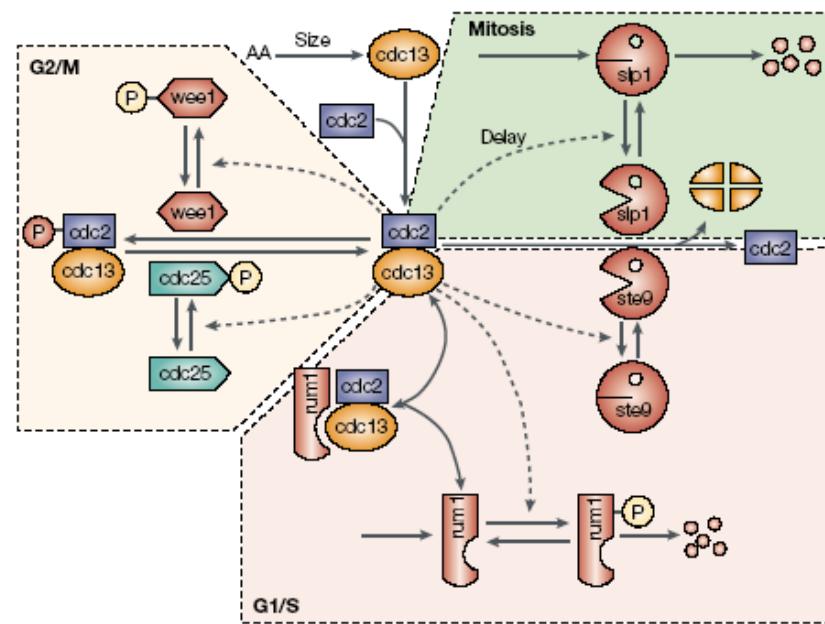
Regulatory gene network for
endomesoderm development in
sea urchin (from E. Davidson)

Complex biological oscillators

NETWORK DYNAMICS AND CELL PHYSIOLOGY

John J. Tyson*, Kathy Chen* and Bela Novak†

Complex assemblies of interacting proteins carry out most of the interesting jobs in a cell, such as metabolism, DNA synthesis, movement and information processing. These physiological properties play out as a subtle molecular dance, choreographed by underlying regulatory networks. To understand this dance, a new breed of theoretical molecular biologists reproduces these networks in computers and in the mathematical language of dynamical systems.



Tyson et al (1991) *Nature Rev.* 2:908-916.

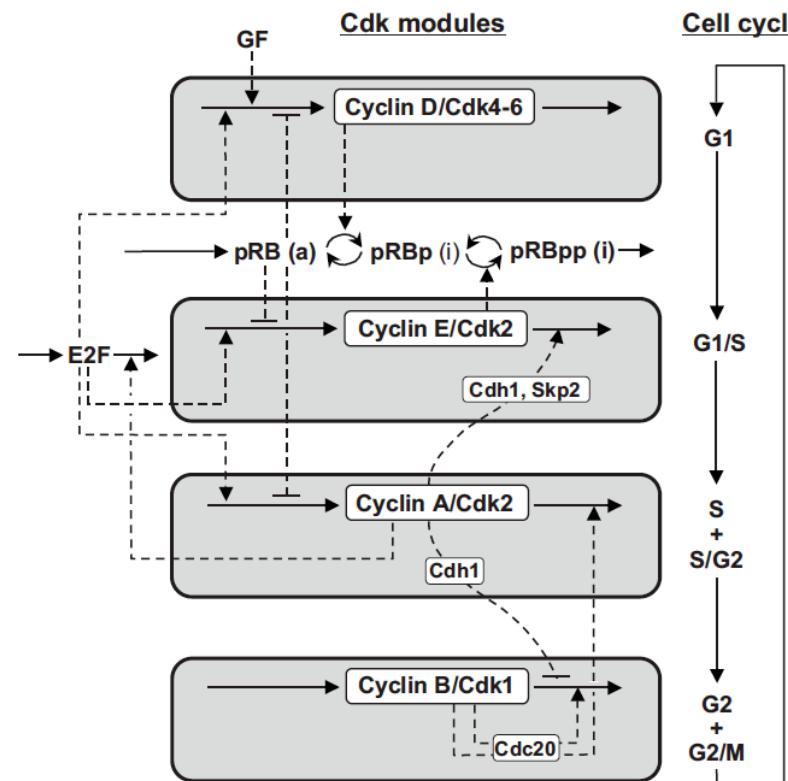
Complex biological oscillators

From simple to complex patterns of oscillatory behavior in a model for the mammalian cell cycle containing multiple oscillatory circuits

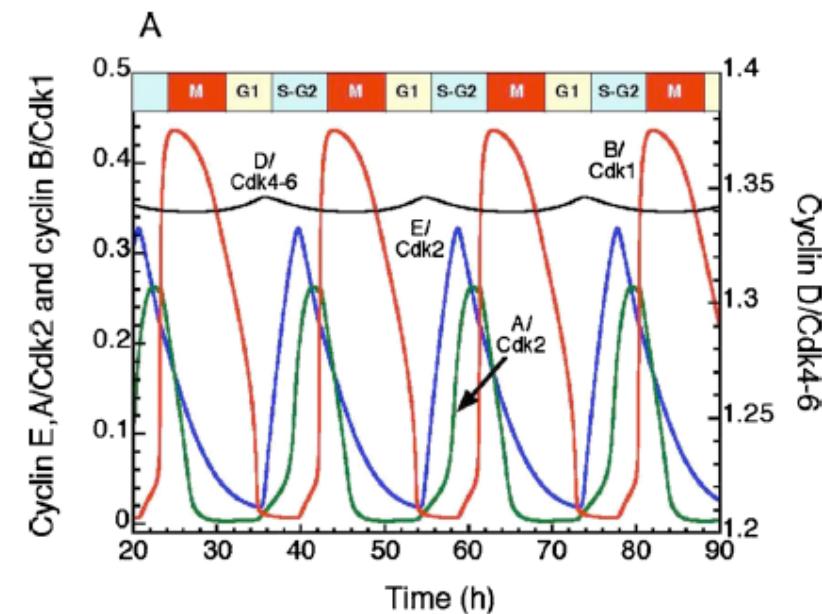
Claude Gérard and Albert Goldbeter^{a)}

Faculté des Sciences, Université Libre de Bruxelles (ULB), Campus Plaine, CP 231, B-1050 Brussels,
Belgium

CHAOS 20, 045109 (2010)



39 variable model



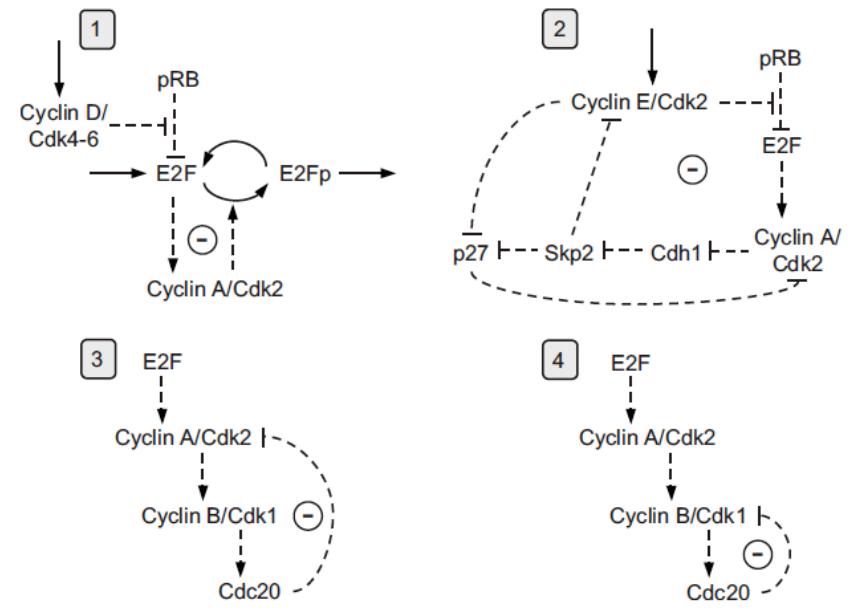
The transitions through the successive phases of the cell cycle are controlled by a complex regulatory network of CDK-cyclin complexes.

Complex biological oscillators

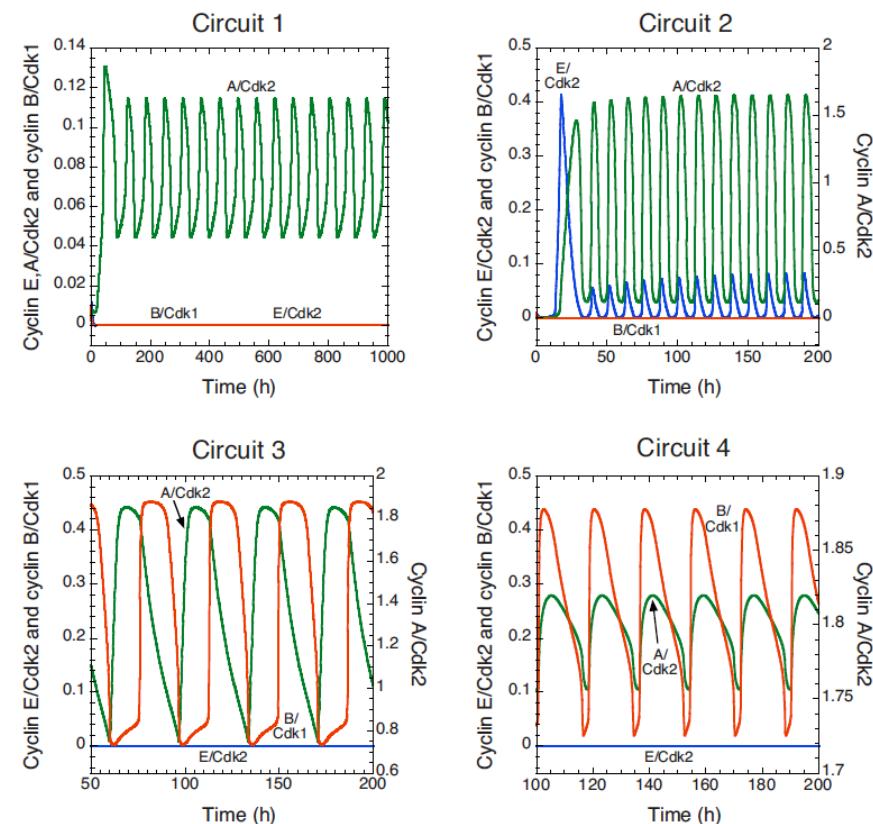
From simple to complex patterns of oscillatory behavior in a model for the mammalian cell cycle containing multiple oscillatory circuits

Claude Gérard and Albert Goldbeter^{a)}

Faculté des Sciences, Université Libre de Bruxelles (ULB), Campus Plaine, CP 231, B-1050 Brussels,
Belgium



Several negative circuits can be identified. They all have the potential to generate oscillations. The question arises as to understand the role of these circuits in the cell cycle regulation.



Complex biological oscillators

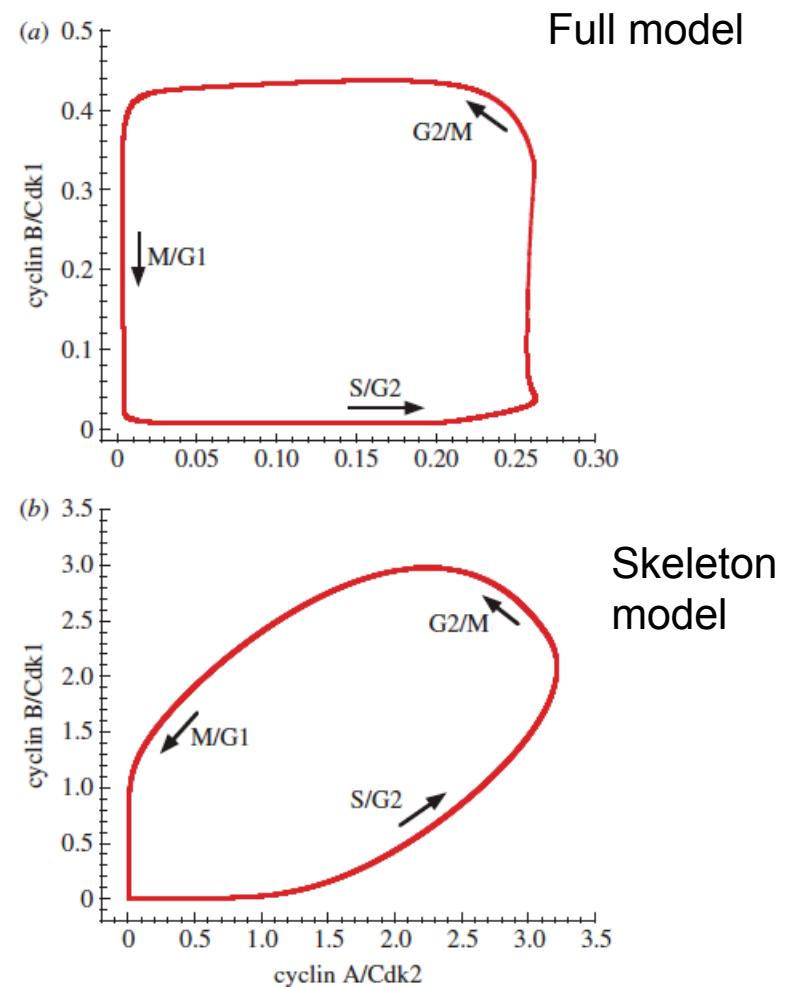
A skeleton model for the network of cyclin-dependent kinases driving the mammalian cell cycle

Claude Gérard and Albert Goldbeter*

Faculté des Sciences, Université Libre de Bruxelles (ULB), Campus Plaine, CP 231,
1050 Brussels, Belgium

Interface Focus (2011) 1, 24–35

In the cell cycle model, several positive feedback loops can also be identified. A comparison of the "full" model with a "skeleton" model that does not incorporate these positive circuits show that these additional loops may be used to make the transitions between the successive activation of the CDK-cyclin complex (and thereby the transitions between the successive phases of the cell cycle) more abrupt.



Synthetic oscillator: *Repressilator*

A synthetic oscillatory network of transcriptional regulators

Elowitz, Leibler (2000) *Nature* 403:335-338

Networks of interacting biomolecules carry out many essential functions in living cells, but the 'design principles' underlying the functioning of such intracellular networks remain poorly understood, despite intensive efforts including quantitative analysis of relatively simple systems. Here we present a complementary approach to this problem: the design and **construction of a synthetic network** to implement a particular function. We used **three transcriptional repressor systems** that are not part of any natural biological clock to build an **oscillating network**, termed the **repressilator**, in *Escherichia coli*. The network periodically induces the synthesis of green fluorescent protein as a readout of its state in individual cells. The resulting oscillations, with typical periods of hours, are slower than the cell-division cycle, so the state of the oscillator has to be transmitted from generation to generation. This artificial clock displays noisy behaviour, possibly because of stochastic fluctuations of its components. Such rational network design may lead both to the engineering of new cellular behaviours and to an improved understanding of naturally occurring networks.

Synthetic oscillator: Repressilator

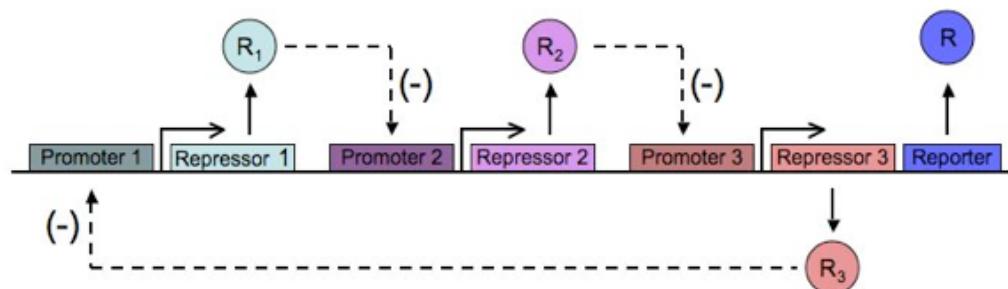
A synthetic oscillatory network of transcriptional regulators

Elowitz, Leibler (2000) *Nature* 403:335-338

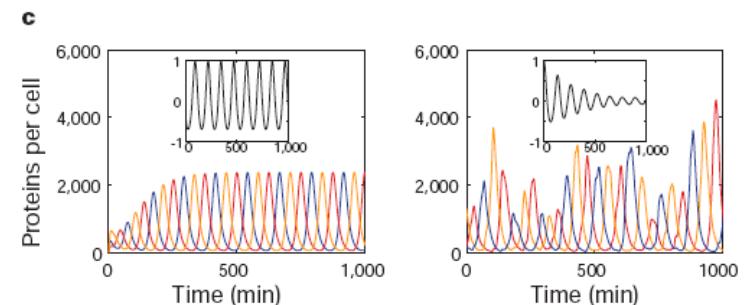
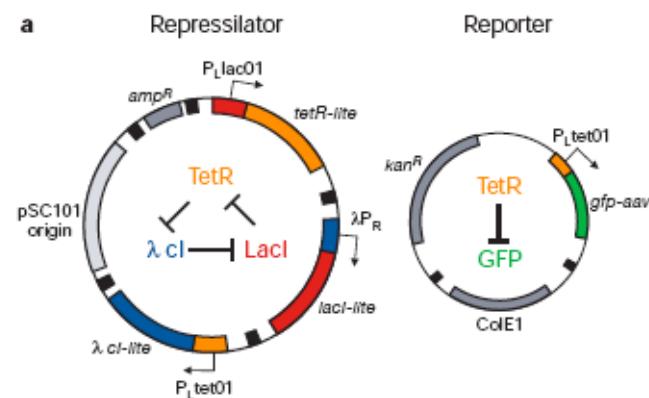
Mathematical model

$$\begin{aligned}\frac{dm_i}{dt} &= -m_i + \frac{\alpha}{(1+p_j^n)} + \alpha_0 & (i = lacI, tetR, cl) \\ \frac{dp_i}{dt} &= -\beta(p_i - m_i) & (j = cl, lacI, tetR)\end{aligned}$$

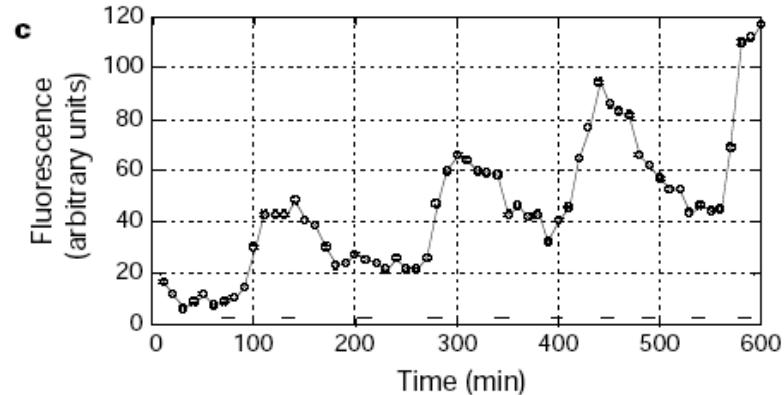
Design of the genetic regulatory network



Genetic construction in *E. coli*



In vivo measurement



Synthetic oscillators

A fast, robust and tunable synthetic gene oscillator

Jesse Stricker^{1*}, Scott Cookson^{1*}, Matthew R. Bennett^{1,2*}, William H. Mather¹, Lev S. Tsimring² & Jeff Hasty^{1,2}

One defining goal of synthetic biology is the development of engineering-based approaches that enable the construction of gene-regulatory networks according to ‘design specifications’ generated from computational modelling^{1–6}. This approach provides a systematic framework for exploring how a given regulatory network generates a particular phenotypic behaviour. Several fundamental gene circuits have been developed using this approach, including toggle switches⁷ and oscillators^{8–10}, and these have been applied in new contexts such as triggered biofilm development¹¹ and cellular population control¹². Here we describe an engineered genetic oscillator in *Escherichia coli* that is fast, robust and persistent, with tunable oscillatory periods as fast as 13 min. The oscillator was designed using a previously modelled network architecture comprising linked positive and negative feedback loops^{1,13}. Using a microfluidic platform tailored for single-cell microscopy, we precisely control environmental conditions and monitor oscillations in individual cells through multiple cycles. Experiments reveal remarkable robustness and persistence of oscillations in the designed circuit; almost every cell exhibited large-amplitude fluorescence oscillations throughout observation runs. The oscillatory period can be tuned by altering inducer levels, temperature and the media source. Computational modelling demonstrates that the key design principle for constructing a robust oscillator is a time delay in the negative feedback loop, which can mechanistically arise from the cascade of cellular processes involved in forming a functional transcription factor. The positive feedback loop increases the robustness of the oscillations and allows for greater tunability. Examination of our refined model suggested the existence of a simplified oscillator design without positive feedback, and we construct an oscillator strain confirming this computational prediction.

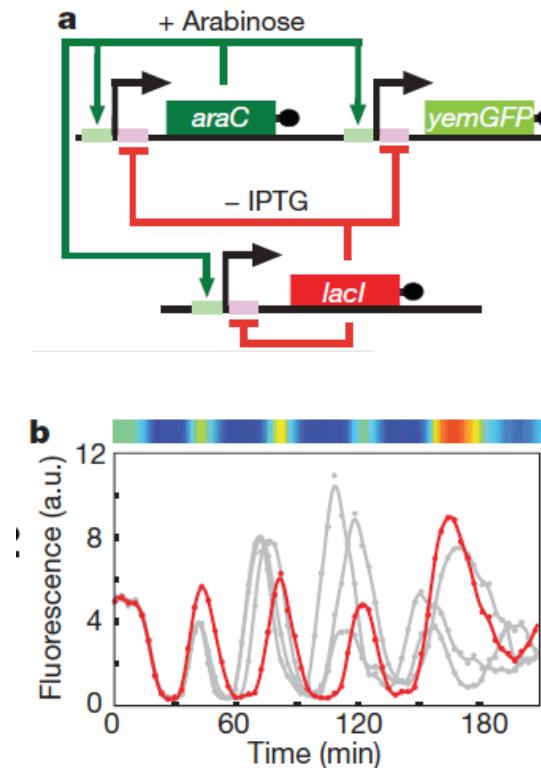
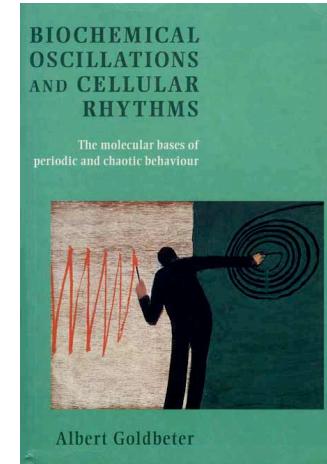


Figure 1 | Oscillations in the dual-feedback circuit. **a**, Network diagram of the dual-feedback oscillator. A hybrid promoter $\text{P}_{\text{lac}}/\text{ara-1}$ drives transcription of *araC* and *lacI*, forming positive and negative feedback loops. **b**, Single-cell fluorescence trajectories induced with 0.7% arabinose and 2 mM IPTG.

References

Books

- Goldbeter (1996) *Biochemical Oscillations and Cellular Rhythms: The molecular bases of periodic and chaotic behaviour*, Cambridge University Press, Cambridge.
- Glass & Mackey (1988) *From Clocks to Chaos: The Rhythms of Life*, Princeton Univ Press.
- Strogatz (1994) *Nonlinear dynamics and chaos*, Perseus Books.



Review papers

- Goldbeter (2001) Computational approaches to cellular rhythms. *Nature* 420:238-45.
- Ferrell JE Jr (2002) Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability. *Curr Opin Cell Biol.* 14:140-8.
- Tyson *et al* (2008) Biological switches and clocks, *J. R. Soc. Interface*.
- Novak, Tyson (2008) Design principles of biochemical oscillators. *Nature review* 9:981-91.
- Purcell O, Savery NJ, Grierson CS, di Bernardo M. (2010) A comparative analysis of synthetic genetic oscillators. *J R Soc Interface*. 7:1503-24.