Structure of small genetic networks and evolution *in silico*

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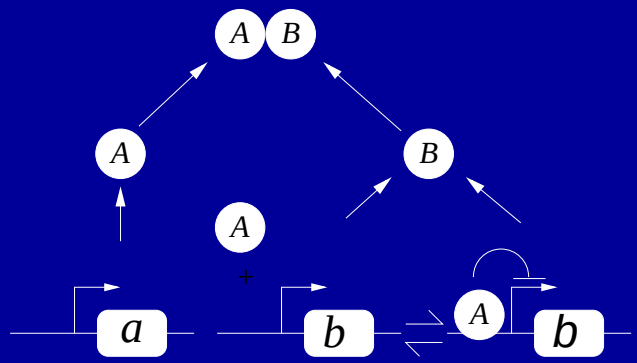


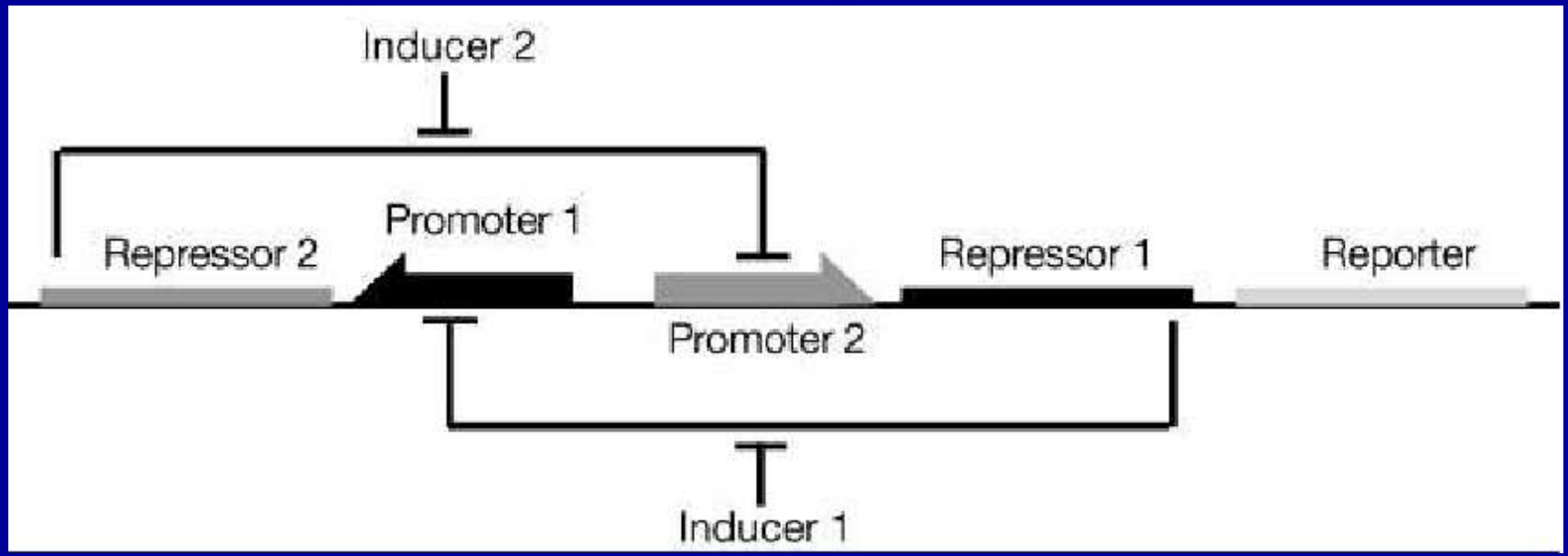
Les Houches, april 07.

Genetic networks.

- Dynamics in a cell: bistability, oscillations (circadian , ...)
- Spatial patterns (C. elegans, somites,...)
- Coordinated evolution of several genes/proteins.
- Design of synthetic modules.

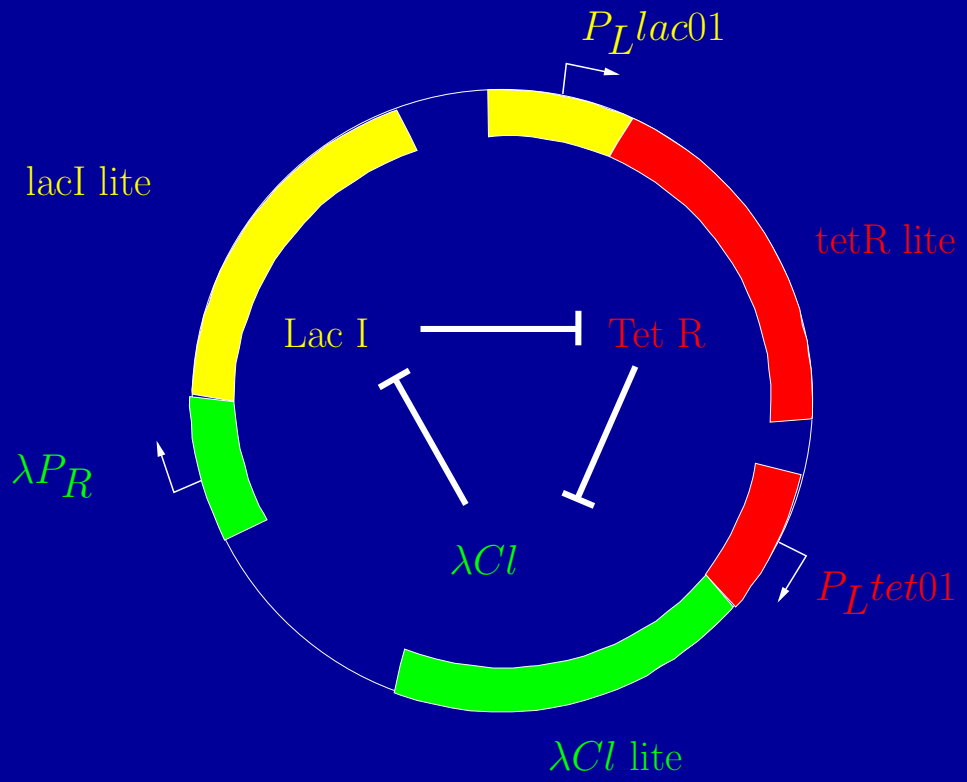
A synthetic genetic switch

Two genes *a* and *b* that inhibit each other. Two stable steady states : [A] high with [B] low, and [B] high with [A] low.



Switching can be induced by an IPTG or a temperature pulse.
Gardner et al, *Nature* 403:339-342 (2000)
Bistability requires dimerizations (or other interactions).

A synthetic genetic ring oscillator



The oscillation is based on three genes that repress each other in a circle ("rock-scissor-paper").

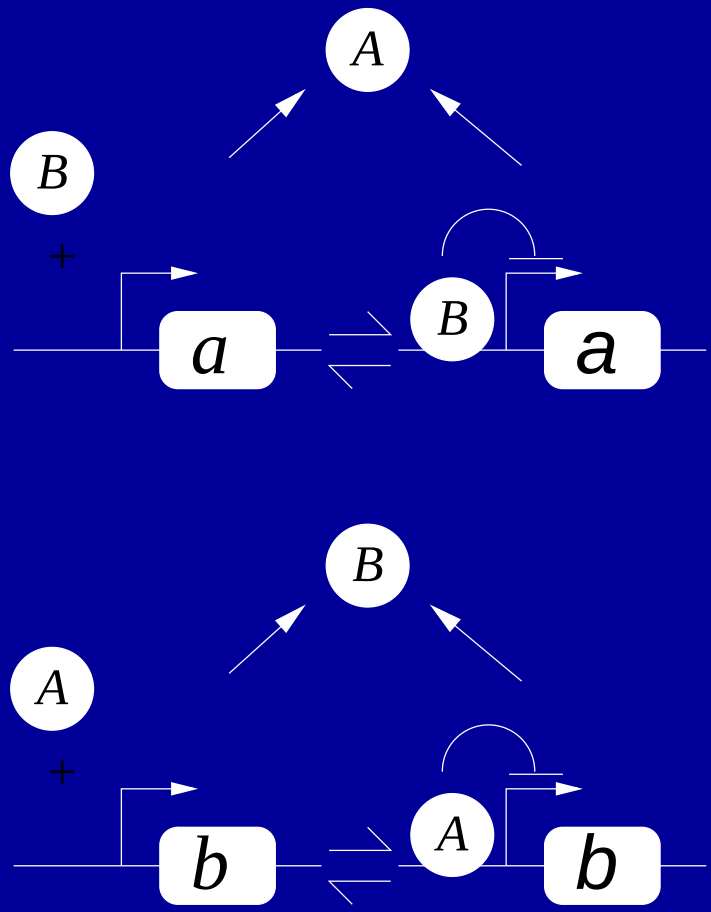
M. Elowitz and S. Leibler, *Nature* 403:335-338 (2000)

'Toggle' switch : mathematical analysis

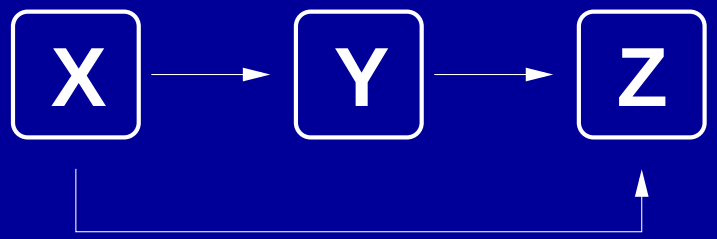
$$\frac{\mathrm{d}A}{\mathrm{d}t} = \frac{\alpha}{B_0 + B^\nu} - \delta_A A$$

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \frac{\beta}{A_0 + A^\mu} - \delta_B B$$

ν μ must be strictly higher than 1 to have bistability, which requires at least *four* (and not two) elementary reactions. [Cherry and Adler, J. Theor. Biol. (2000)]



An overrepresented motif in transcriptional networks



The "feedforward loop" is overrepresented in the transcriptional networks of *E. Coli* and *S. Cerevisiae*
(Milo et al., *Science* 298: 824-827(2002)).

Function: a persistence detector?

- What are the designs that achieve a given function?
- Can one sample them and add desired constraints (robustness,...) ?
- Easyness of creation, evolvability,...?
- Blueprints of useful networks.

Design by evolution/selection *in silico.*

The inverse of the statistical approach: from the desired task to the network.

To design modules performing given tasks (e.g. switches and oscillators), without imposing *a priori* any structure to the network, one evolves a collection of virtual "cells".

P. François and V. Hakim, *PNAS*, **101** 580-585 (2004).

One computer 'cell' consists in

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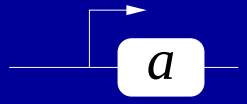
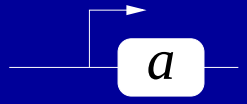
- a collection of genes 

- and associated proteins 

First implementation: transcription and translation condensed in one single step.

mRNA are included in the present version.

One computer 'cell' consists in

- a collection of genes 

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First implementation: transcription and translation condensed in one single step.

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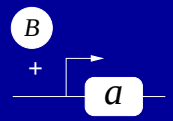
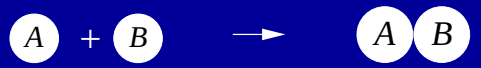
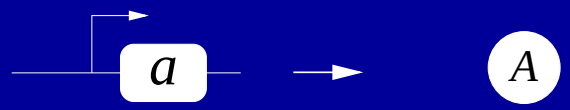
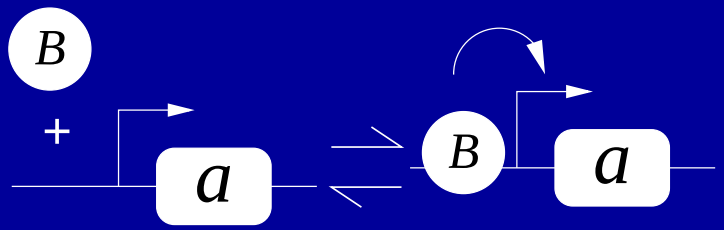
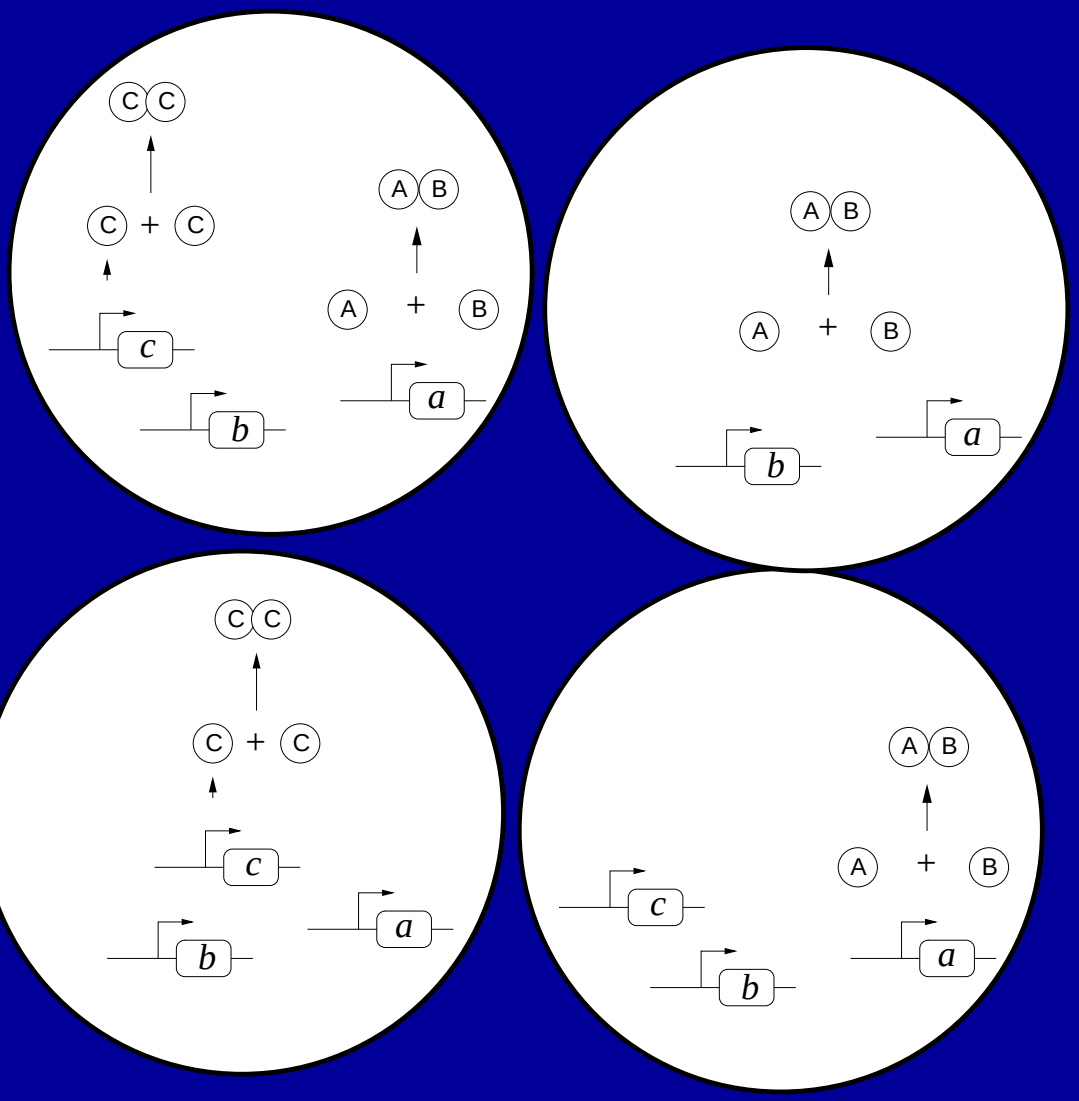
- transcriptional regulations 

- post-transcriptional regulations. 

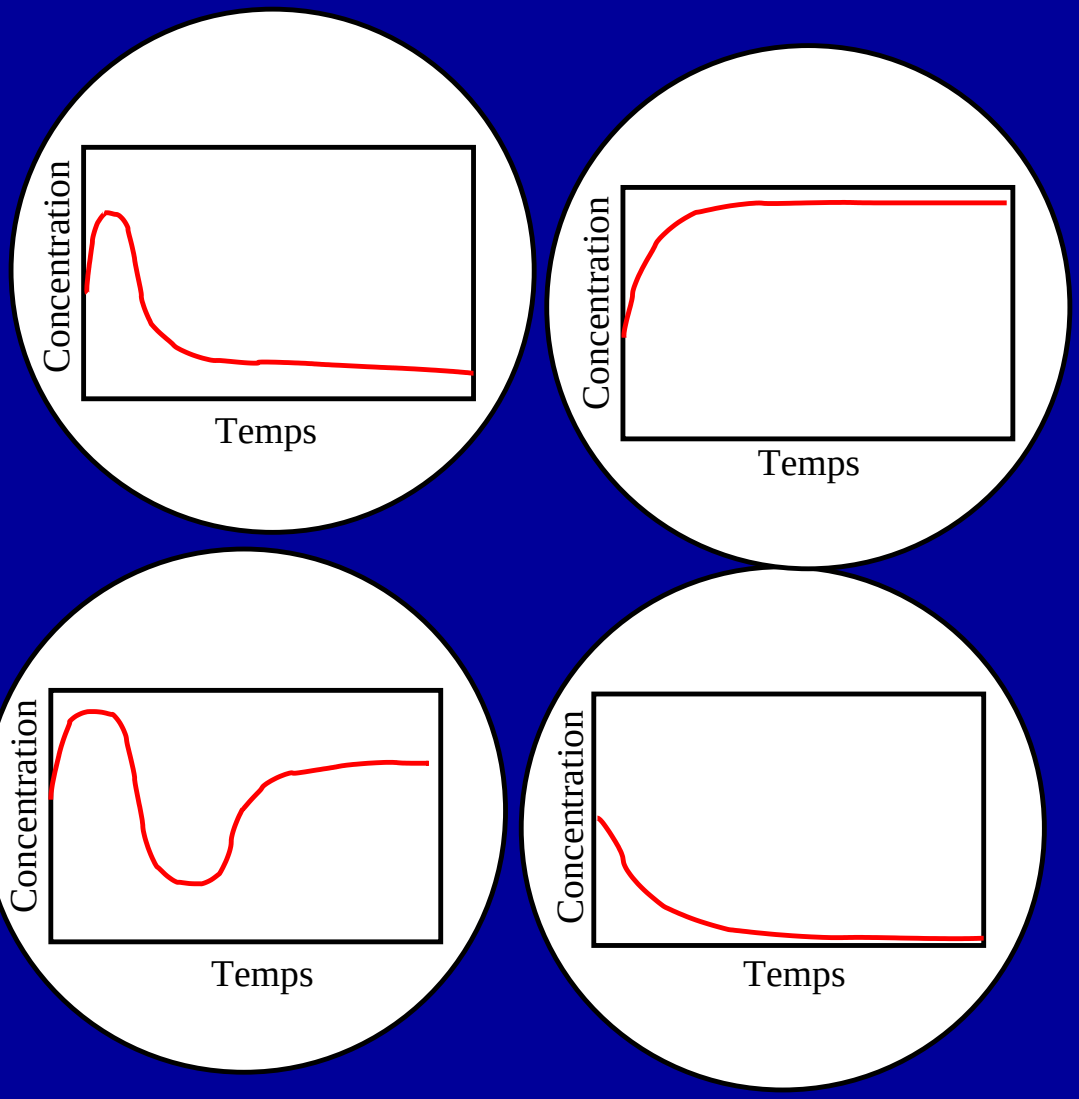
Representation	Corresponding equations
	$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{A}] = \tau_A[a] - \delta_A[\mathrm{A}]$
	$\frac{\mathrm{d}}{\mathrm{d}t}[a] = \theta[a:\mathrm{B}] - \gamma[a][\mathrm{B}]$ $\frac{\mathrm{d}}{\mathrm{d}t}[a:\mathrm{B}] = \gamma[a][\mathrm{B}] - \theta[a:\mathrm{B}]$ $\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{A}] = \tau_A[a] + \tau'_A[a:\mathrm{B}]$

The evolution *in silico*.



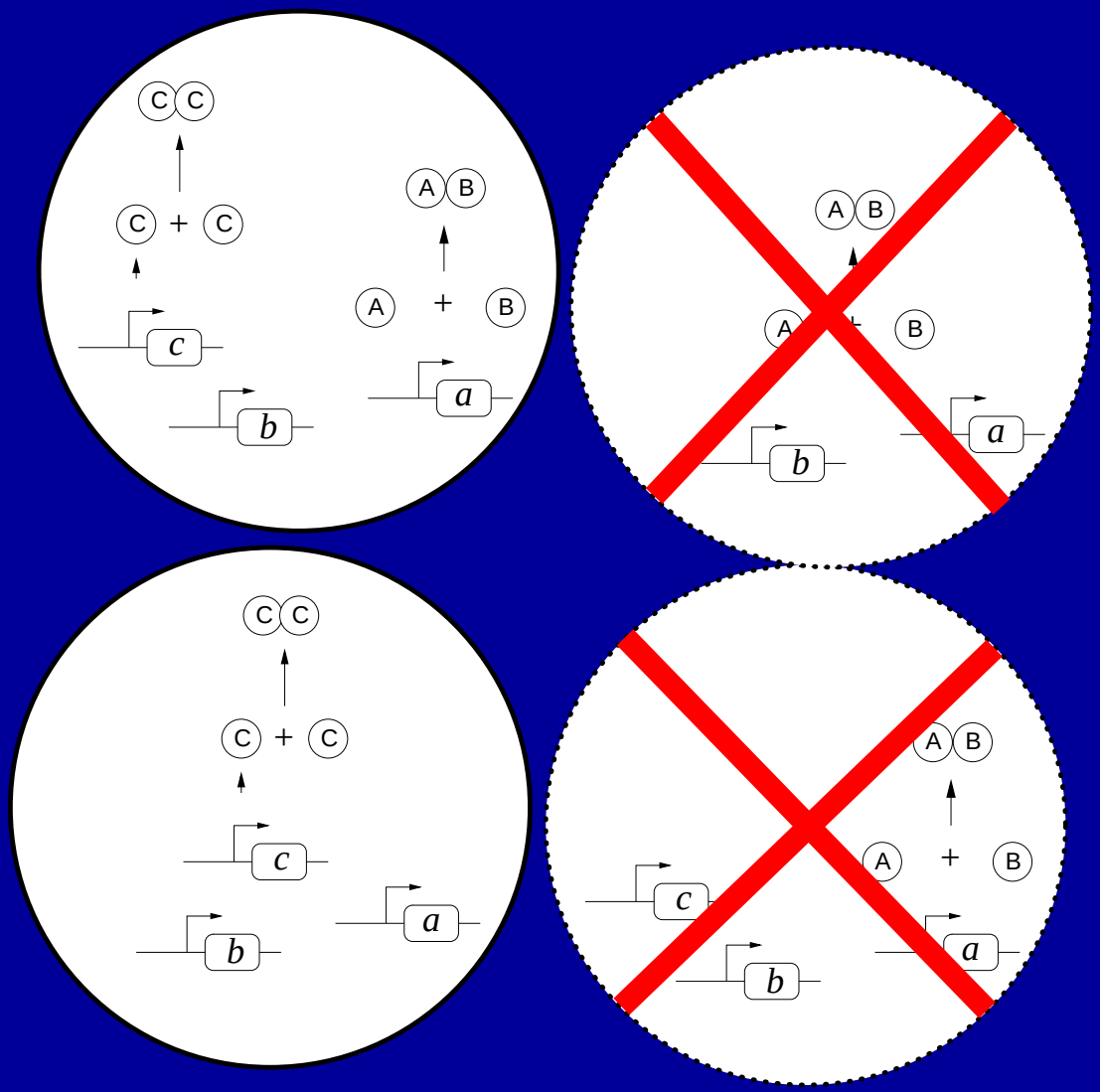
Cells

The evolution *in silico*.



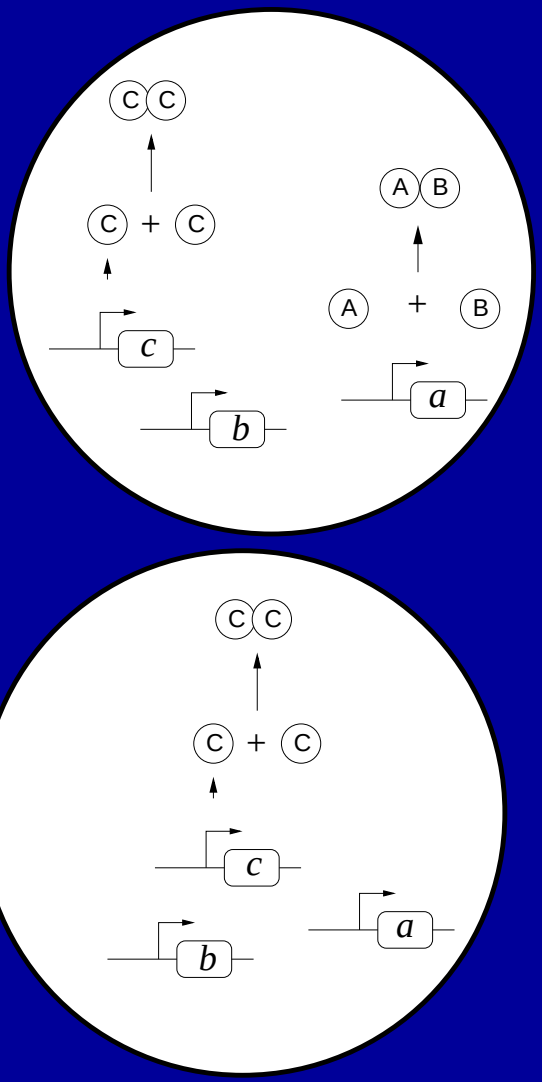
Integration of ODEs

The evolution *in silico*.



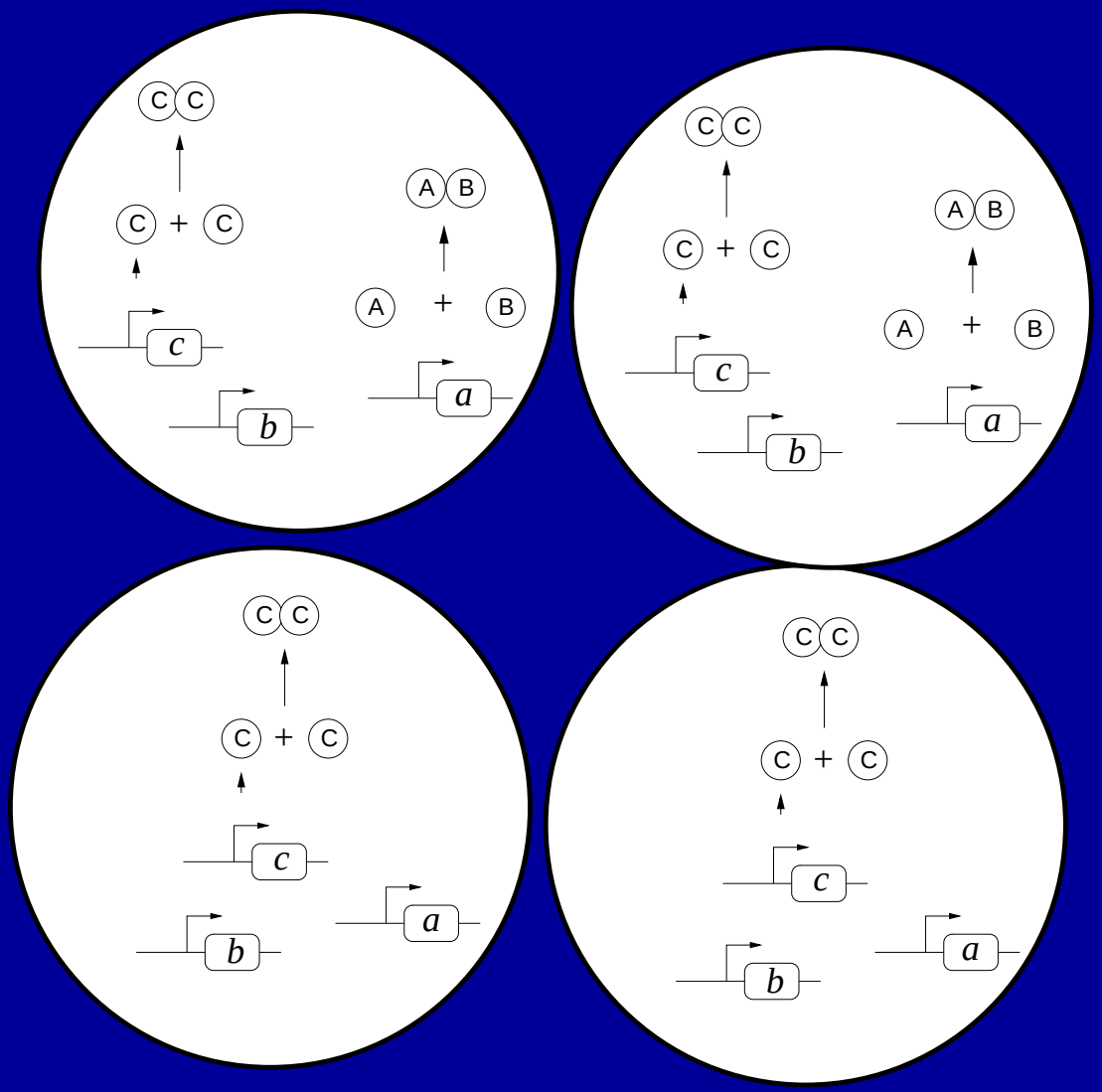
Selection

The evolution *in silico*.



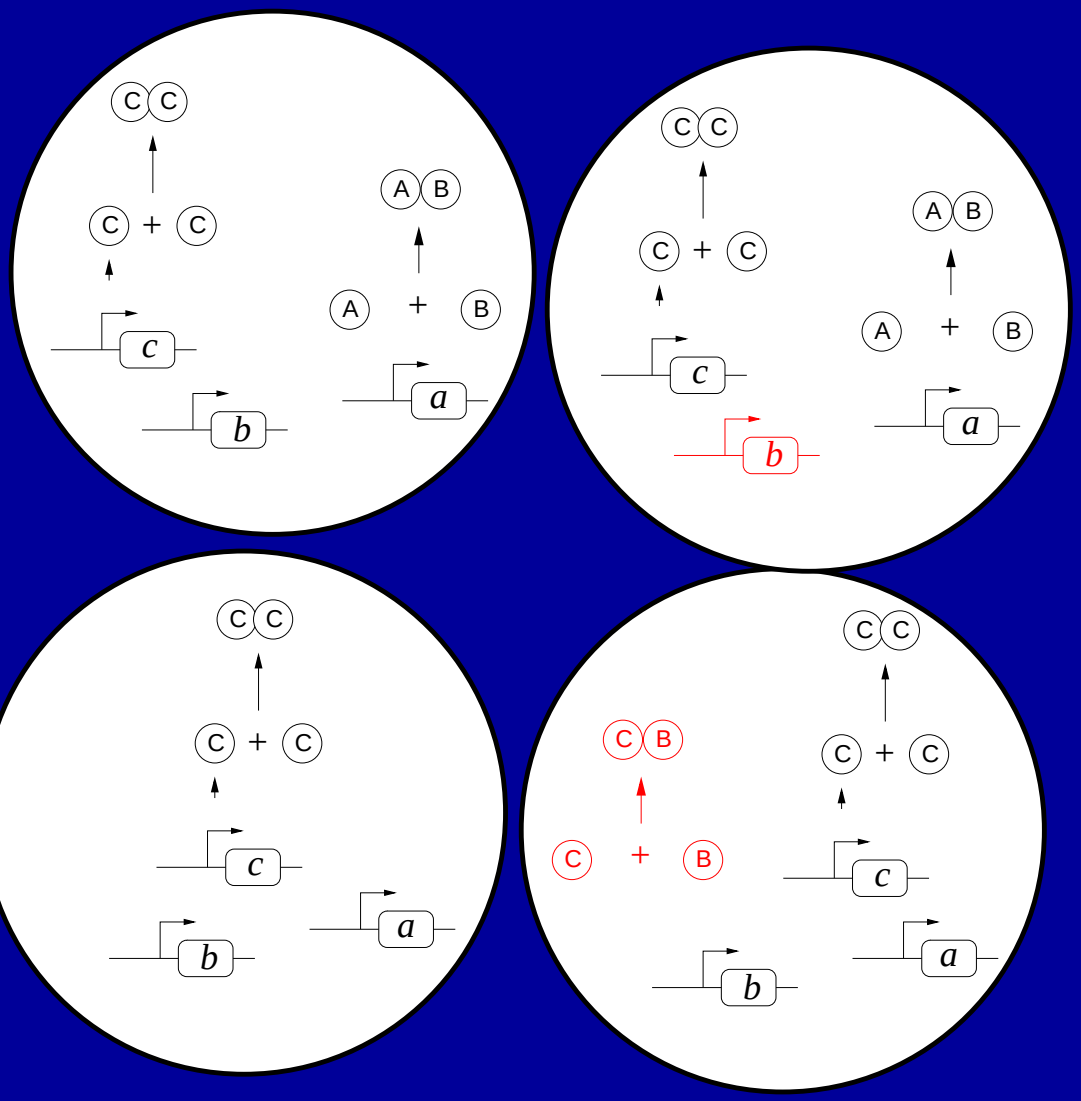
Elimination

The evolution *in silico*.



Duplication

The evolution *in silico*.



Mutations

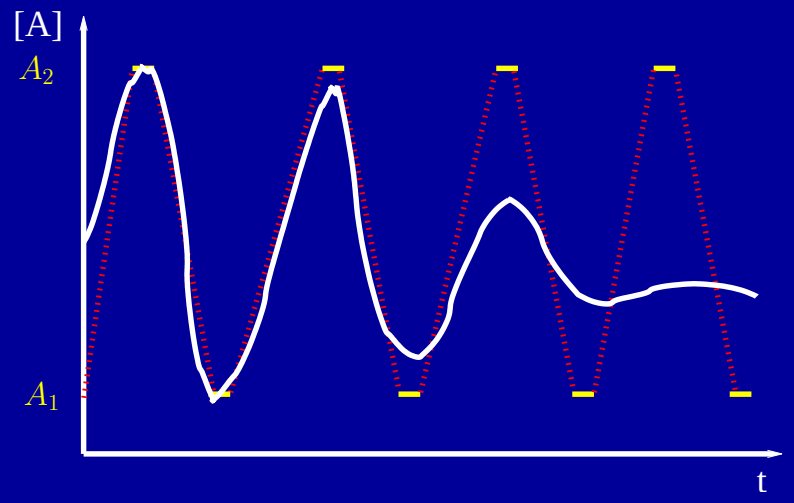
Possible mutations

- The modification of a kinetic constant in an existing reaction

  or the addition of
- A new transcriptional regulation
- A new post-transcriptional regulation
- A new gene

The process is iterated over several generations.

Fitness function for oscillators



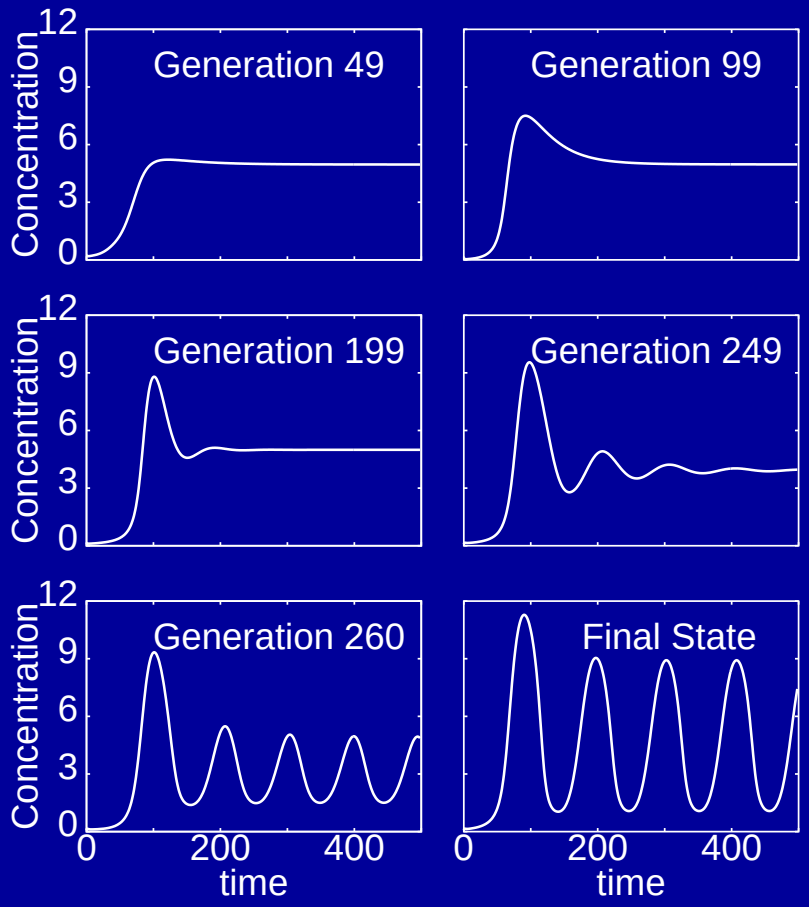
Two concentrations are fixed A_1 and A_2.

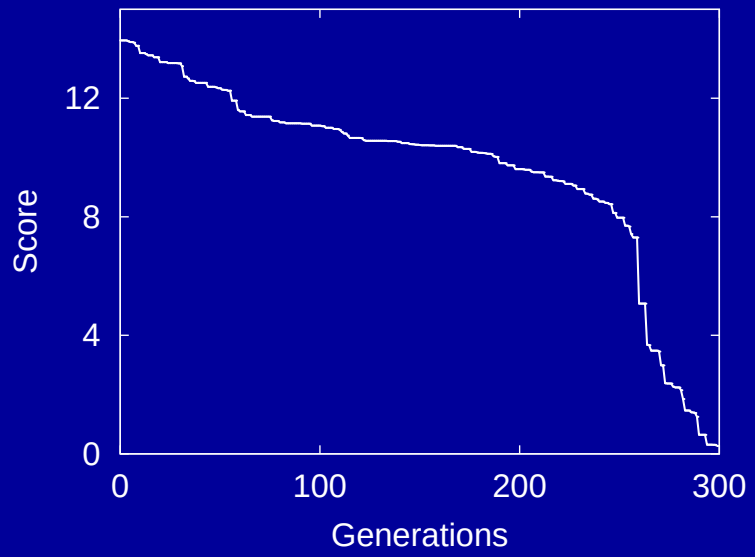
ODEs are integrated

For $t = T/2, 3T/2, 5T/2...$ fitness is given by the integral $(A - A_1)^2$.

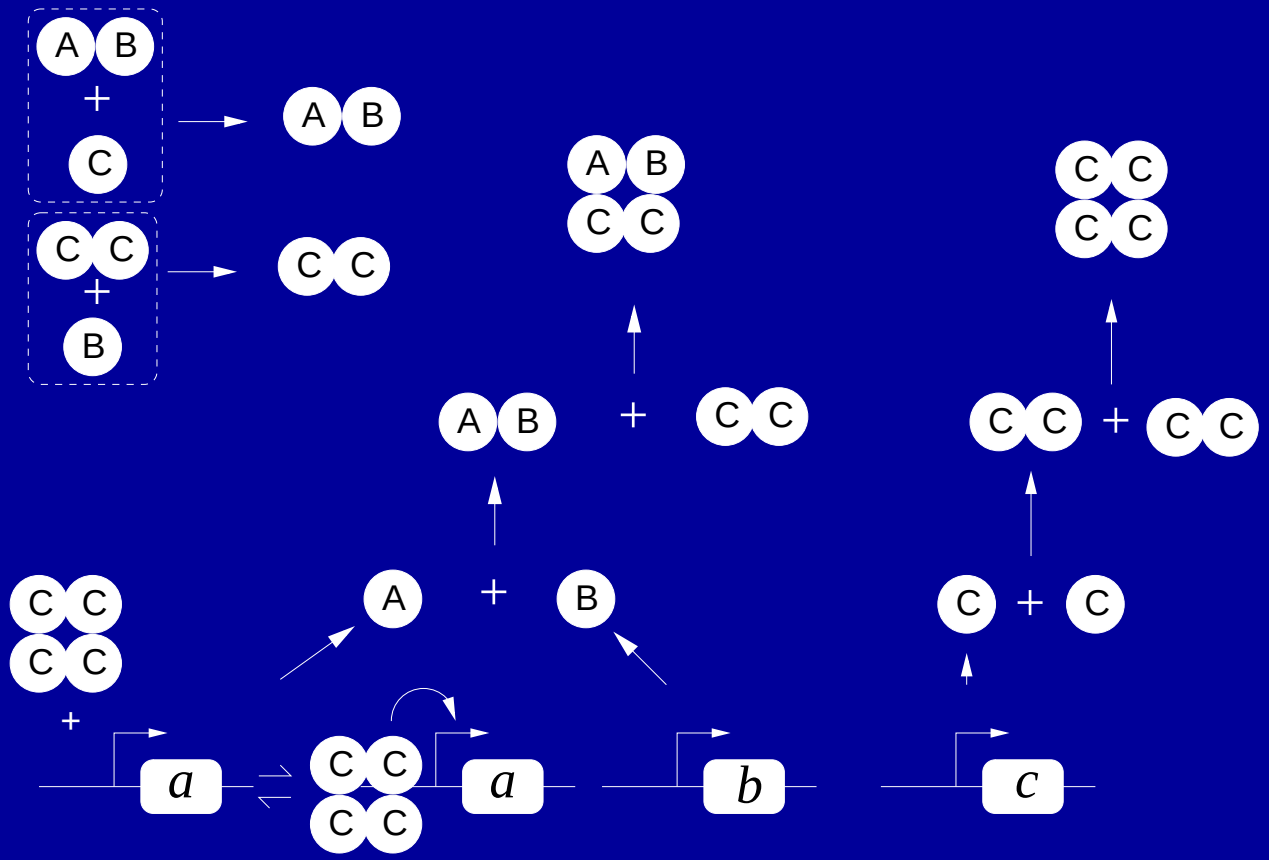
For $t = T, 2T, 3T...$ fitness is given by the integral $(A - A_2)^2$.

Fitness evolution

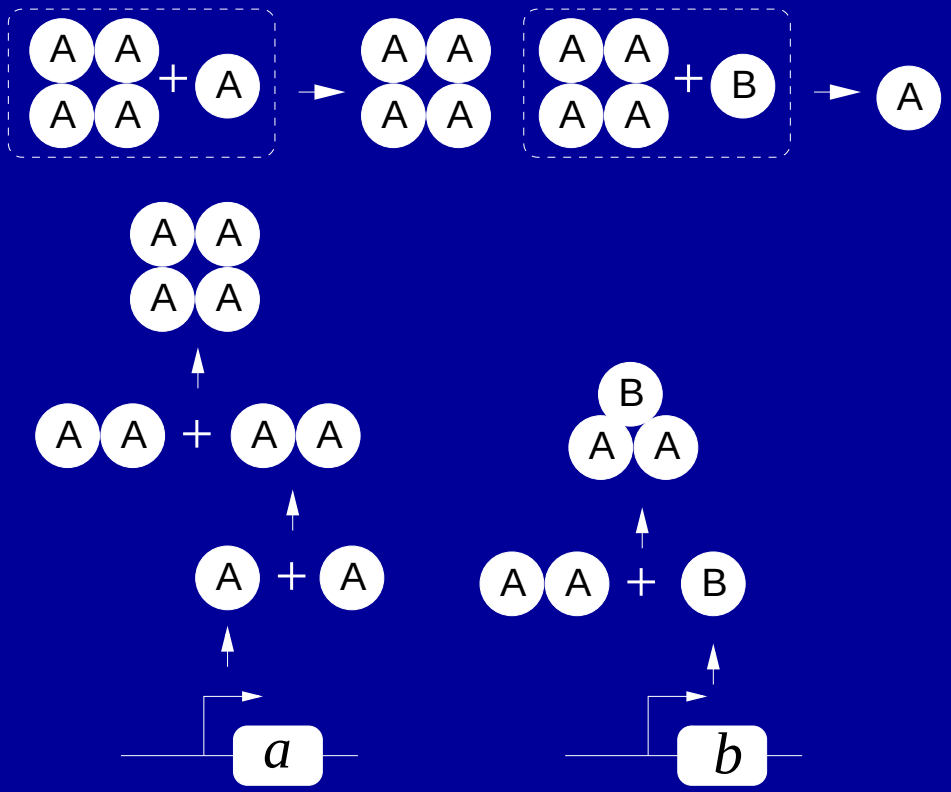




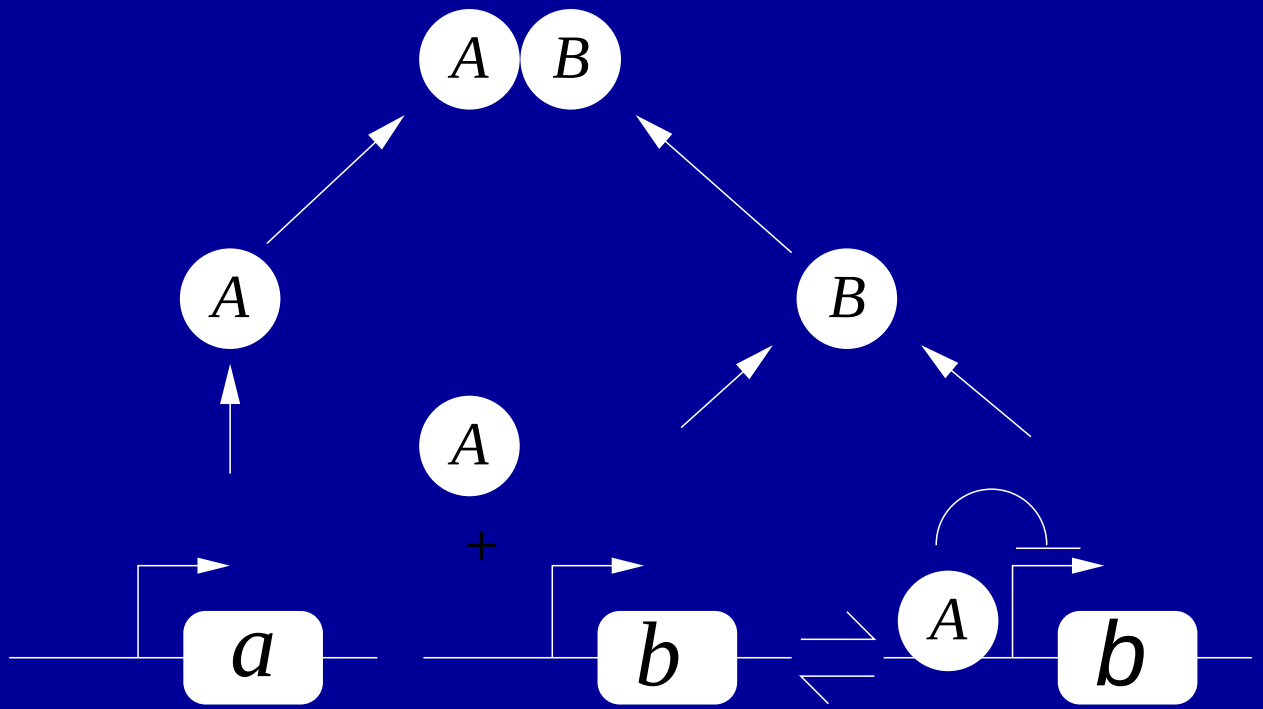
The oscillating network



A purely biochemical oscillator

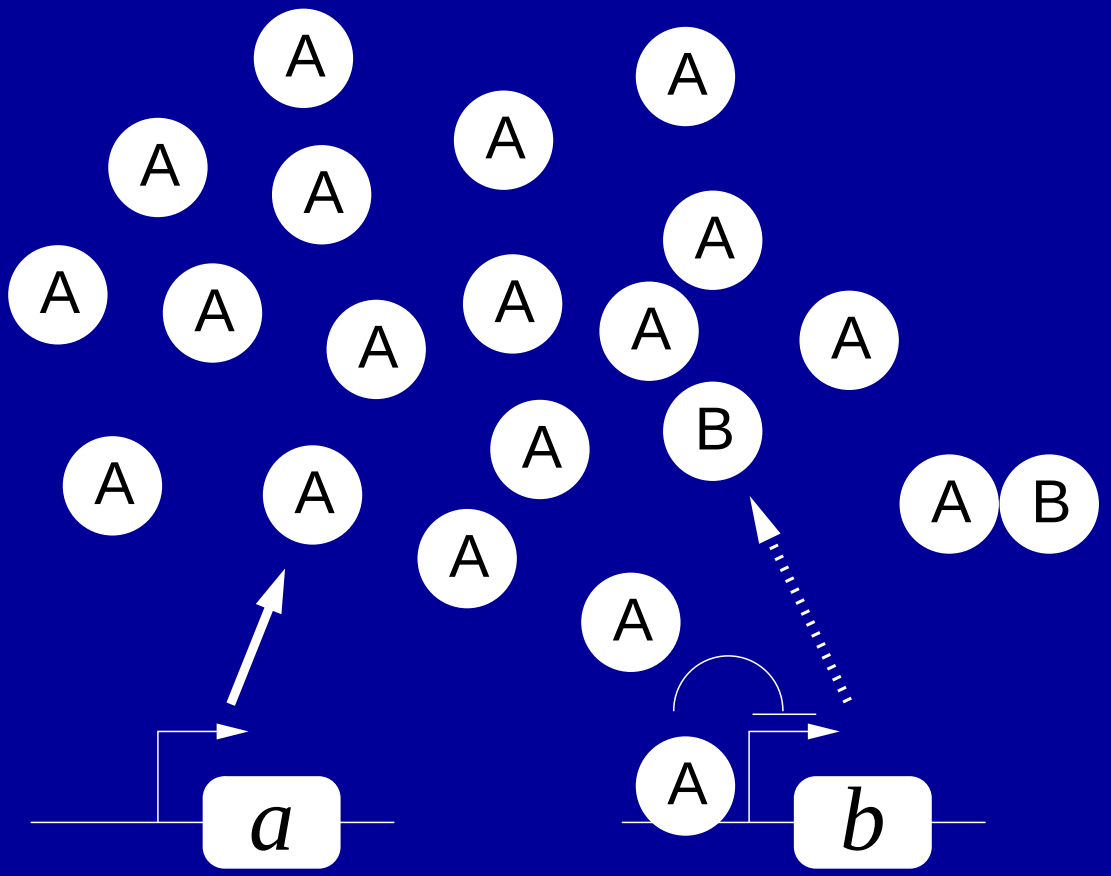


A created bistable switch



Very different from two genes with reciprocal inhibition

A created bistable switch



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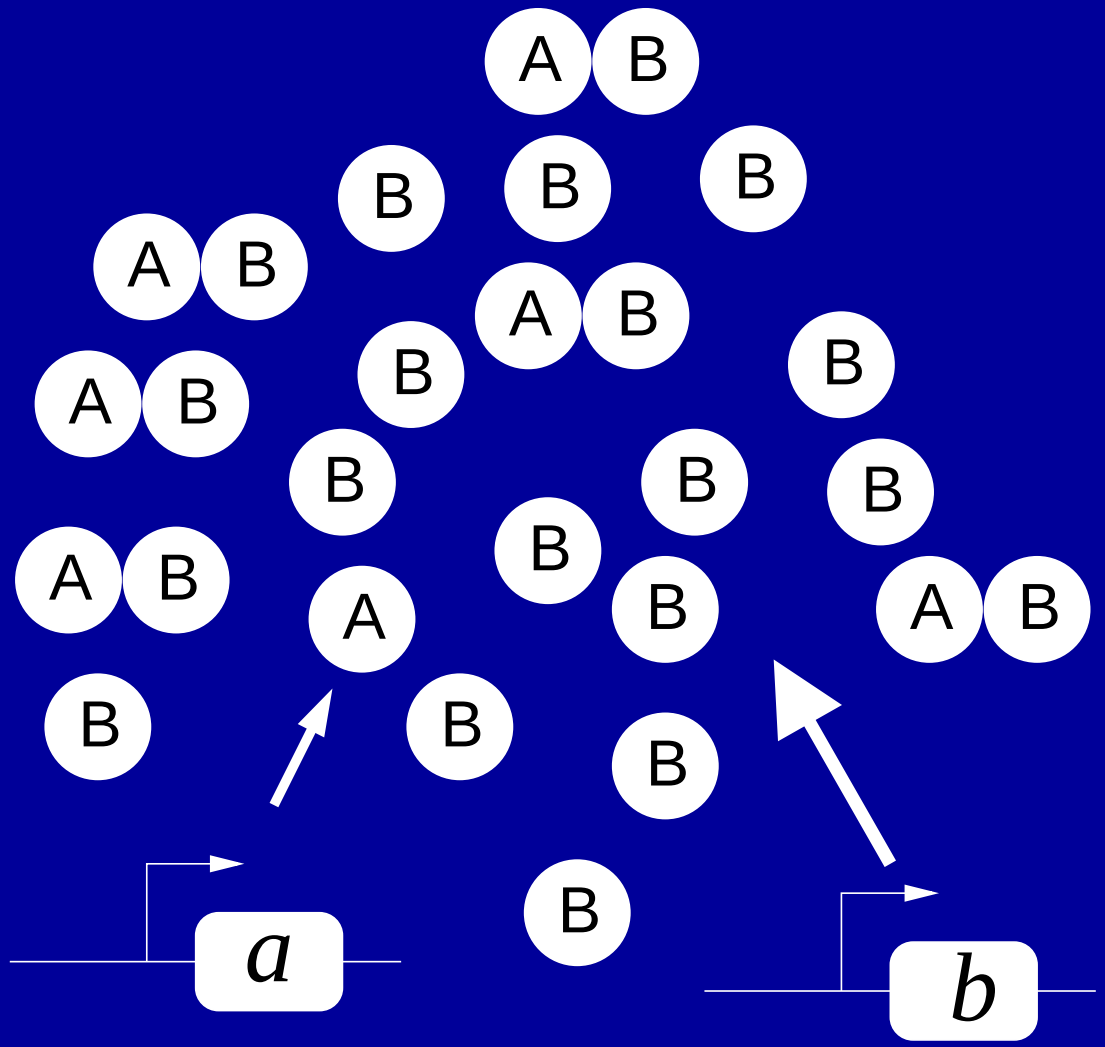
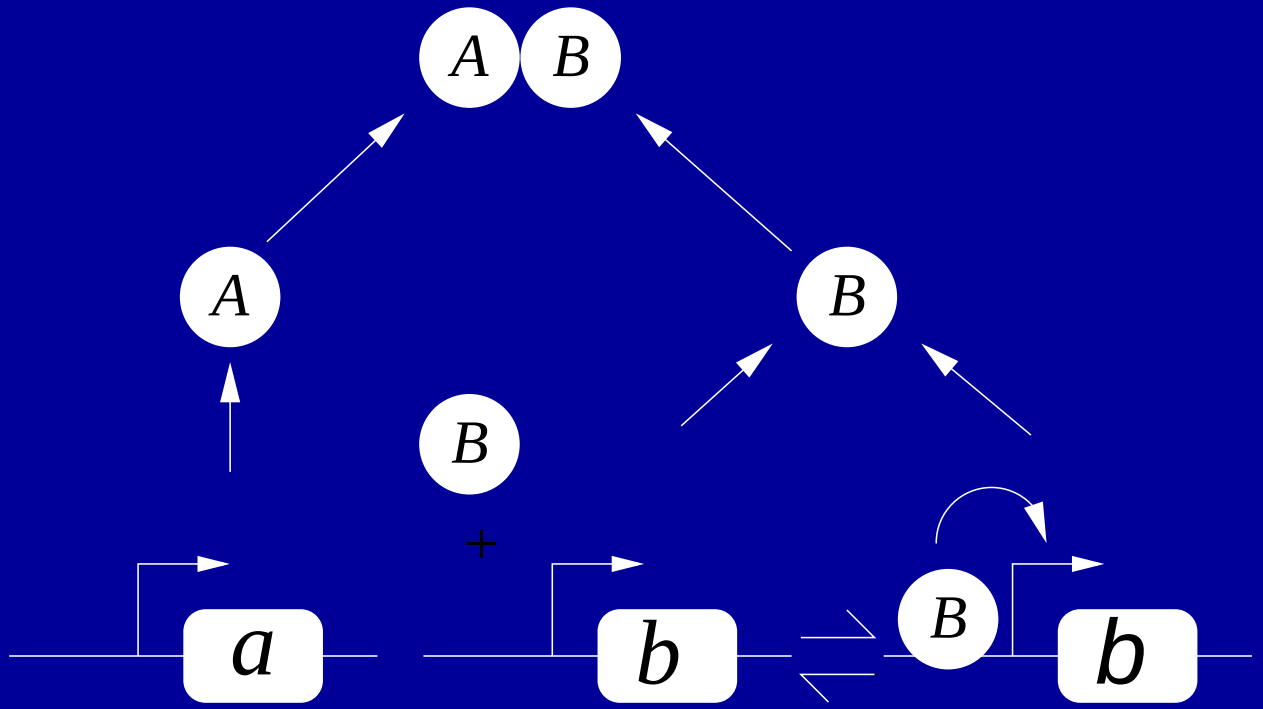


A second type of switch



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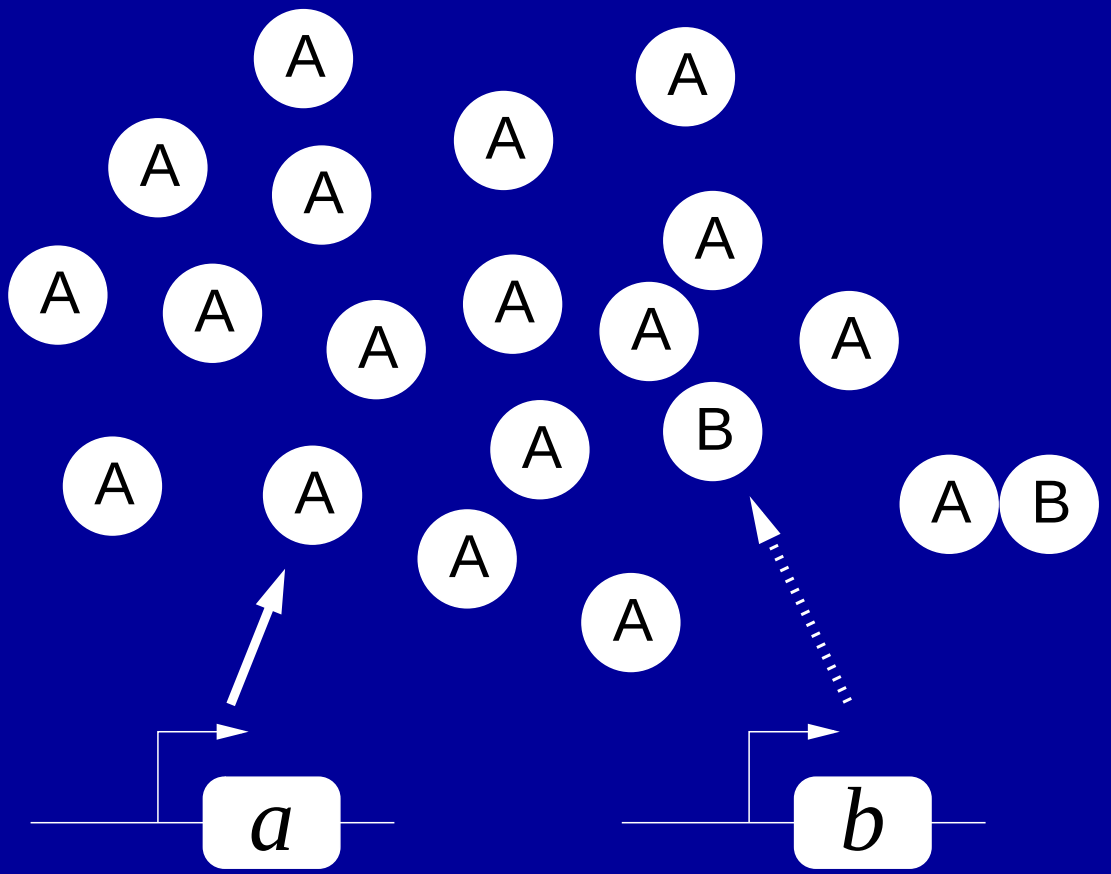
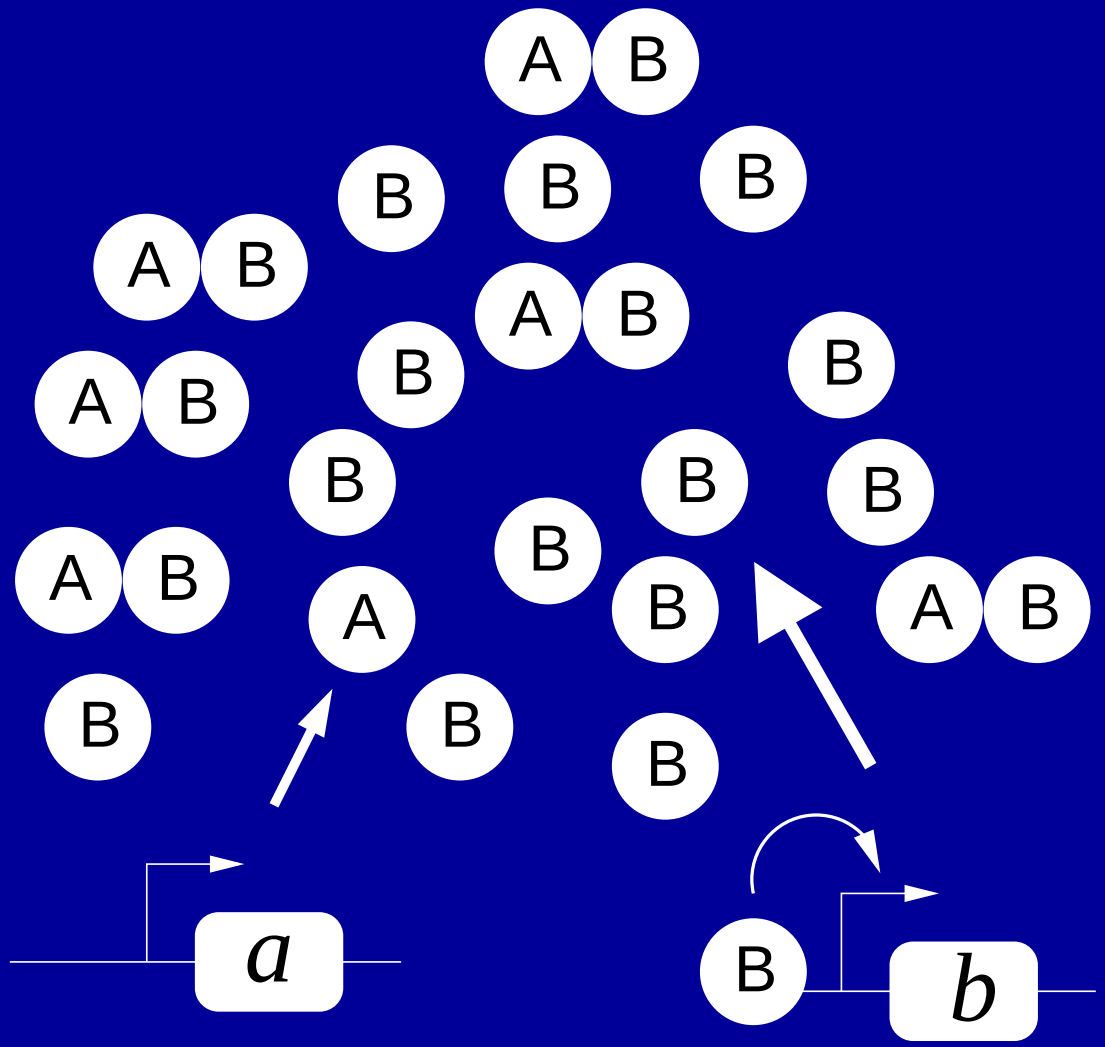
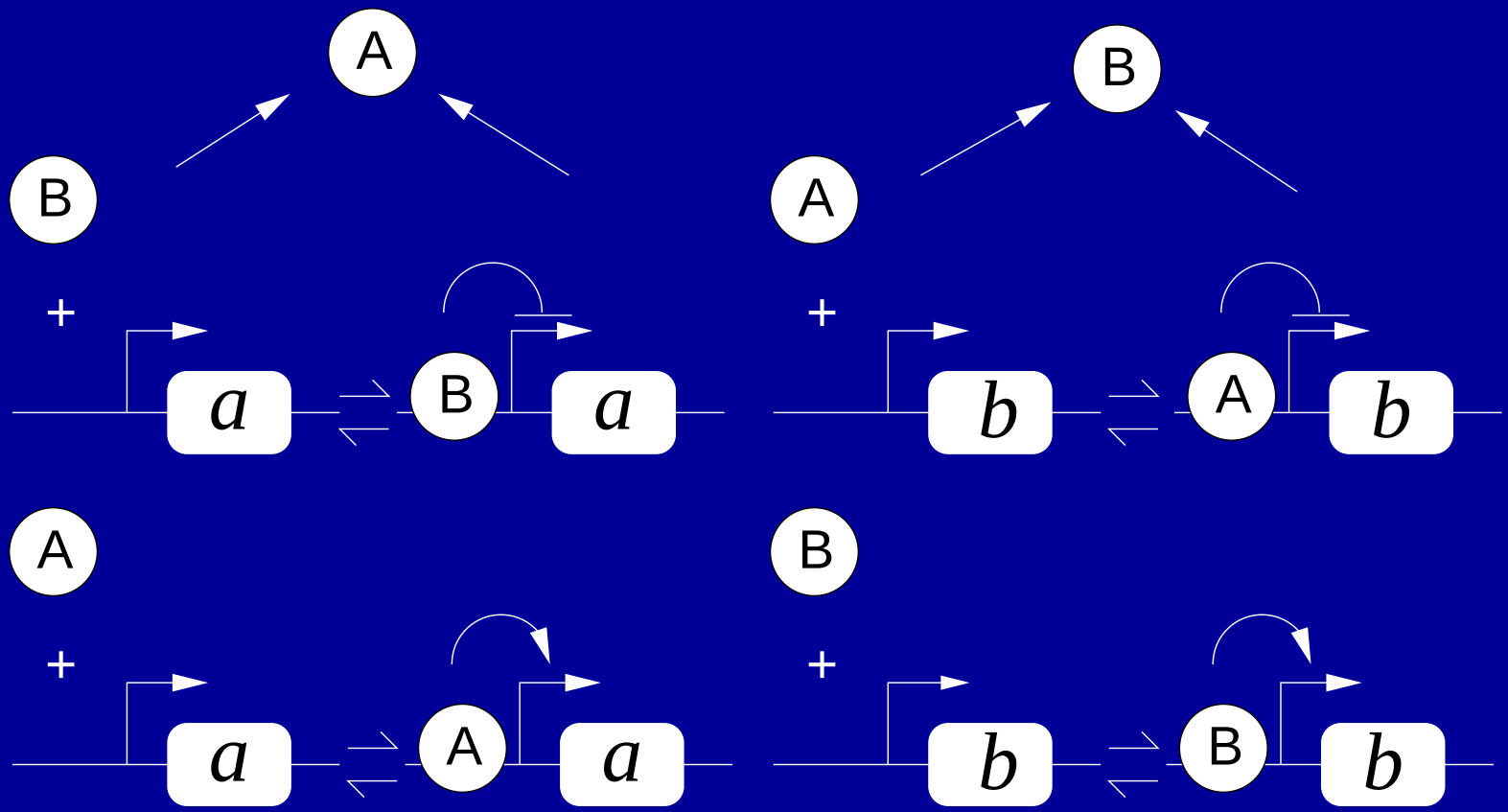


A second type of switch

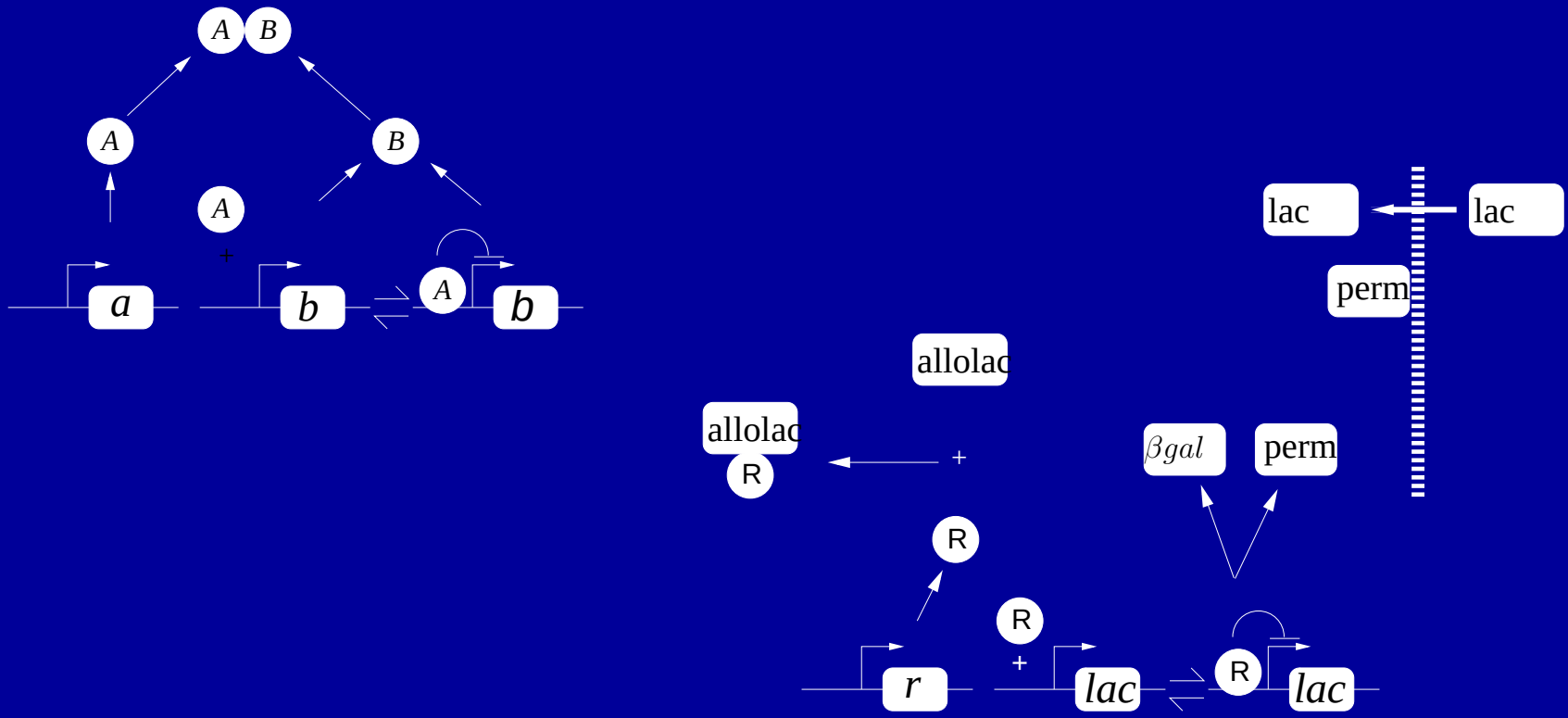


Transcriptional switches



Comparison with real networks

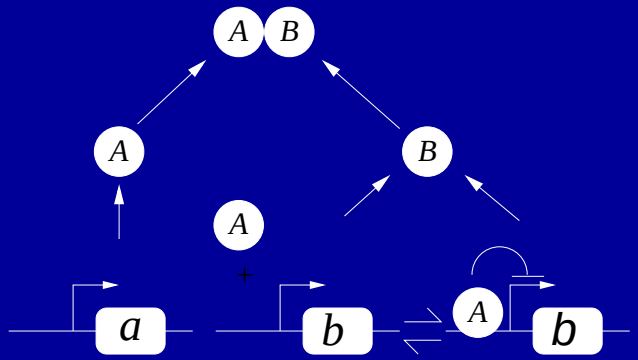
First switch: lactose operon, with allolactose binding to lac repressor.



Proposed in 1961 by Monod and Jacob (based on Lac operon) as an alternative to reciprocal inhibition (Delbrück, 1949) !

Comparison with real networks

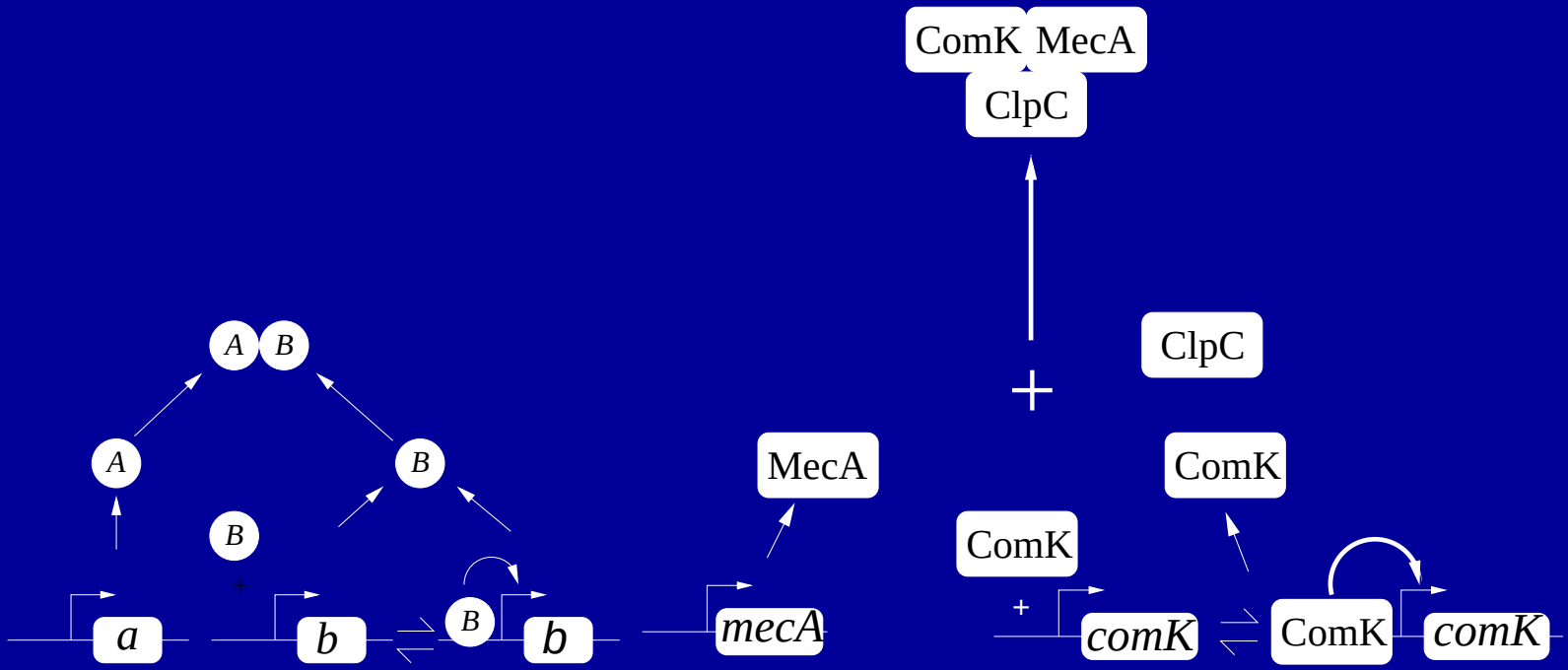
First switch: lysis/lysogeny switch in a "simple" phage



Lactococcal phage TPS901-1 (K. Hammer et al J. Virology (2003) and unpublished) A: CI repressor, B: Mor
CI transcriptionally represses Mor, Mor lifts this repression by binding to CI and preventing CI DNA binding.
An ingenious refinement: CI represses itself so as to avoid continuous production of repressor. This works because CI is a very stable protein and also because self-repression of CI is much weaker than CI repression of Mor.

Comparison with real networks

Second switch: developement of competence in *B.subtilis* , Comk activates itself and is repressed by MecA.

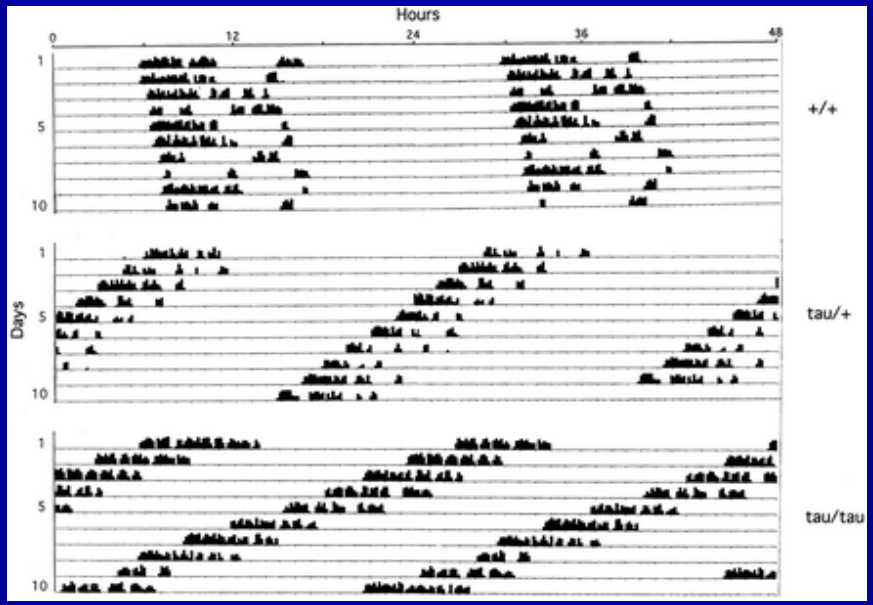


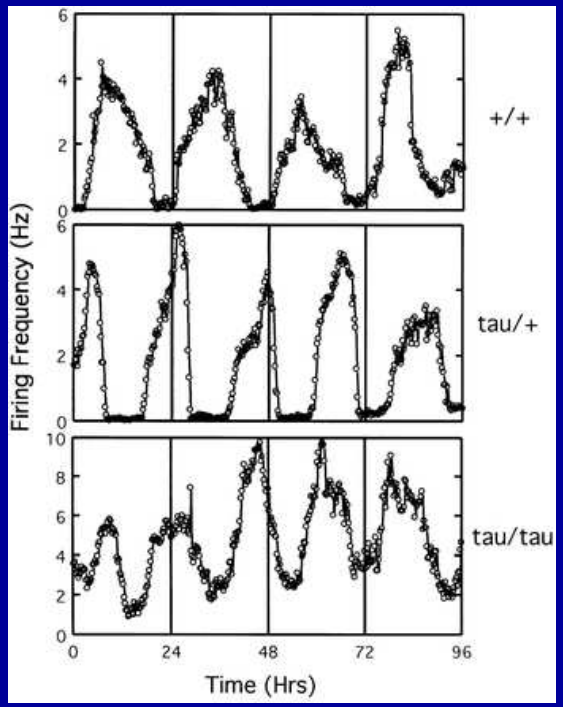
Smits et al (2005), Maamar and Dubnau (2005): experimental demonstration that this stripped network is indeed a switch!

Endogeneous oscillator : the circadian clock

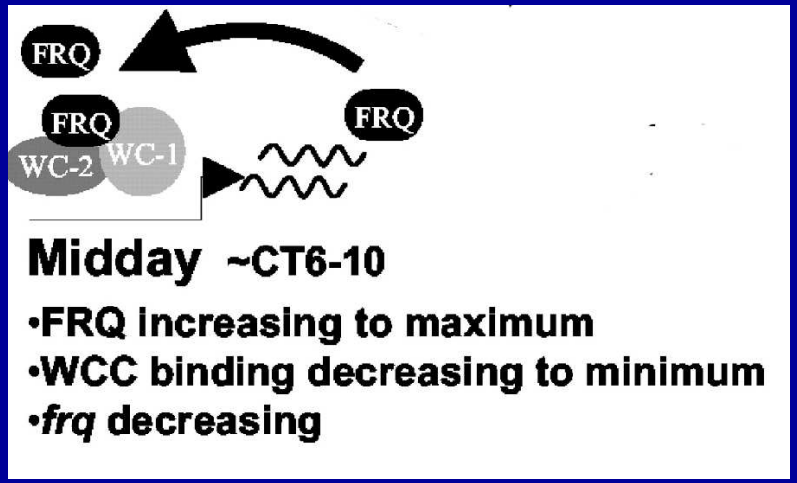
Circadian activities of whole animals and single cells

Liu et al, Cell (1997)





The core structure of circadian clocks



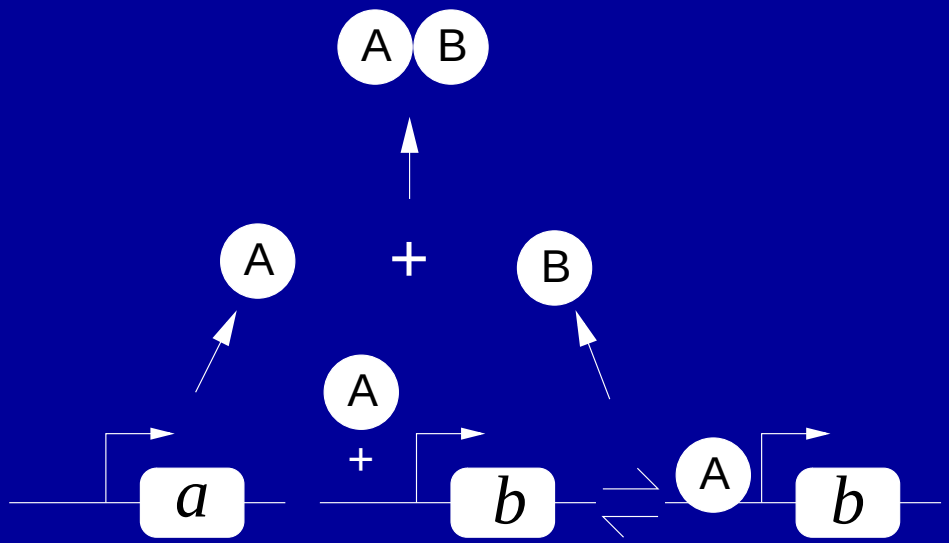
Froehlich et al, PNAS (2003)

Organism	Activators A	Repressors B
Neurospora Crassa	WC-1, WC-2	FRQ
Drosophila	dCLK	PER, TIM
Mammals	CLOCK, BMAL	PER, CRY

The created networks are working examples without delays or high Hill coefficients $\Rightarrow$ motivation for **new models** of the circadian rhythms [for *Neurospora*, P. François Biophys. J. **88**, 2369 (2005)].

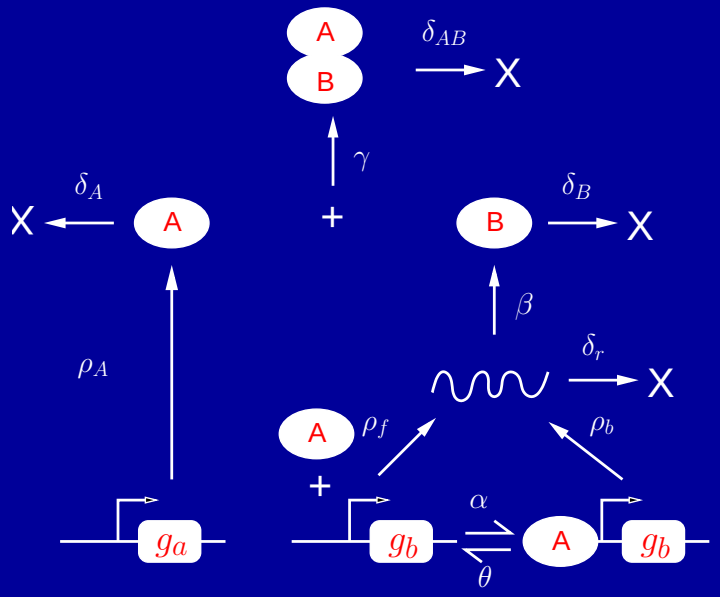
A core genetic circuit: the Mixed Feedback Loop

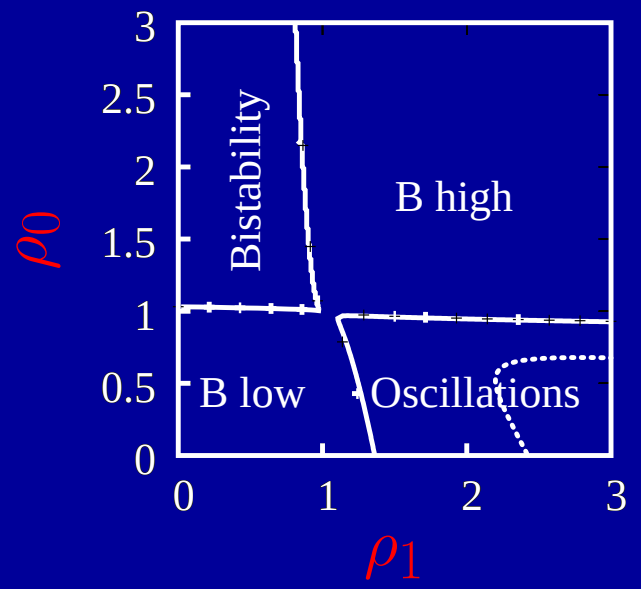
A loop combining transcriptional and post-transcriptional interaction (i.e. protein-protein interaction) is **at the core** of several of these networks.



This **Mixed Feedback Loop** has now been found to be **over-represented** in S. Cerevisiae and E.Coli (Yeger-Lotem et al, PNAS 2004).

Mathematical analysis of the MFL





Reduced parameters: $\rho_0 = \beta\rho_f/(\rho_A\delta_r), \rho_1 = \beta\rho_b/(\rho_A\delta_r)$

A small parameter: $\delta_r/\sqrt{\rho_A\gamma}$

(P. François and V. Hakim, PRE (2005)

The algorithm finds known (with complete description) and original designs.

An important lesson: The post-transcriptional interactions play a crucial role: the function of the networks cannot be understood at all by focusing only on the transcriptional regulations (protein sequestration in a complex appears to be a particularly important mechanism).

From single cells to multicellular pattern formation

- Can one evolve more complicated structures?
- Spatial patterns, morphogenesis?
- A test case: segmentation

Segmentation in a static gradient

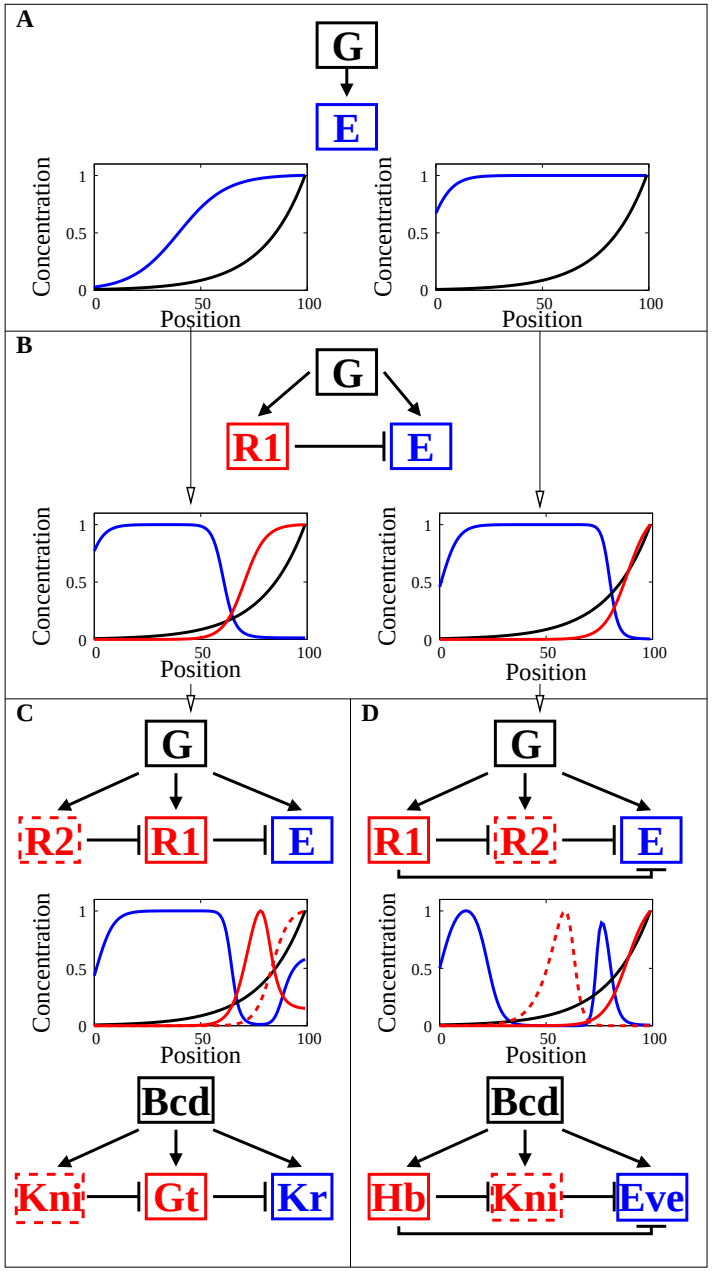
- Drosophila early embryogenesis is very well studied (Thieffry and Perkins's lectures!): a nice bench mark.

- Evolution of a collection of "organisms" (hundred cell each).

- Fitness: maximise the number of "segments": the number of jumps in the concentration of a test protein.

- Only transcriptional interactions for numerical tractability.

Evolution of segmentation in a static gradient



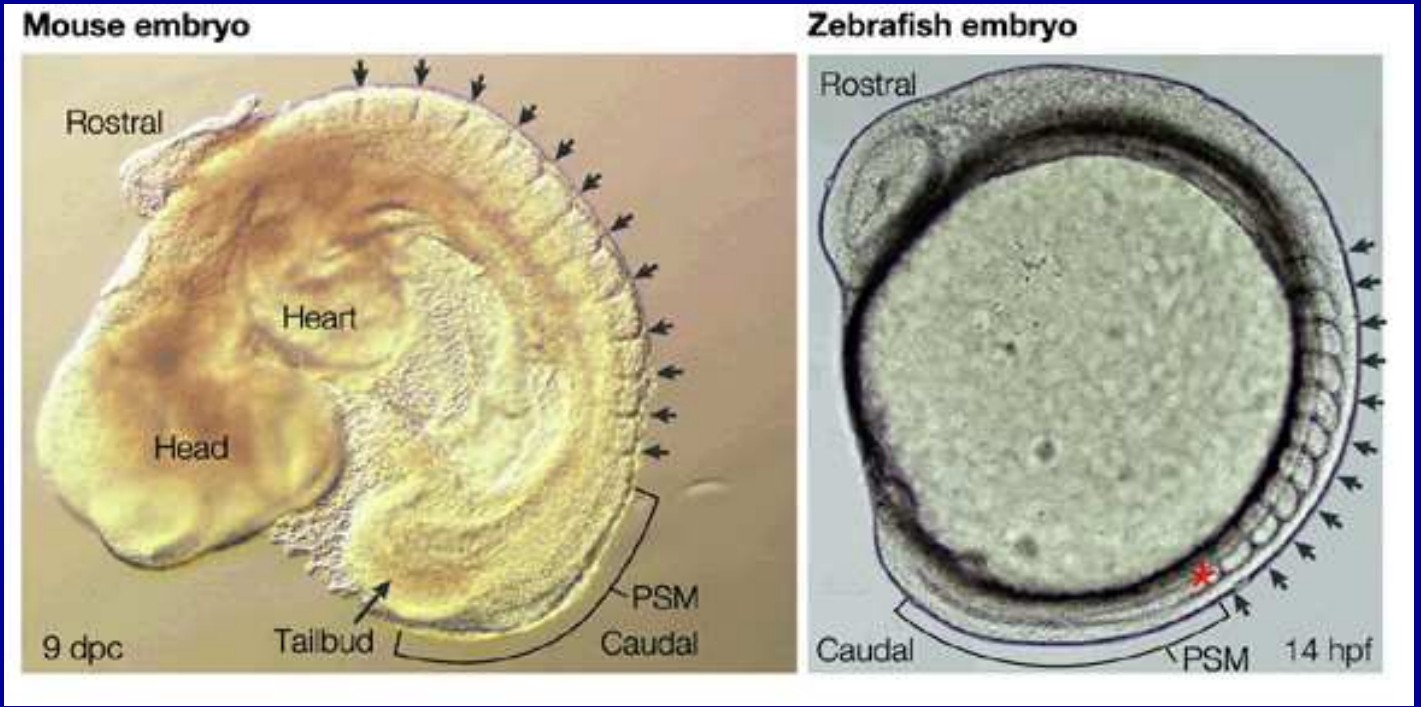
Evolution of segmentation in a static gradient

- Cascades of repressors reminiscent of Drosophila segmentation network.

- Feedforward loop that gives a general way to express a gene at an intermediate gradient concentration
Real example: Dorsal activates rhomboid and less efficiently snail. Snail represses rhomboid. Rhomboid expressed at intermediate Dorsal concentration (neurogenic ectoderm)

- Reasonable results. What does one find for a dynamic gradient (short germ insects, somites,...)?

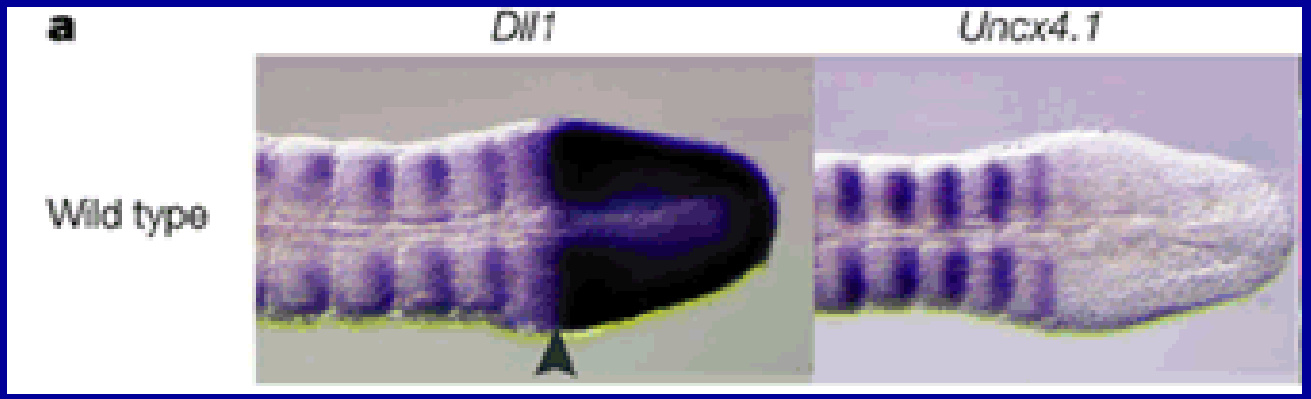
Segmentation in vertebrates: somite formation

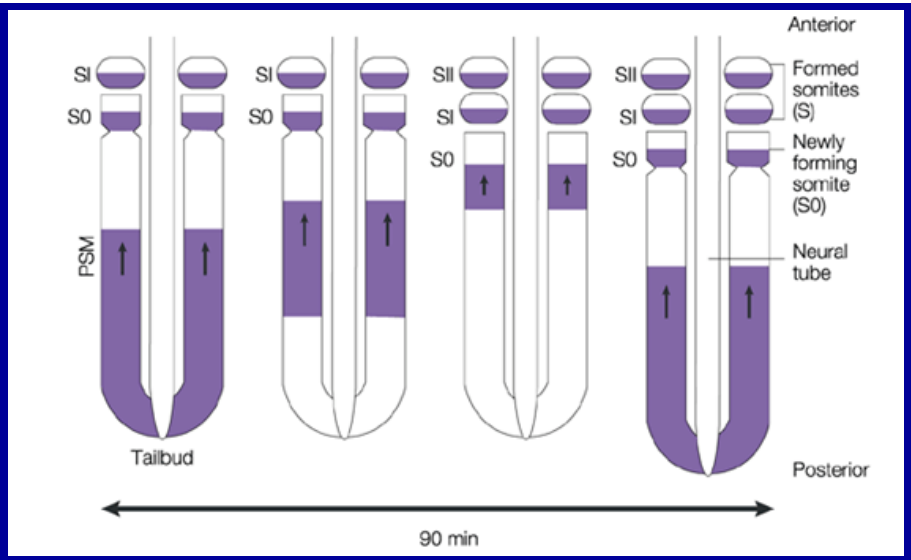


Y. Saga, Nat. Rev. Gen. (2001)

Segmentation and oscillations

(Cooke & Zeeman (1976) $\rightarrow$ Palmeirim et al (1997))





Y. Saga, Nat. Rev. Gen. (2001)

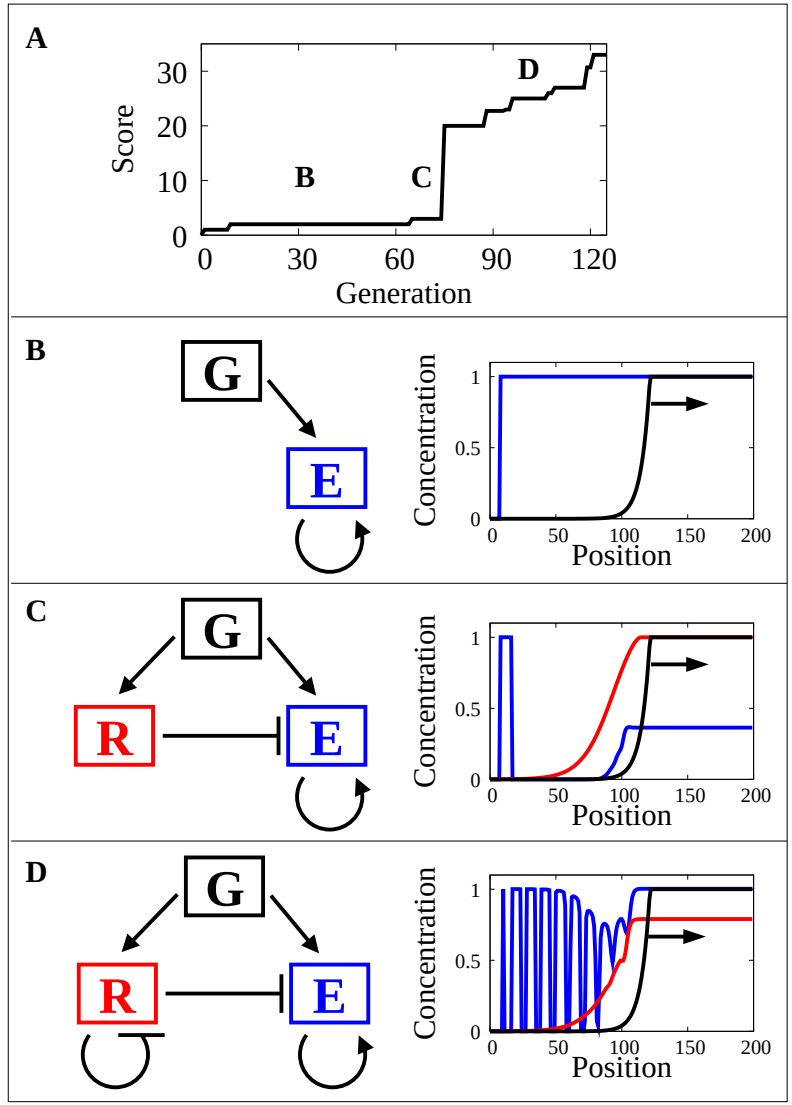
Evolution of segmentation in a temporal gradient

- Dynamics as the gradient sweeps across the array.

- Score: numbers of jumps in a concentration of a protein after the gradient has disappeared.

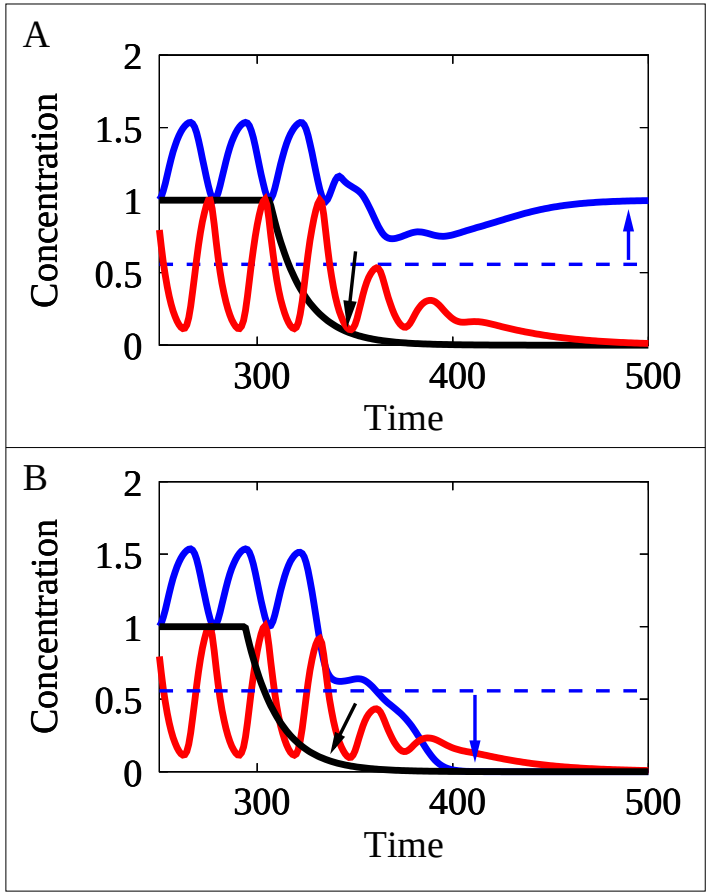
- Again, only transcriptional interactions for numerical tractability (delays to account for intermediate steps).

Evolution of segmentation in a temporal gradient



Segmentation as an oscillating/bistable transition?

Oscillations when external signal (FGF8?) is high, bistability when it is low. Bistability encodes the oscillation phase at the time of transition in a binary (and cell autonomous) way.



Some conclusions and perspectives

• A simple model of sequential segmentation. Hopefully, useful in different contexts (somites, short-germ insects, other segmented structures e.g. limbs ?,...)

• Very constrained evolutionary path *in silico*: first bistability to have persistent activation after disappearance of the gradient, then repressors added (for creation of high/low boundaries), finally negative feedback and oscillations. Real evolution?

• Early appearance of sequential segmentation? Multiple interconversion between the two modes of segmentation? (new phylogeny: hymenoptera -long germ wasp Nasonia- at the base of holometabolous insects -include short and long germ).

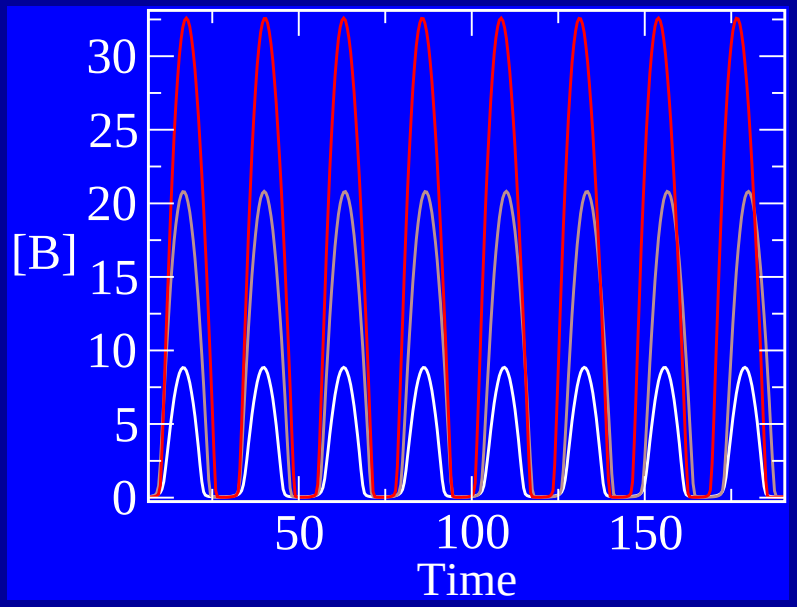
The End (for today).

Thank you!

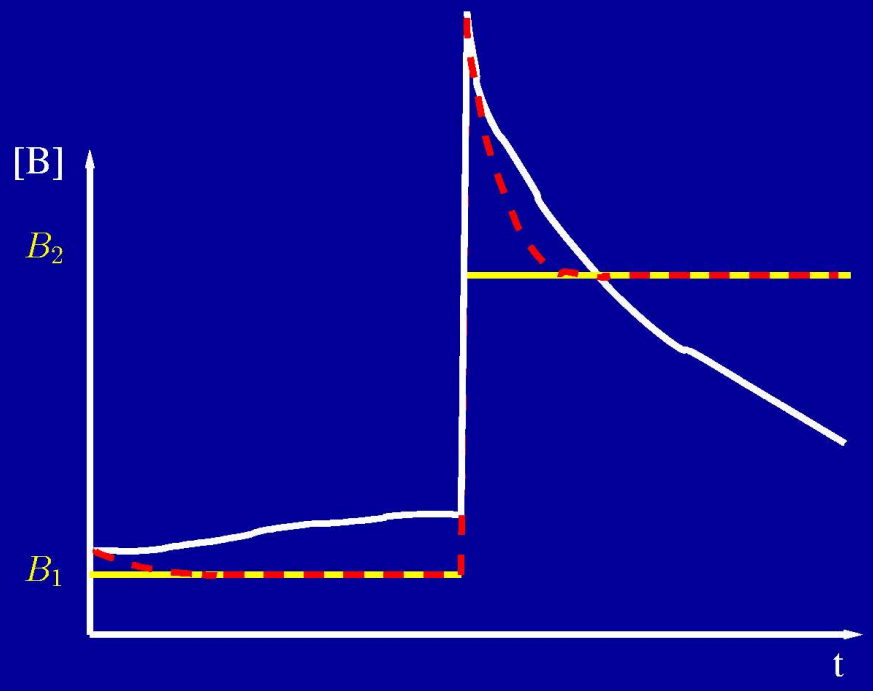
Temperature compensation

Selection of activation energies for temperature compensation:

- a $10^o K$ increase : $T : 300^o K \rightarrow 310^o K$
- the kinetic constants increase $> 30\%$,
- period change $< 3\%$.

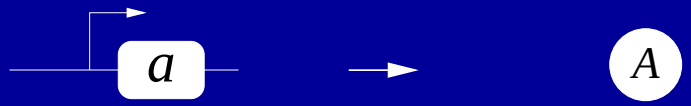


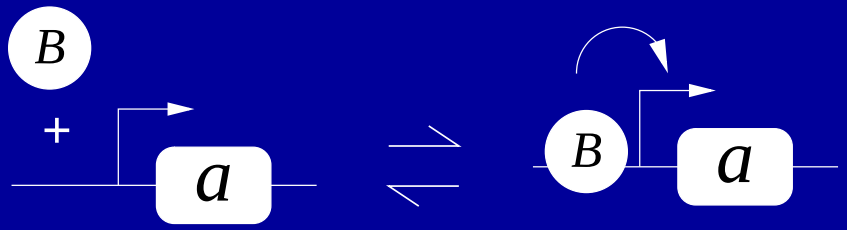
Fitness function for the switches



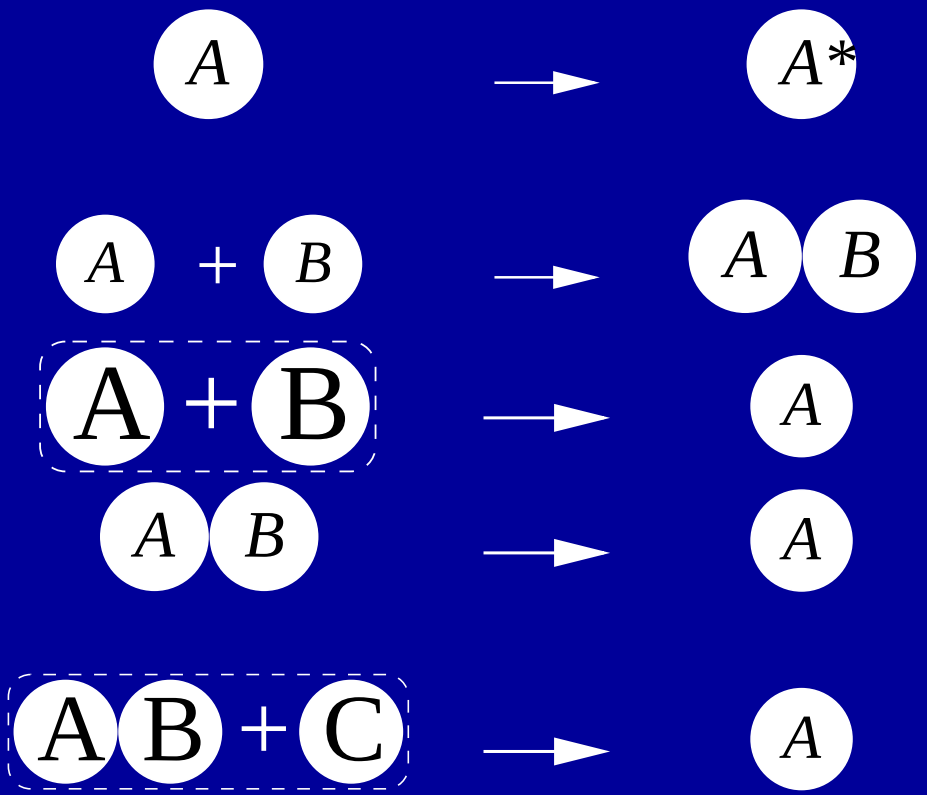
The desired two stable states are chosen (A_1, B_1) and (A_2, B_2) . ODEs are integrated, the "fitness" is given by the integral $(A - A_1)^2 + (B - B_1)^2$. Pulse of B protein ODEs are integrated, the fitness is given by the integral $(A - A_2)^2 + (B - B_2)^2$.

Transcriptional regulations

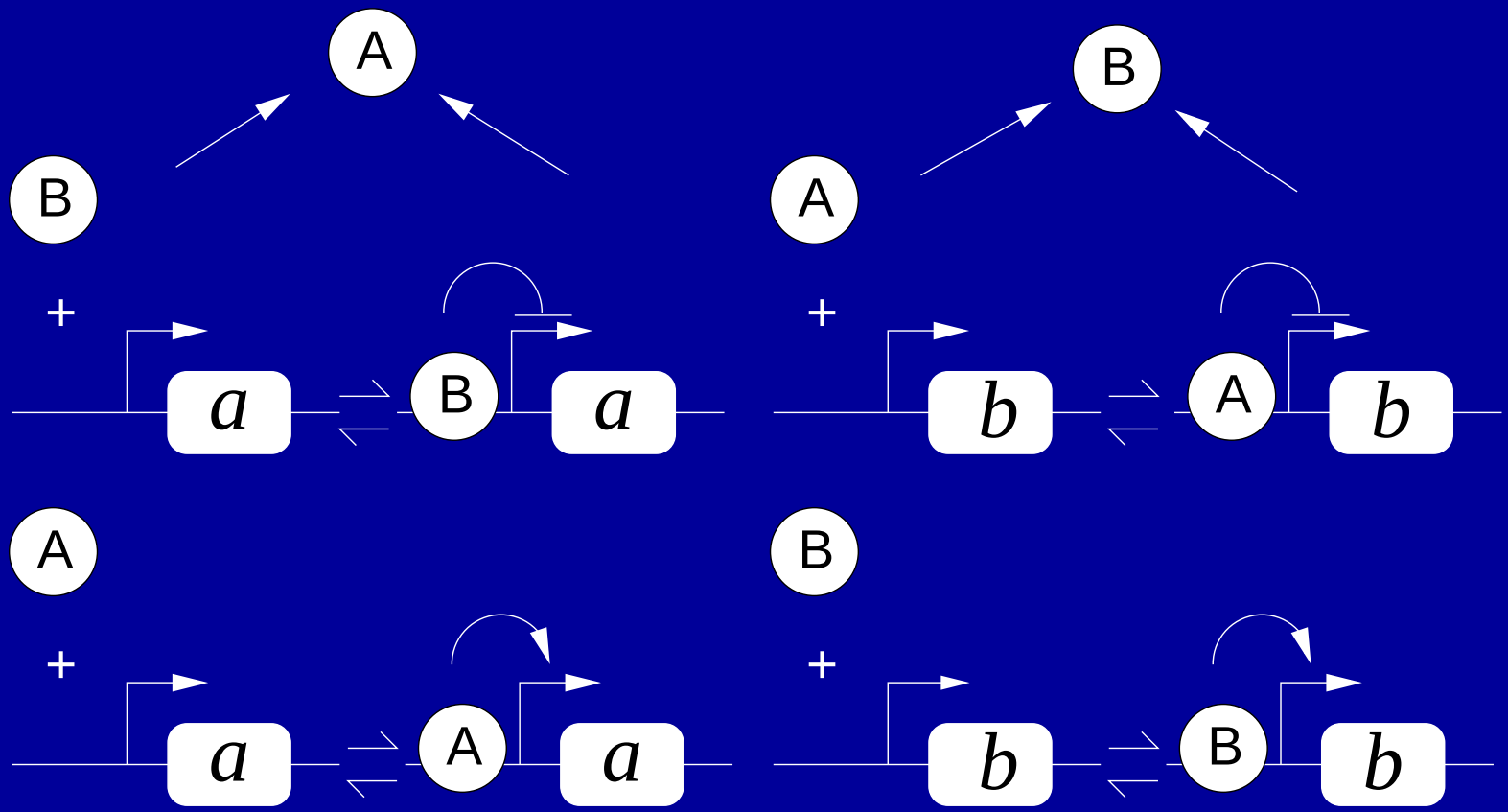




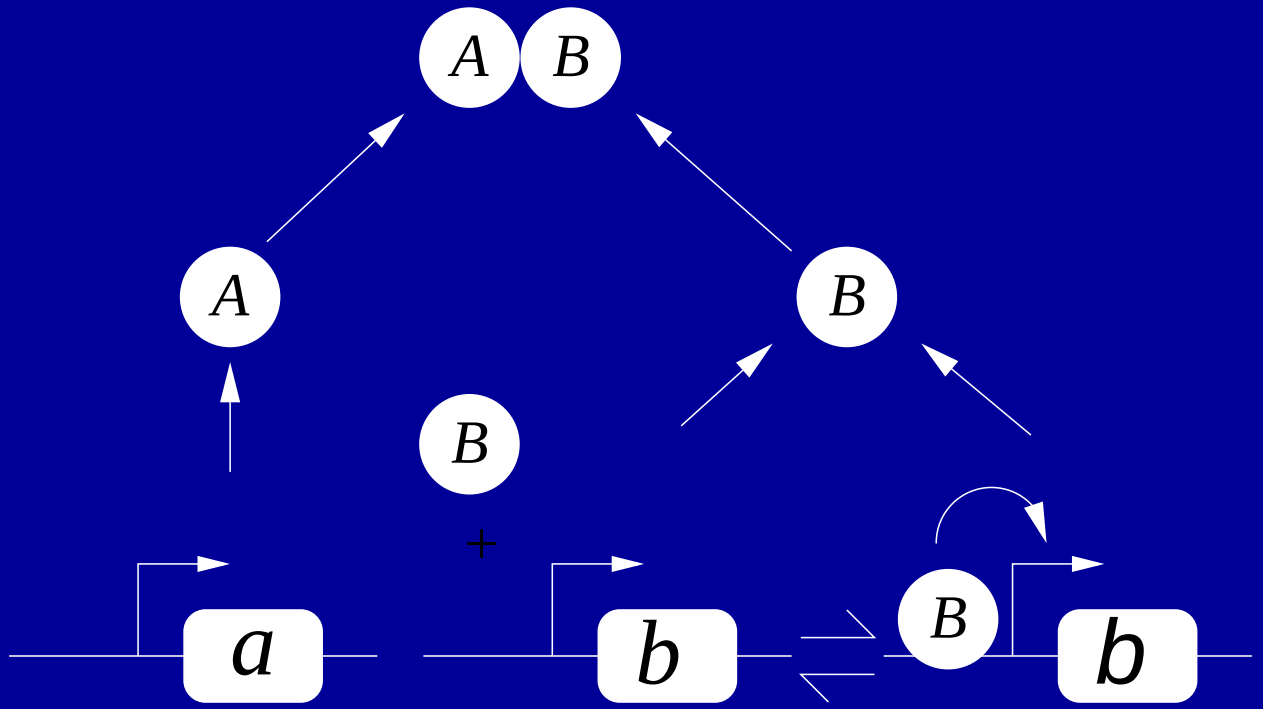
Post-transcriptional regulations



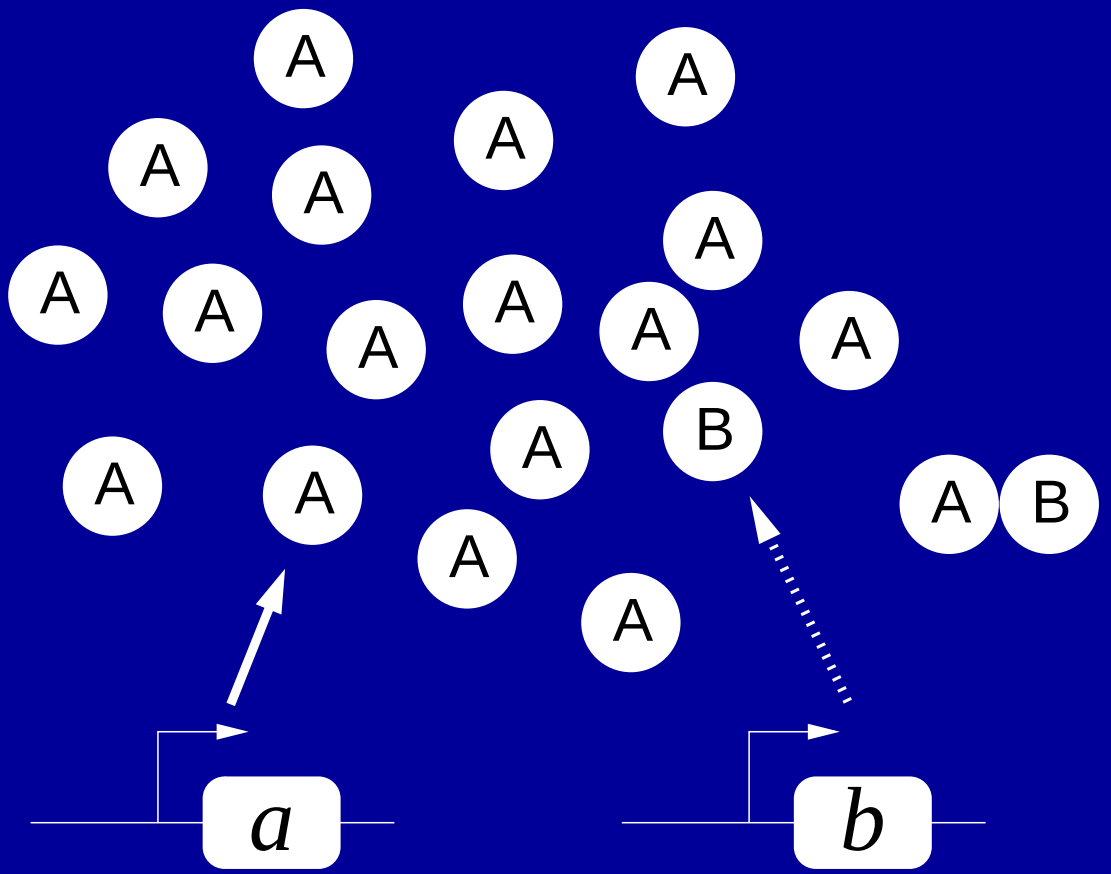
Transcriptional switches



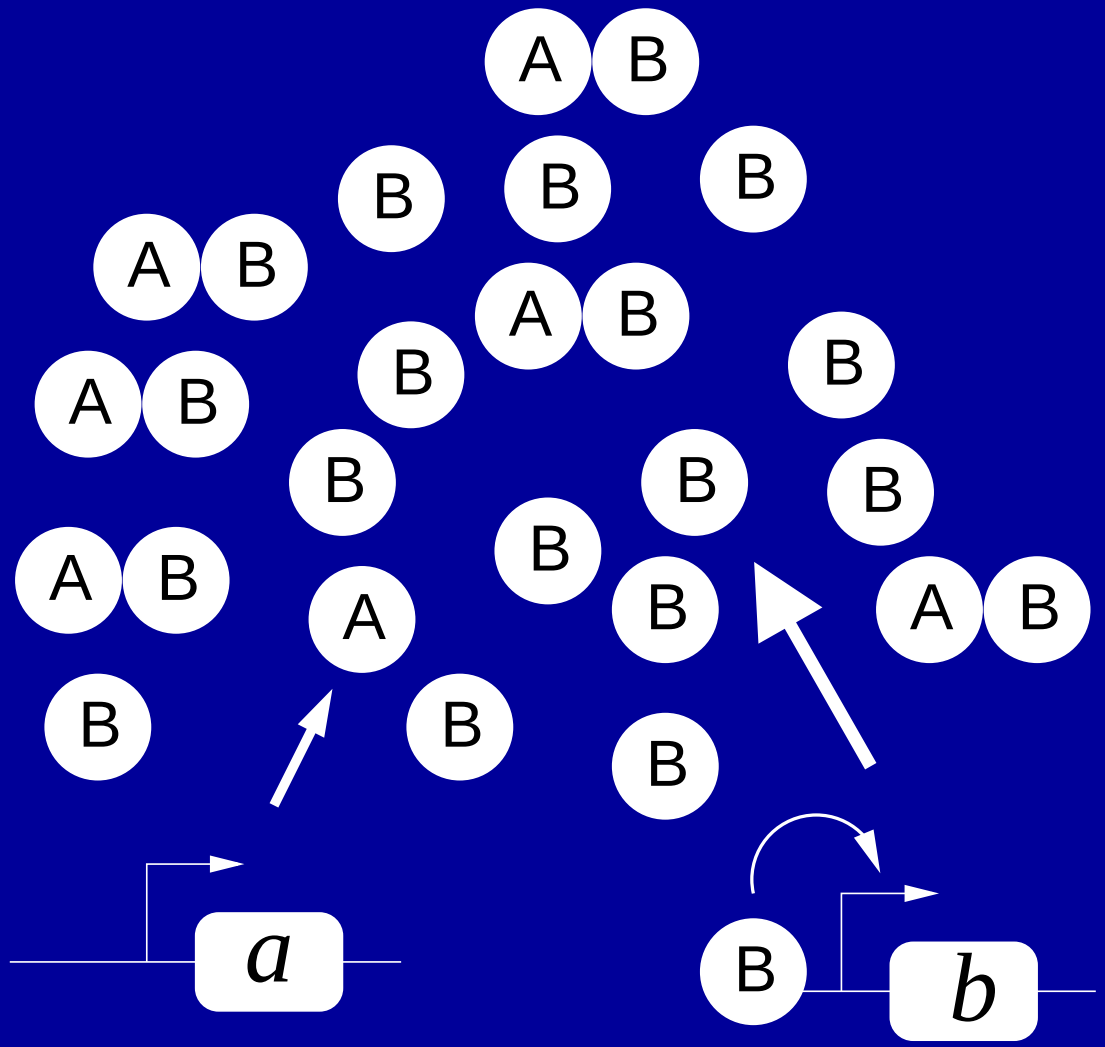
A second type of switch



A second type of switch



A second type of switch



'Toggle' switch : mathematical analysis

$$\frac{\mathrm{d}A}{\mathrm{d}t} = \frac{\alpha}{B_0 + B^\nu} - \delta_A A$$

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \frac{\beta}{A_0 + A^\mu} - \delta_B B$$

ν μ must be strictly higher than 1 to have bistability, which requires at least *four* (and not two) elementary reactions.
[Cherry and Adler, J. Theor. Biol. (2000)]