

# Regulation of Gene Expression

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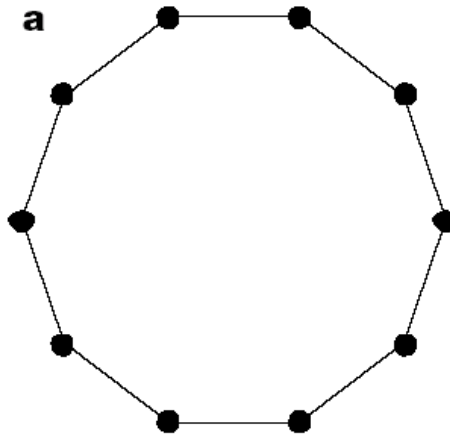
# Régulation globale de l'expression génique

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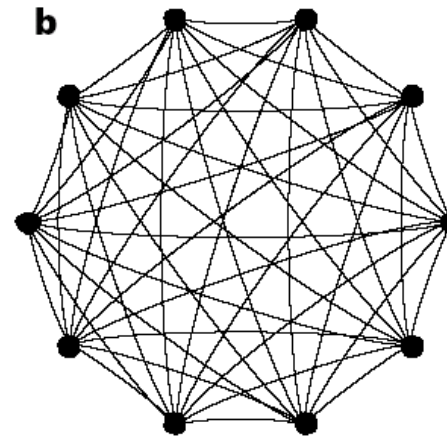
- Les connexions régulatrices dans un réseau de régulation, Topologie, aspect globaux
- Méthodes globales de mesure
- Modules et motifs
  - Fonctionnalité des motifs
  - Analyse de la dynamique d'un motif
- Réseaux de régulation
  - Opéron *lac*, Décision développementale (phage  $\lambda$ )
  - Réseaux artificiels
- Robustesse

# Topologie d'un réseau

- La connectivité même d'un réseau affecte profondément son fonctionnement



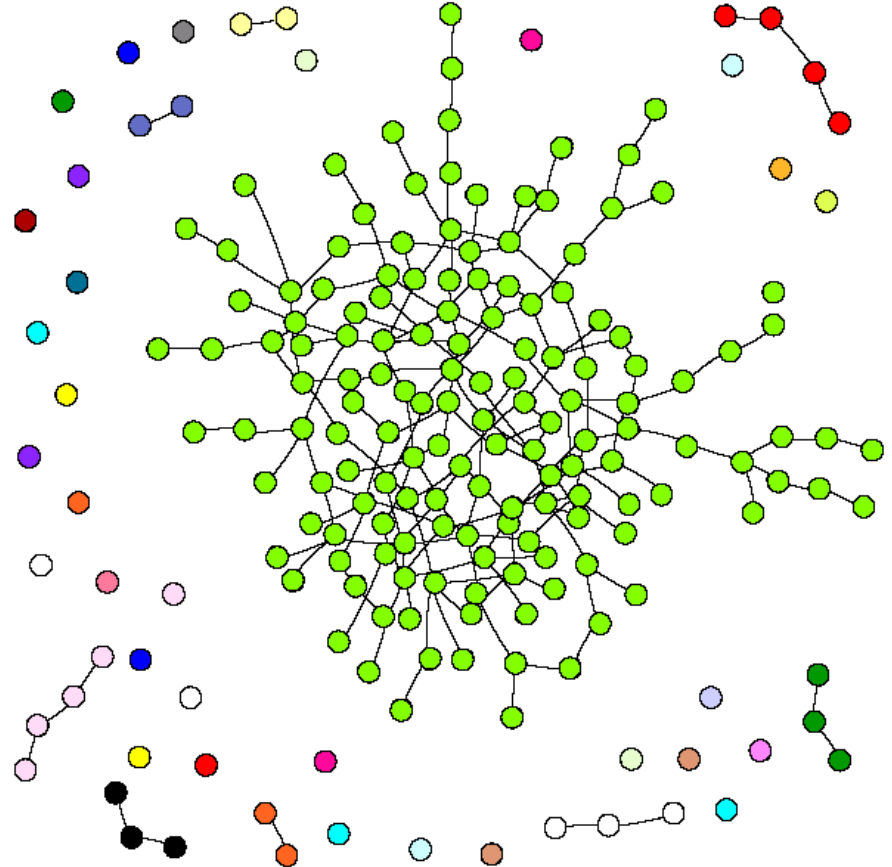
Connexions régulières



Réseau complètement connecté

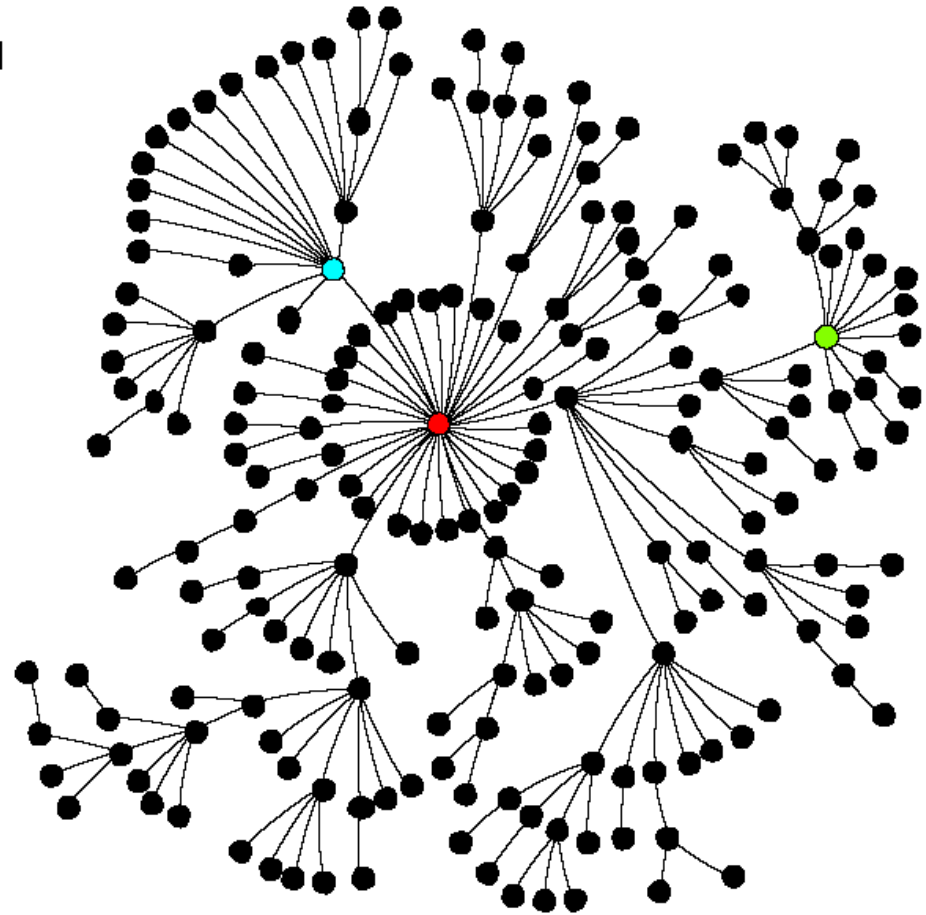
# Topologie d'un réseau

- Un réseau connecté<sup>c</sup> au hasard produit une composante géante fortement connectée



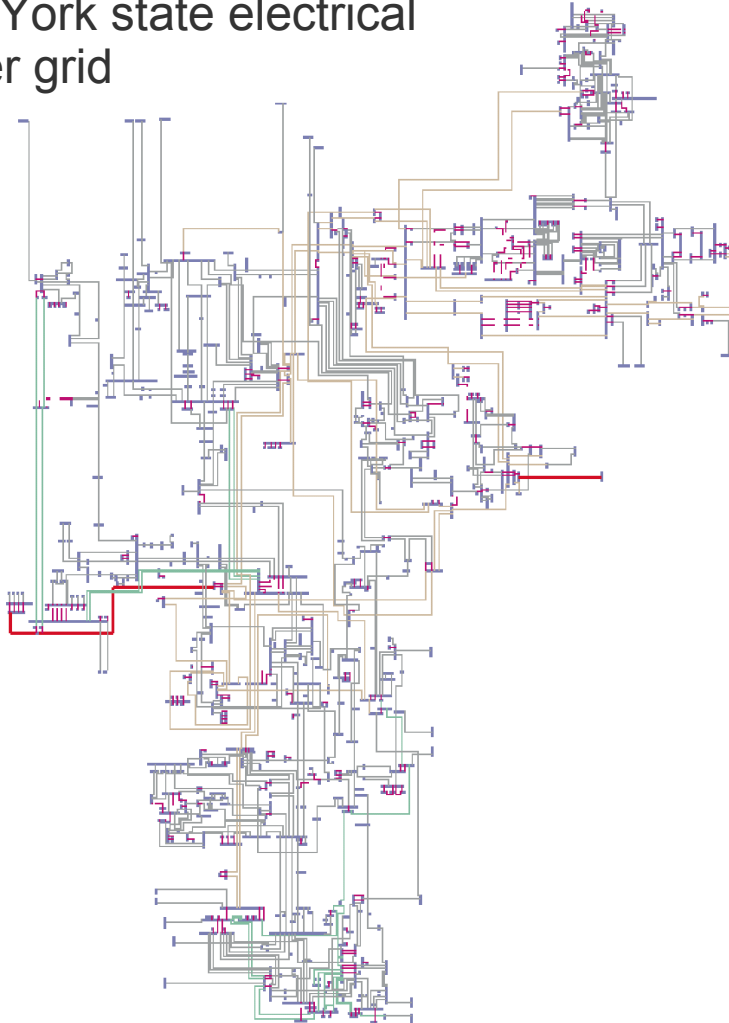
# Topologie d'un réseau

- **Scale-free graph**, grown by attaching new nodes at random to previously existing nodes. The probability of attachment is proportional to the degree of the target node; thus richly connected nodes tend to get richer, leading to the formation of hubs and a skewed degree distribution with a heavy tail.

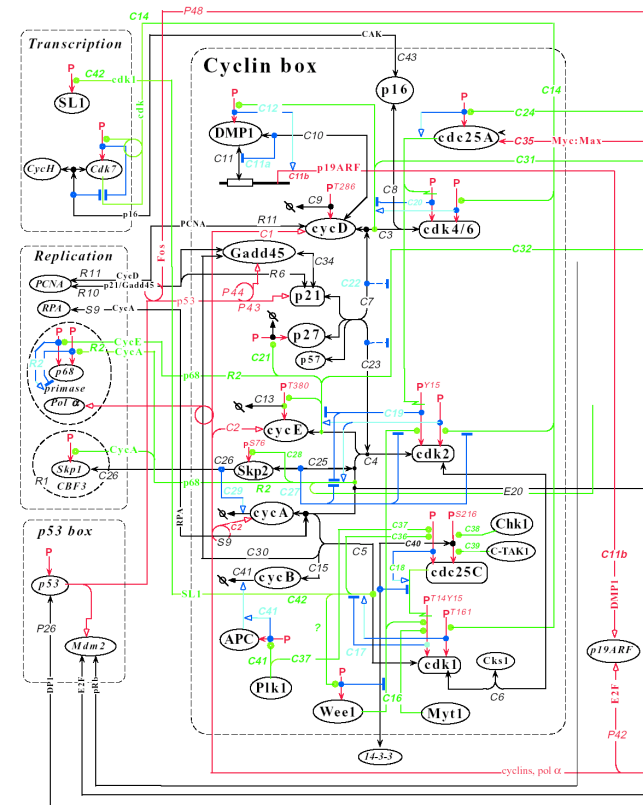


# Engineering and biology

New York state electrical power grid

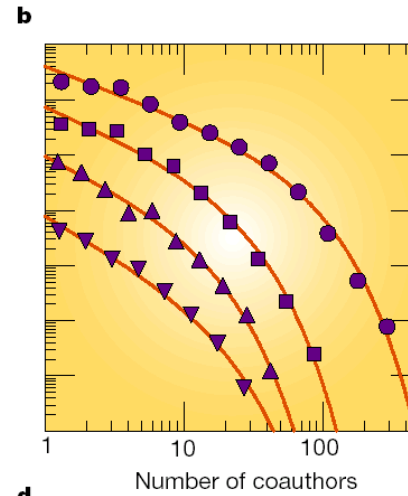
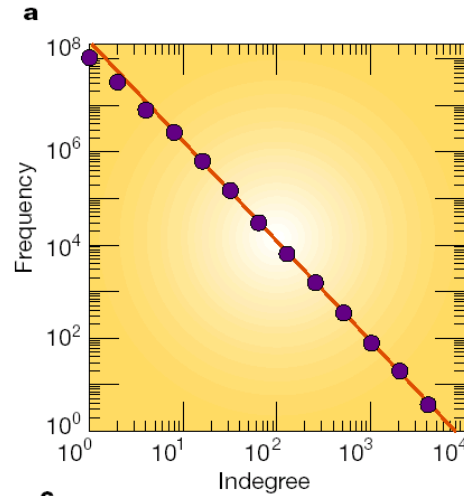


Part of the cell cycle network



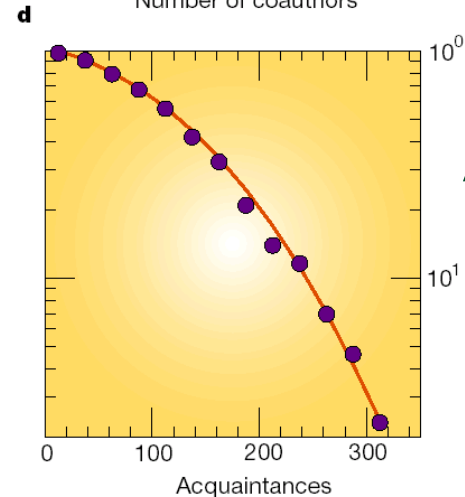
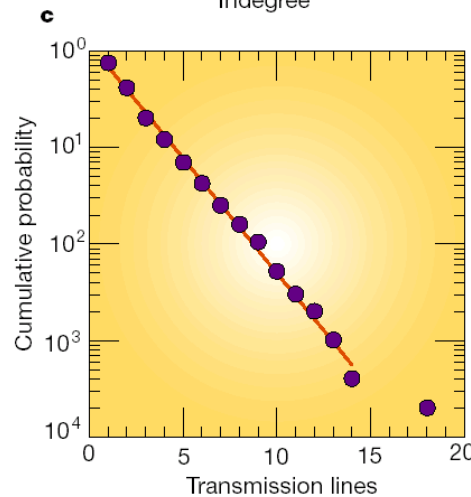
# Degree distributions of real networks

Internet  
Metabolism



Co-authorship

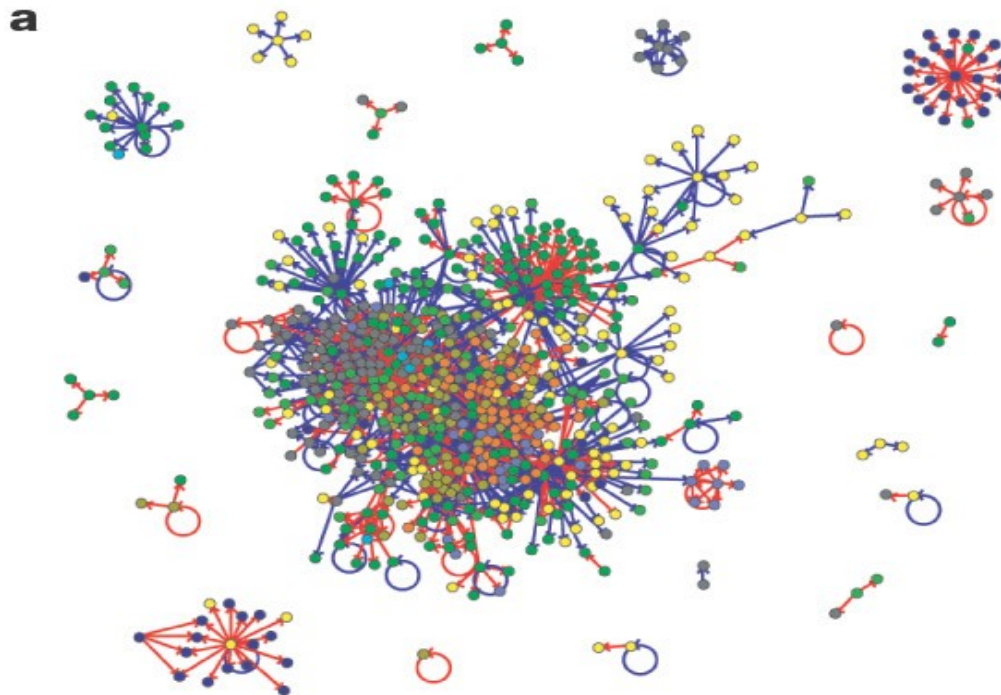
Powergrid of  
the Western US  
and Canada



Acquaintances

# Caractéristiques de réseaux de régulation transcriptionnelle

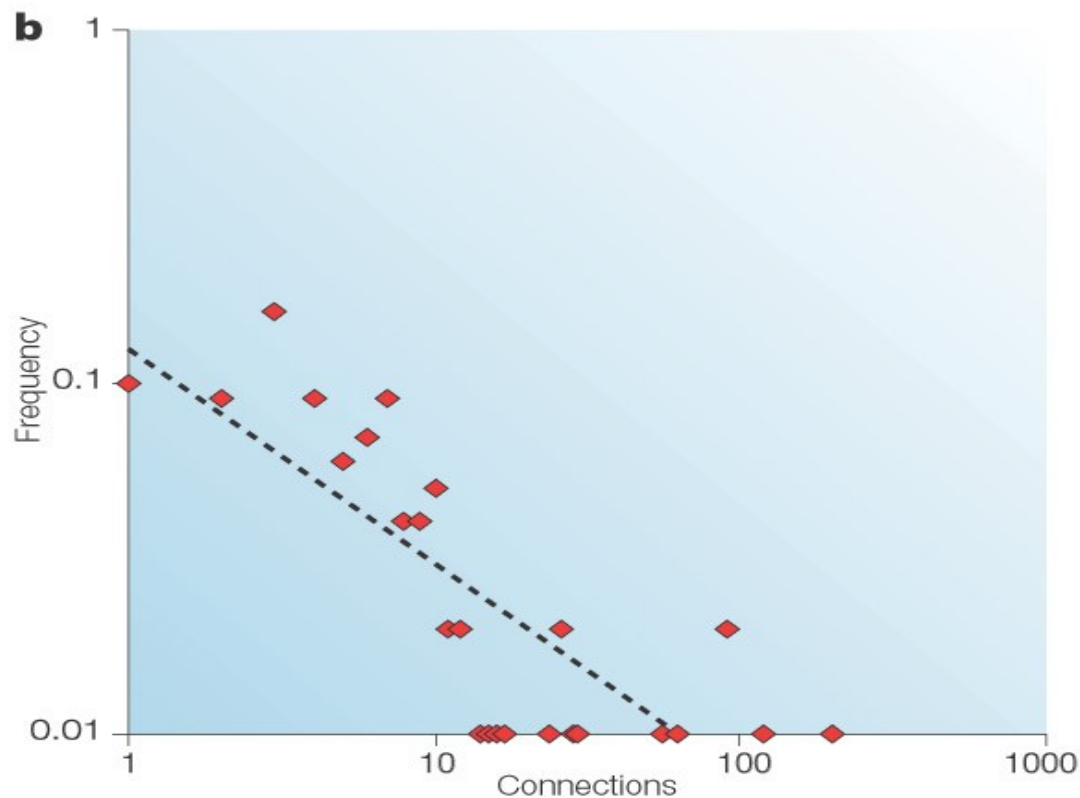
## □ Le réseau d'*Escherichia coli*





# Caractéristiques de réseaux de régulation transcriptionnelle

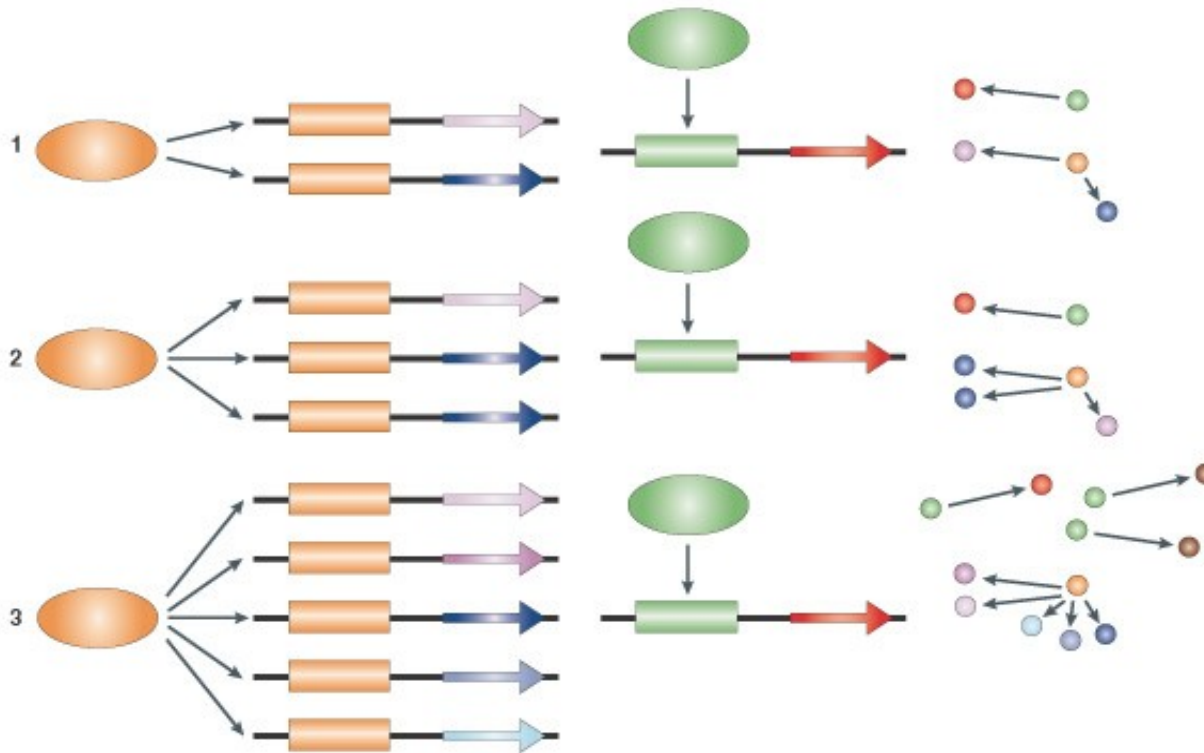
□ Le réseau d'*Escherichia coli* est “scale-free”



# Le principe de Mathieu appliqué aux réseaux de transcription

## □ On ne prête qu'aux riches

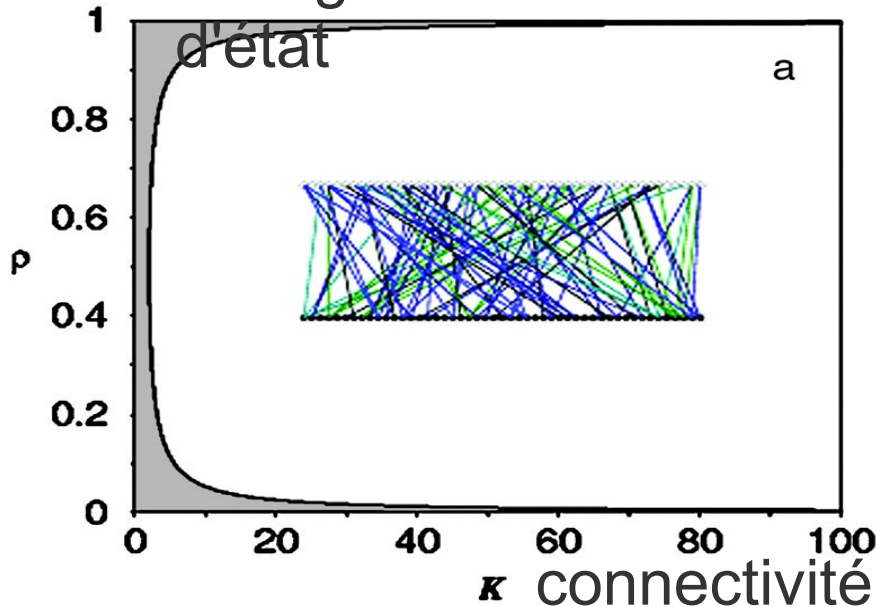
The 'Matthew principle': Matthew 13:12, "...for whosoever hath, to him shall be given, and he shall have more abundance: but whosoever hath not, from him shall be taken away even that he hath..."



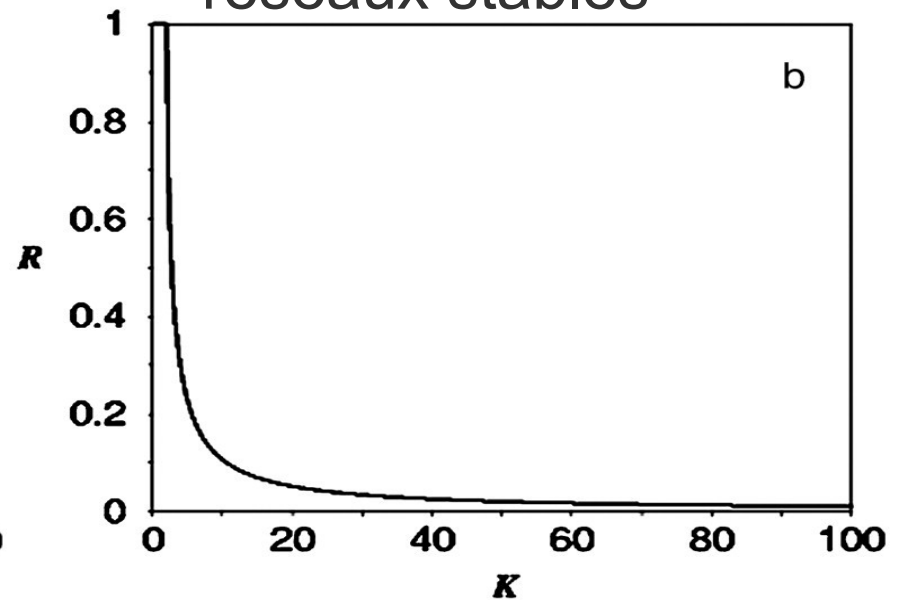
# Robustness d'un réseau aléatoire

- Un réseau aléatoire est chaotique
- Seulement un jeu très particulier de paramètres conduit à un comportement stable

Probabilité de  
changement  
d'état

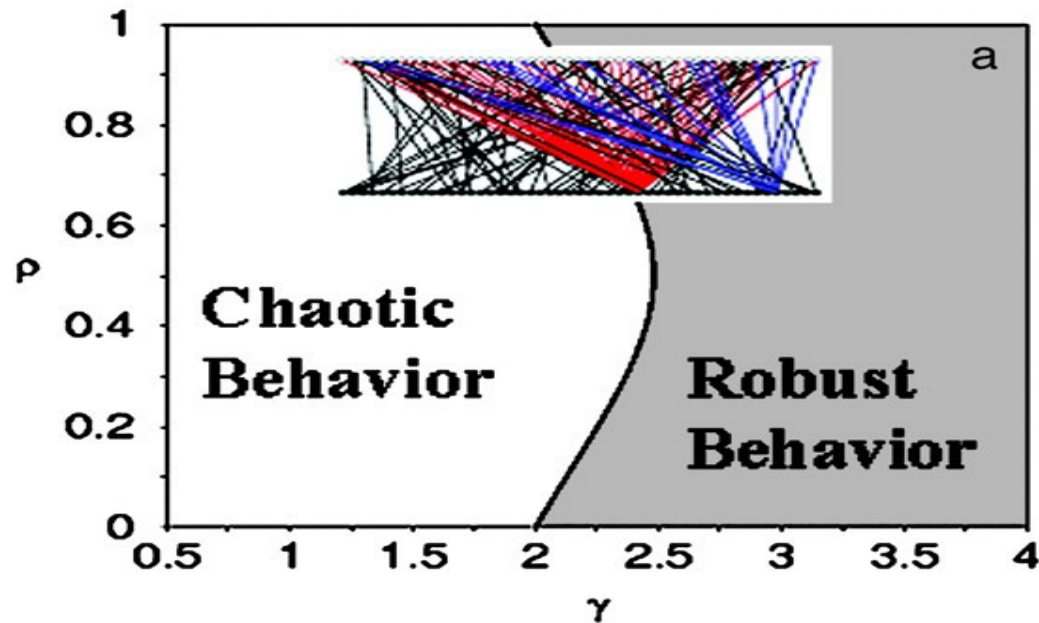


Fraction de  
réseaux stables



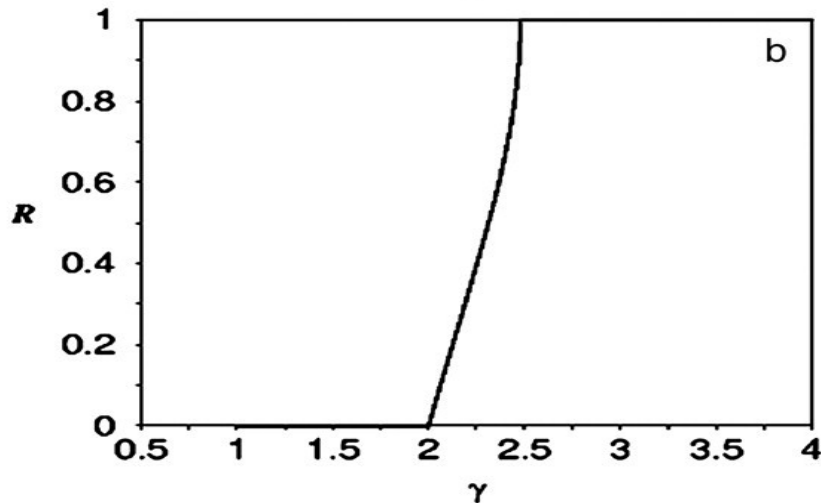
# Robustness d'un réseau aléatoire

- Un réseau aléatoire “scale-free” est robuste

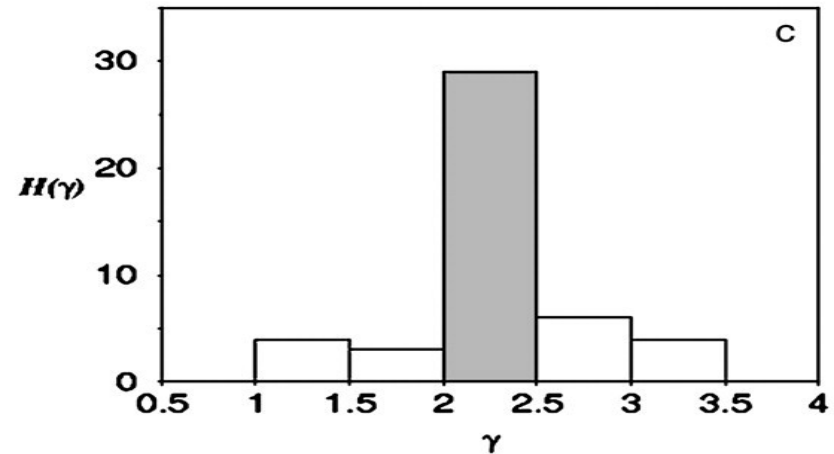


# Robustness d'un réseau aléatoire scale-free

□ Un réseau aléatoire “scale-free” est robuste



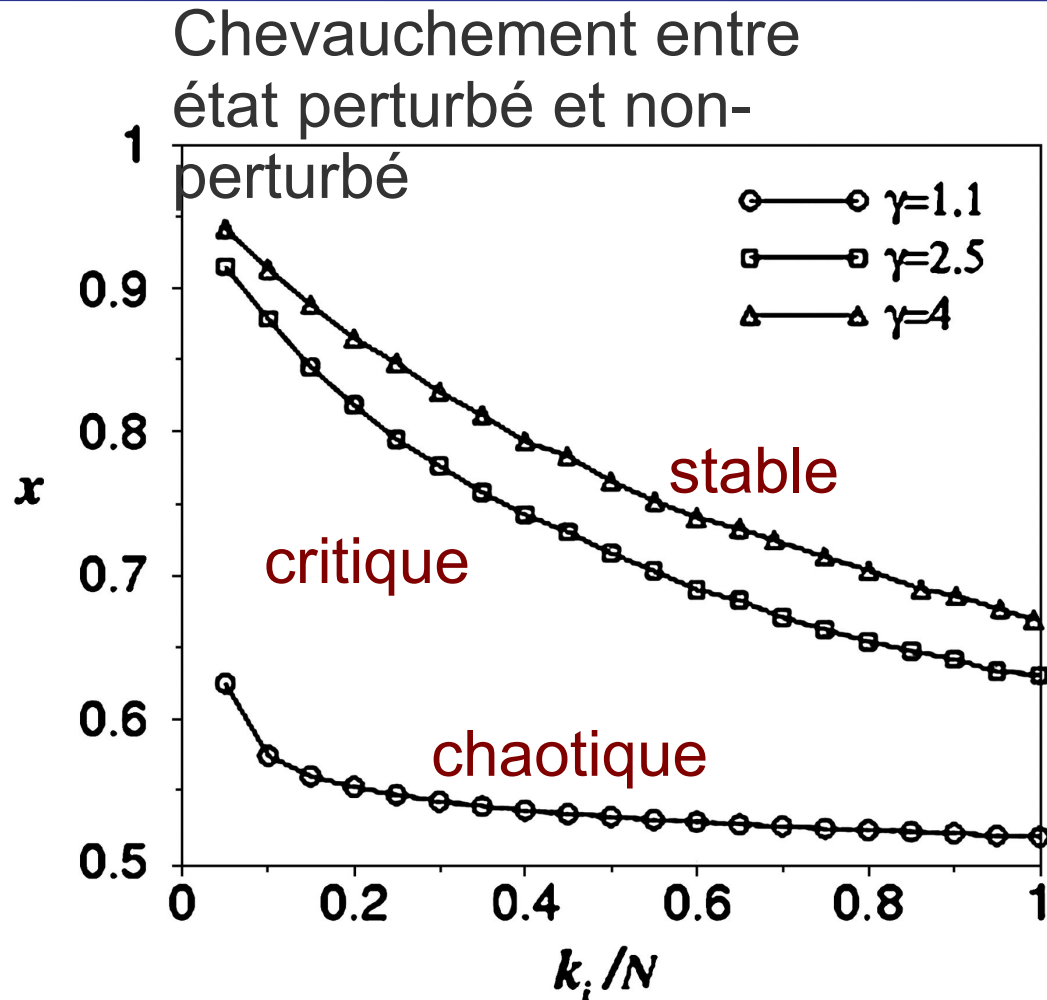
Transition entre chaotique et stable



La plupart des réseaux naturel sont scale-free avec un exposant entre 2 et 2.5

# Stabilité dynamique d'un réseau aléatoire

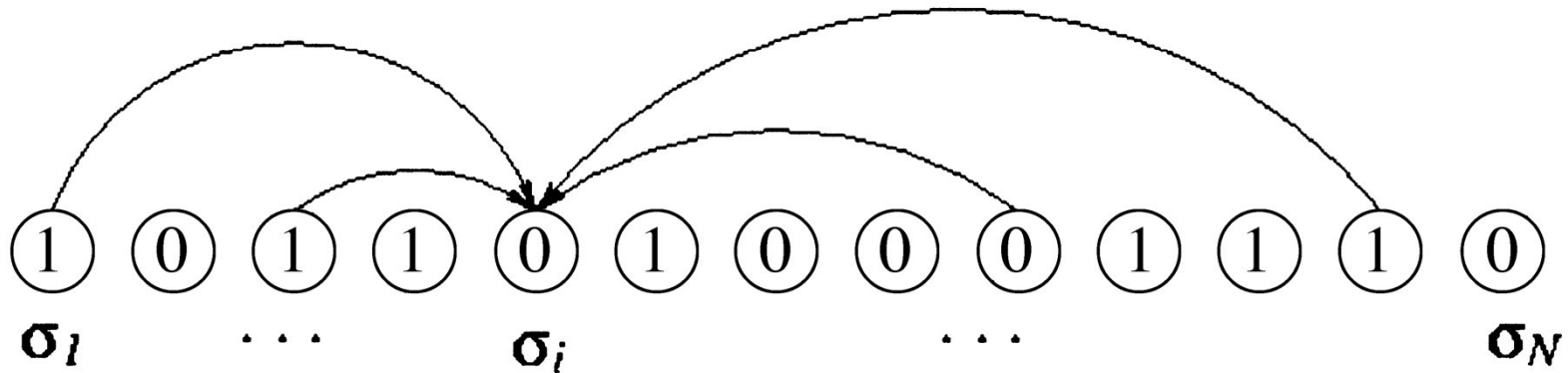
- Un réseau aléatoire "scale-free" est stable
- Plus la connectivité est grande, plus le réseau est instable



# Le modèle de Kauffman

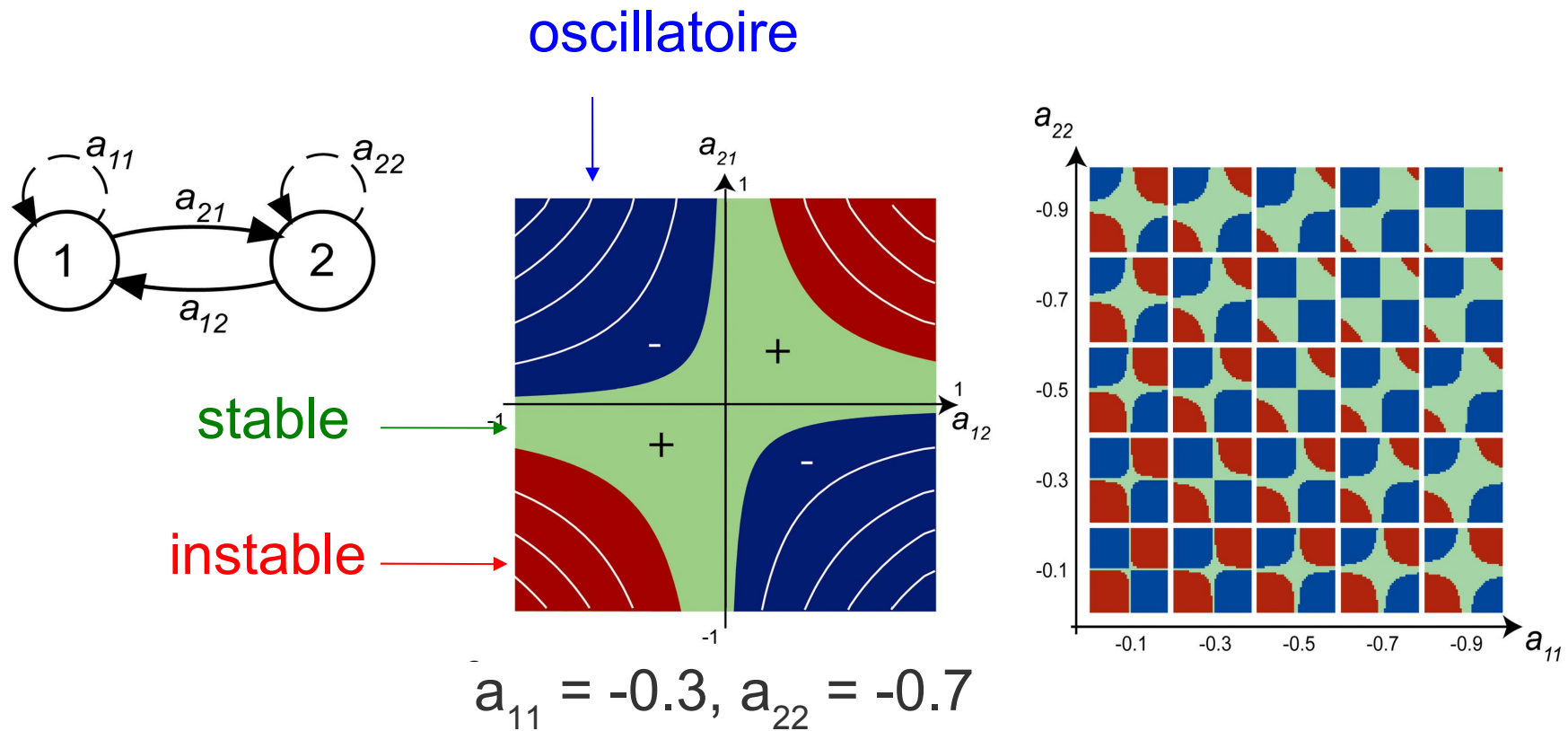
□ Connexions aléatoires

□  $K = 4$



# Dynamique d'un module

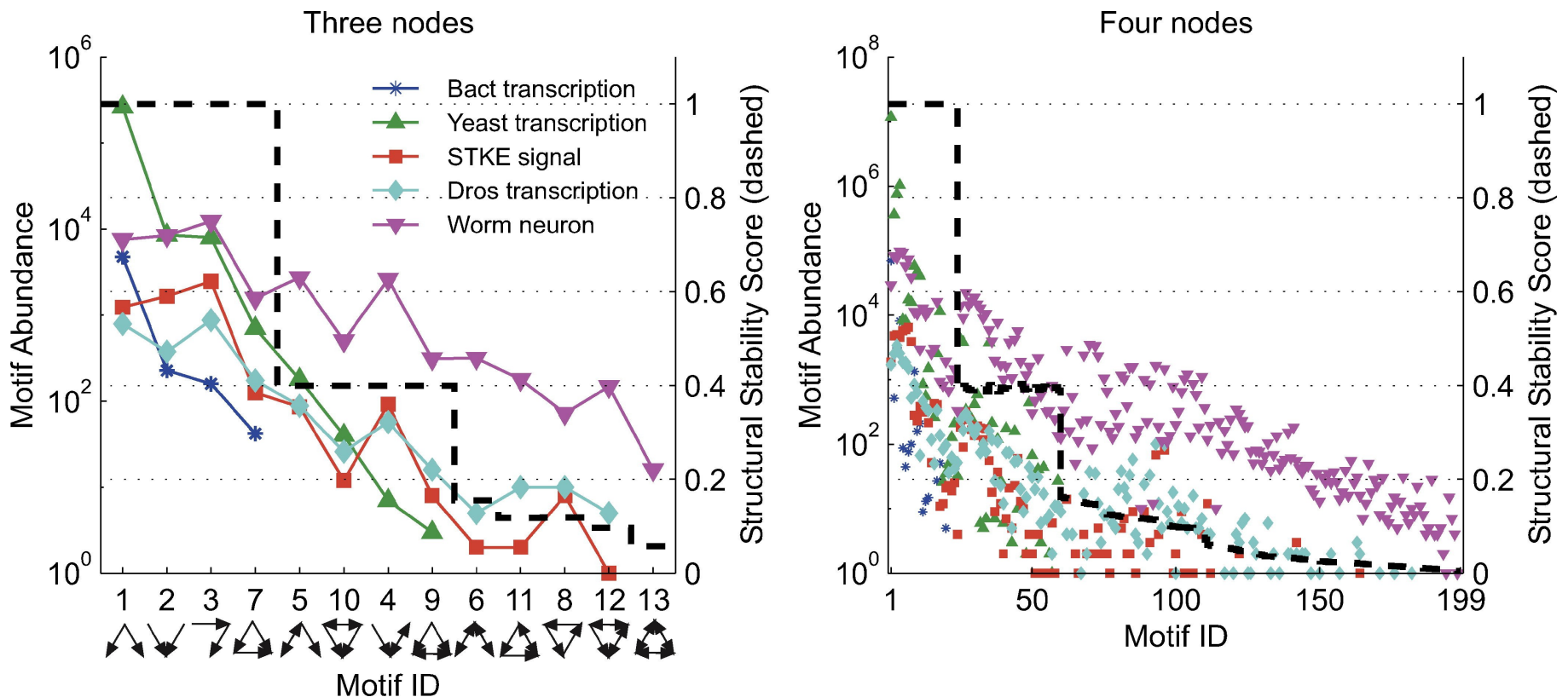
- Même un module simple peut avoir une dynamique complexe





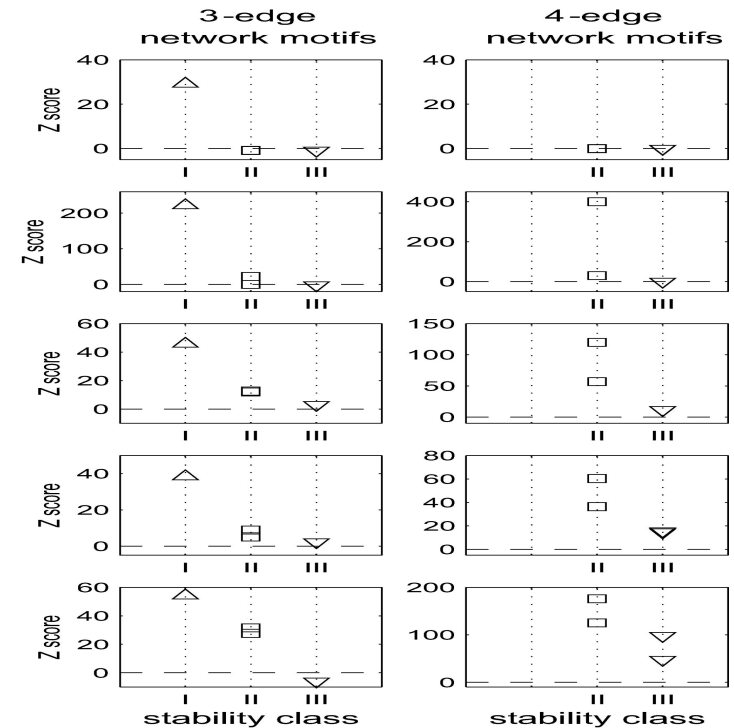
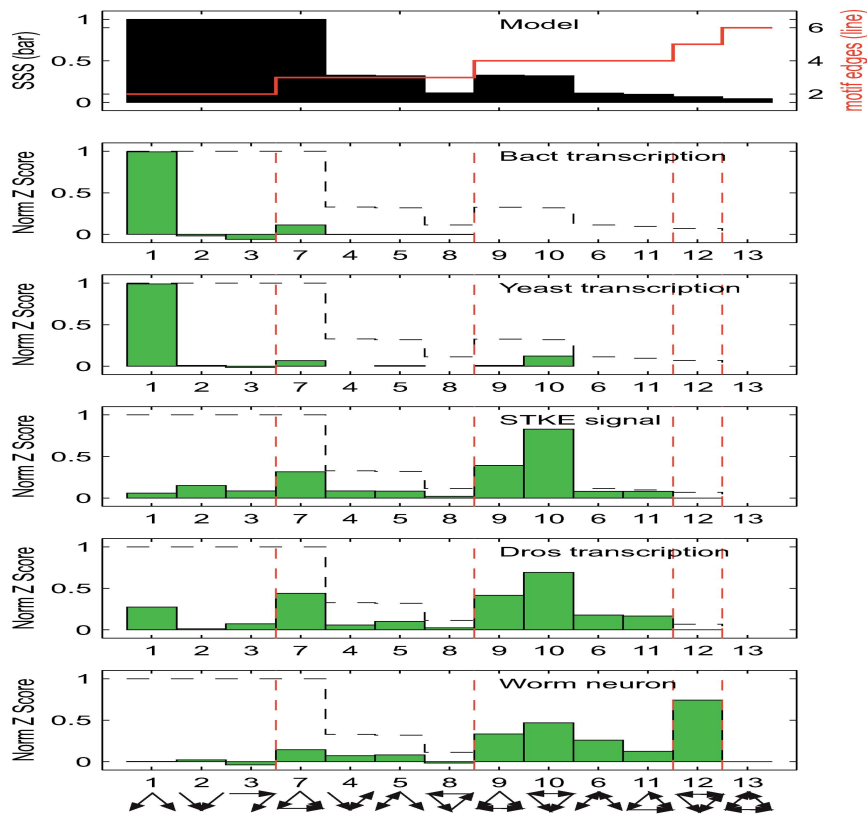
# L'abondance d'un motif est corrélé avec son SSS

□ SSS: structural stability score (probabilité de retourner vers l'état stable après une perturbation)

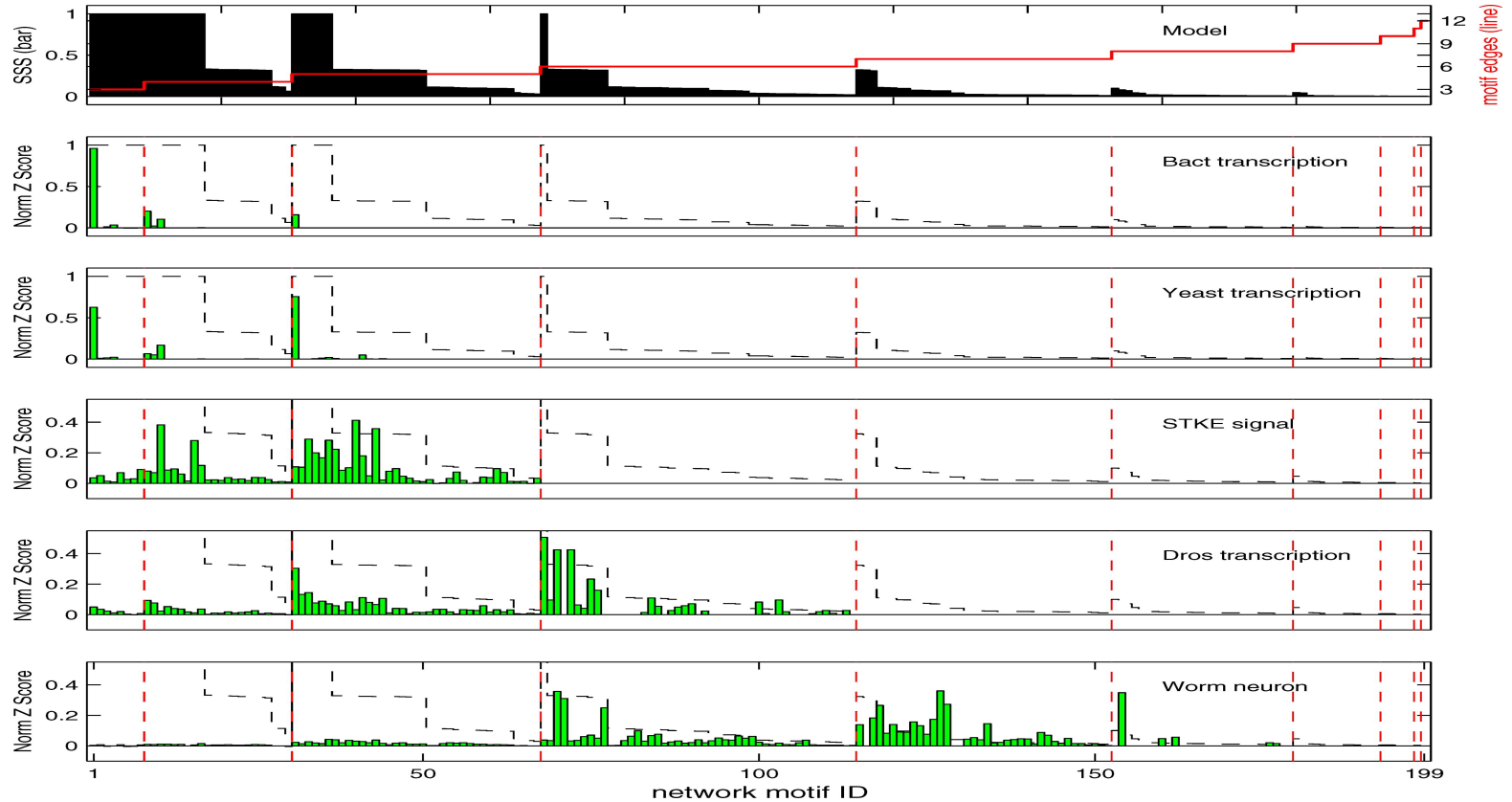


# Le motif le plus stable est préféré

□ Pour un nombre de noeuds et de connexions donné, le motif le plus stable est sur-représenté



# Le motif le plus stable est préféré (4 noeuds)



# Le motif le plus stable est préféré

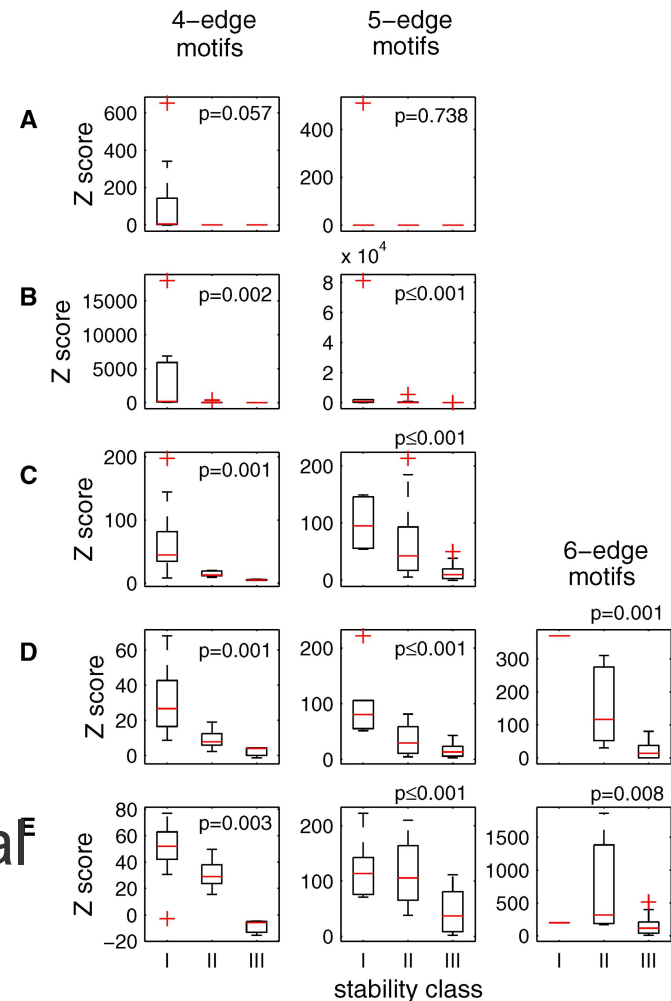
bactéries

levure

Transduction du signal

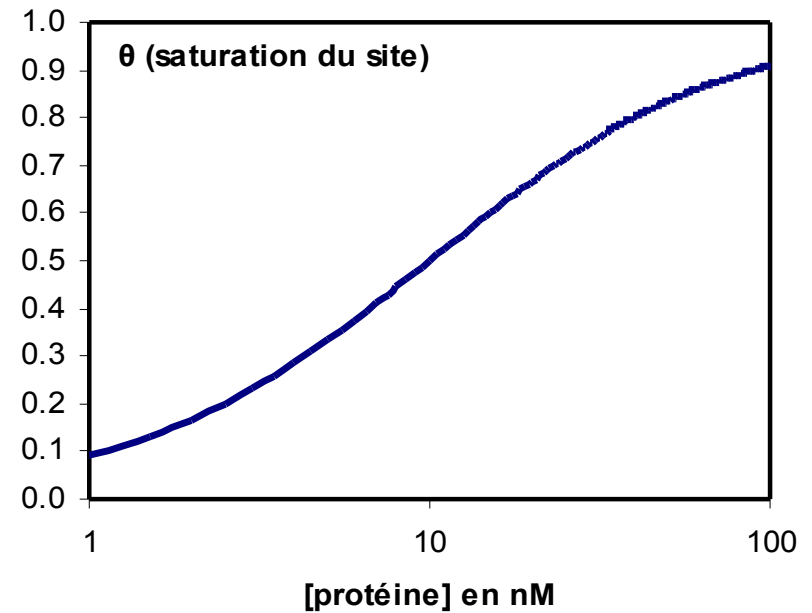
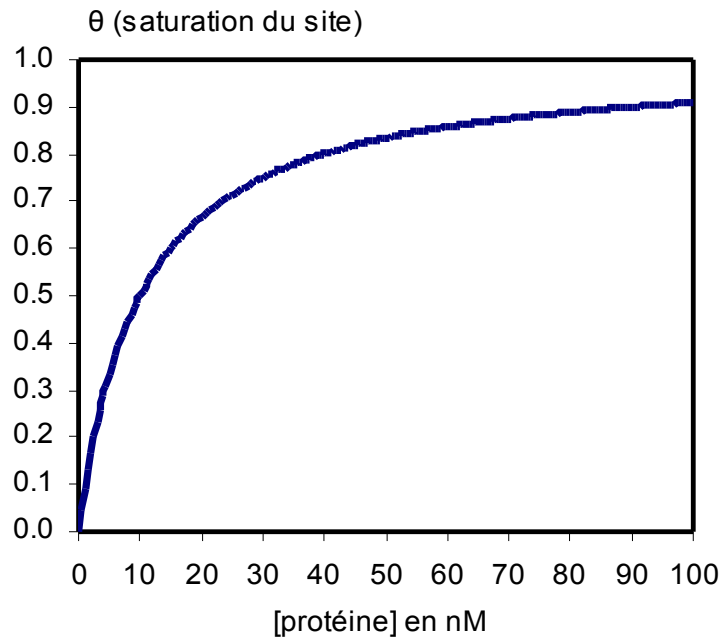
Développement de  
*D. melanogaster*

Développement neuronal  
de *C. elegans*



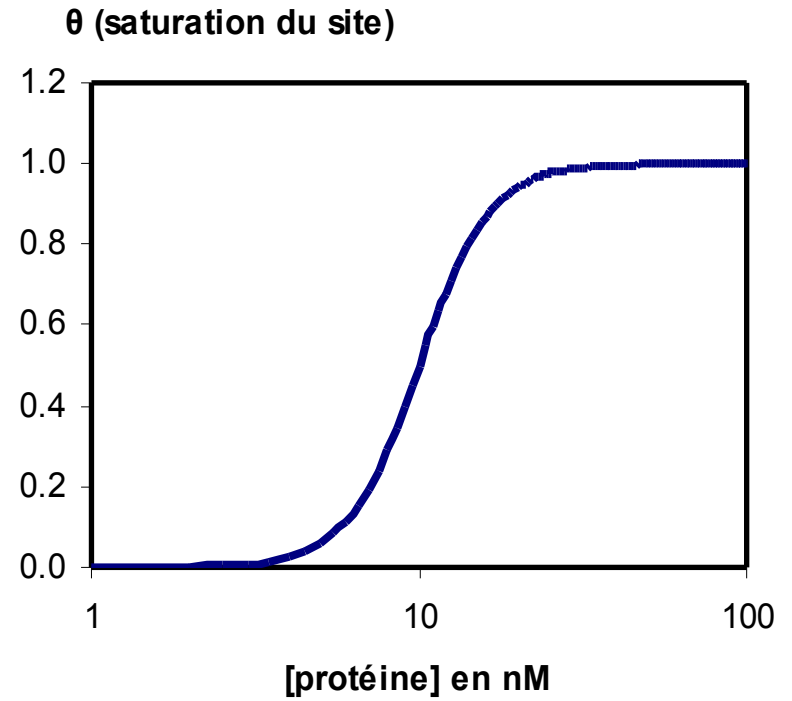
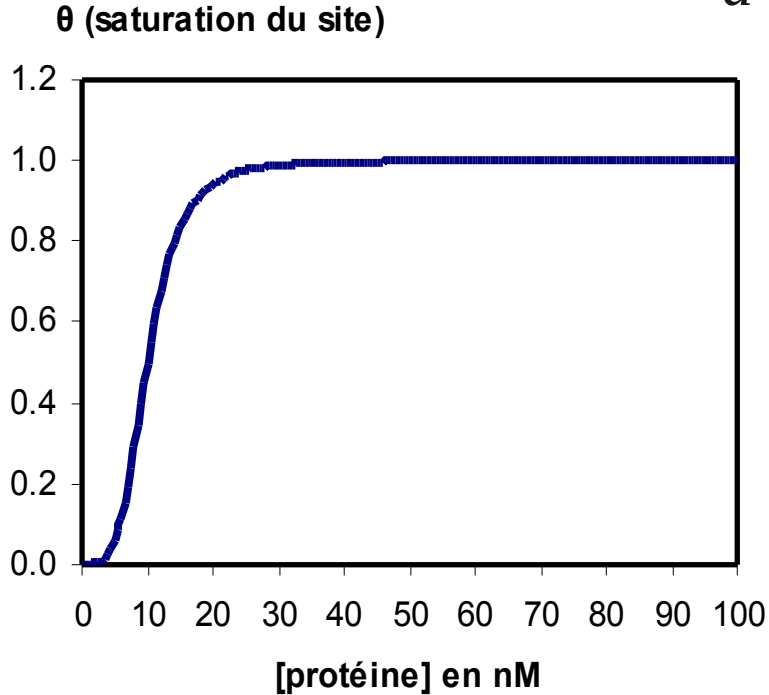
# La liaison simple à l'ADN

$$\theta = \frac{[P]}{K_d + [P]}$$



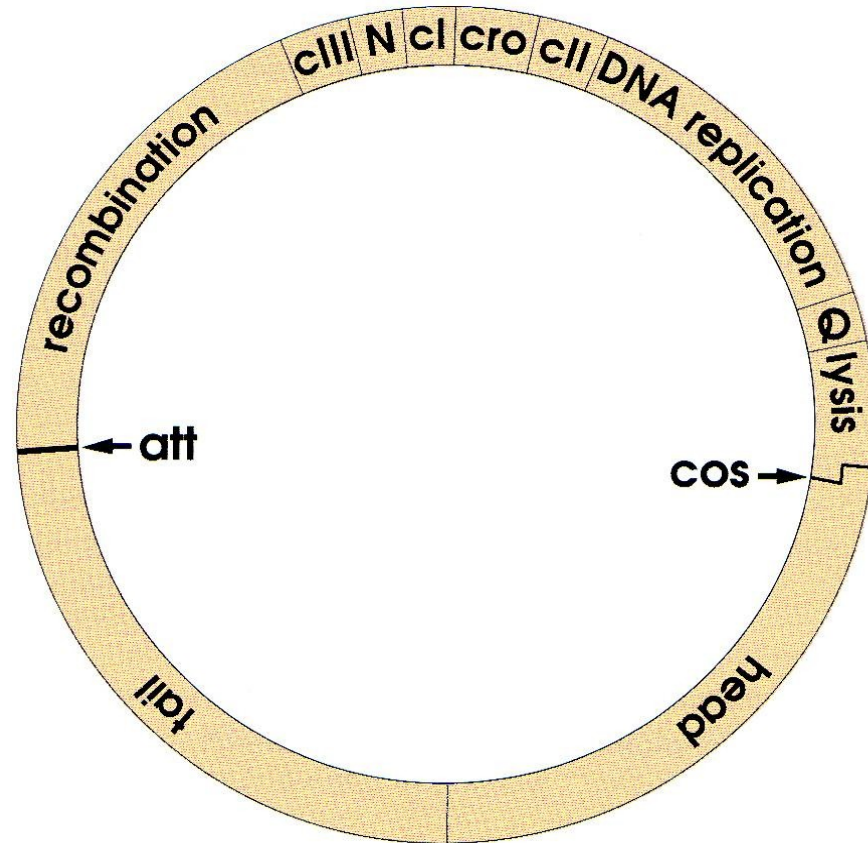
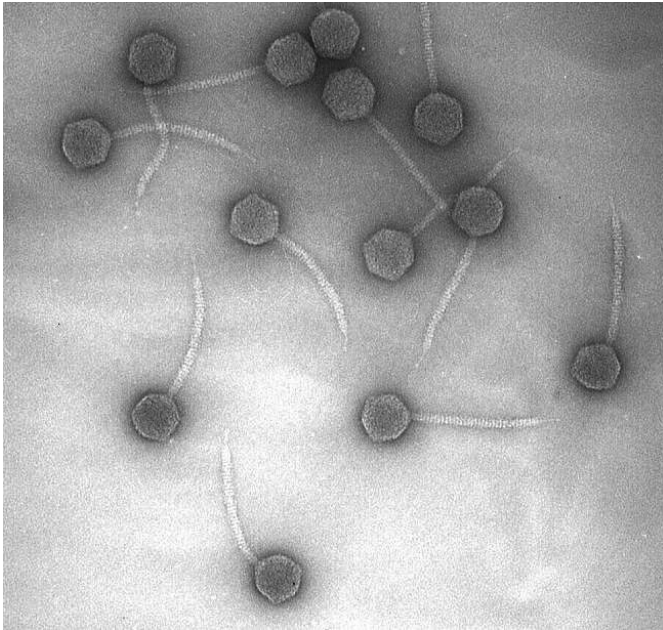
# La liaison coopérative à l'ADN

$$\theta = \frac{[P]^n}{K_d + [P]^n}$$



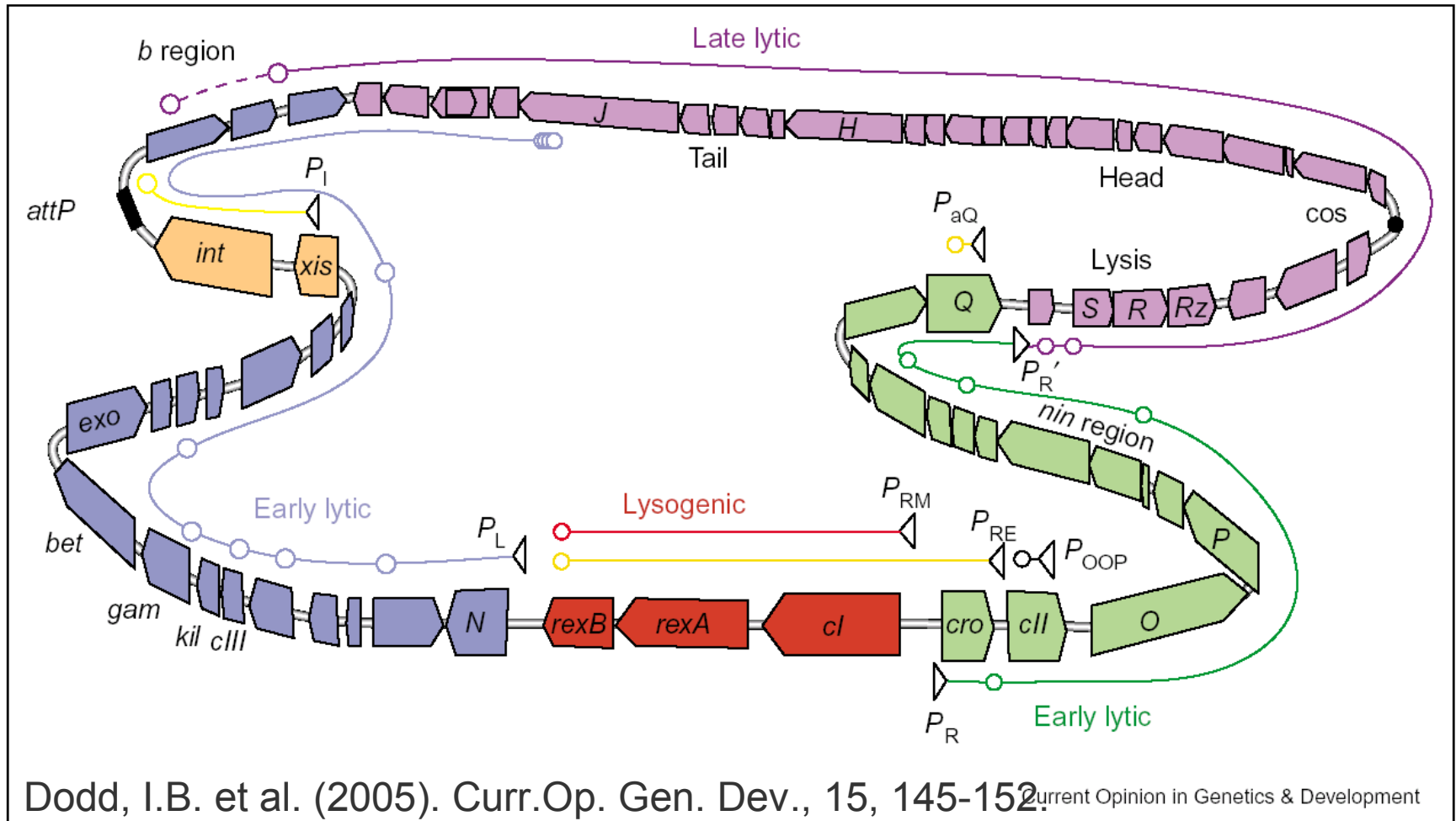
# Le bactériophage $\lambda$

- Génome circulaire
- Lyse ou lysogénie



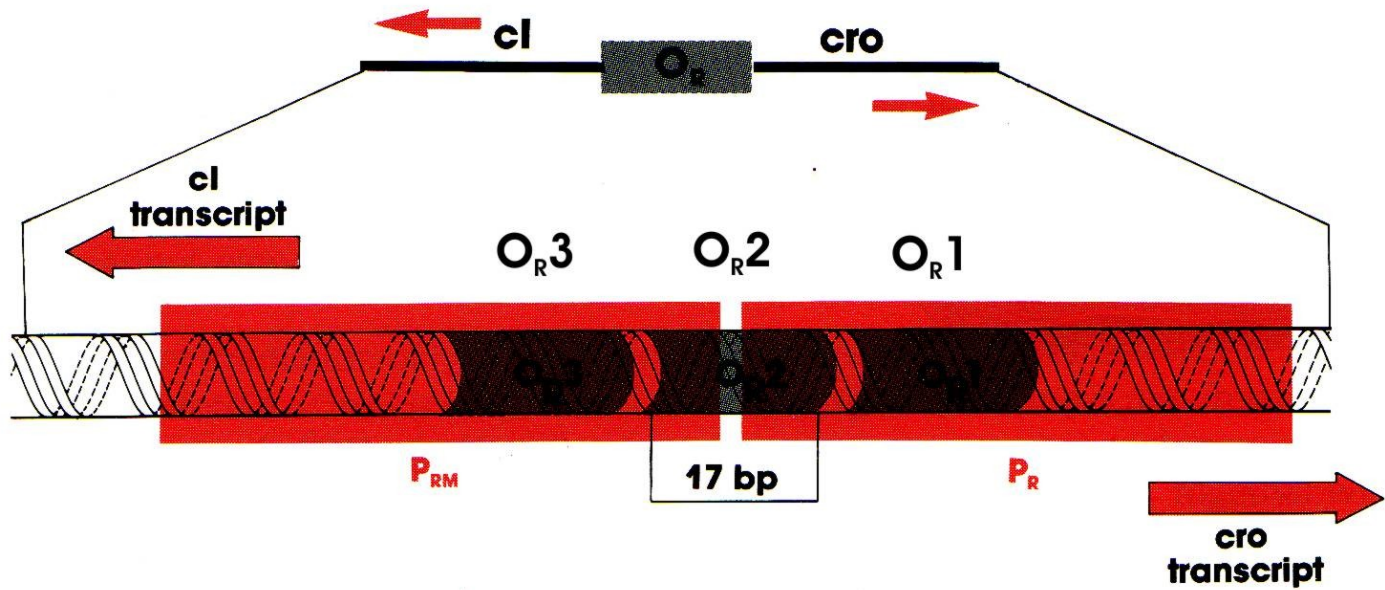
Ptashne, M. (1992). A genetic switch. Cell Press and Blackwell Scientific.

# Le génome de $\lambda$





# La région de contrôle



Ptashne, M. (1992). A genetic switch. Cell Press and Blackwell Scientific.

# Liaison de Cro sur les opérateurs

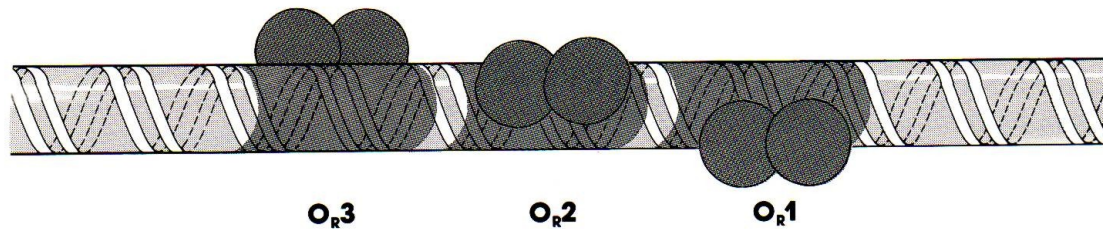
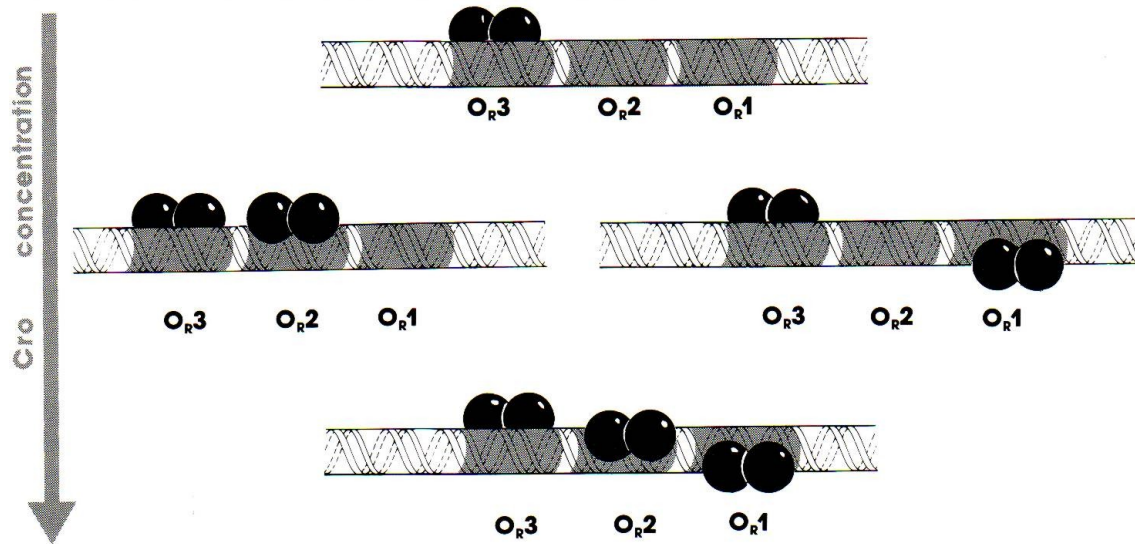
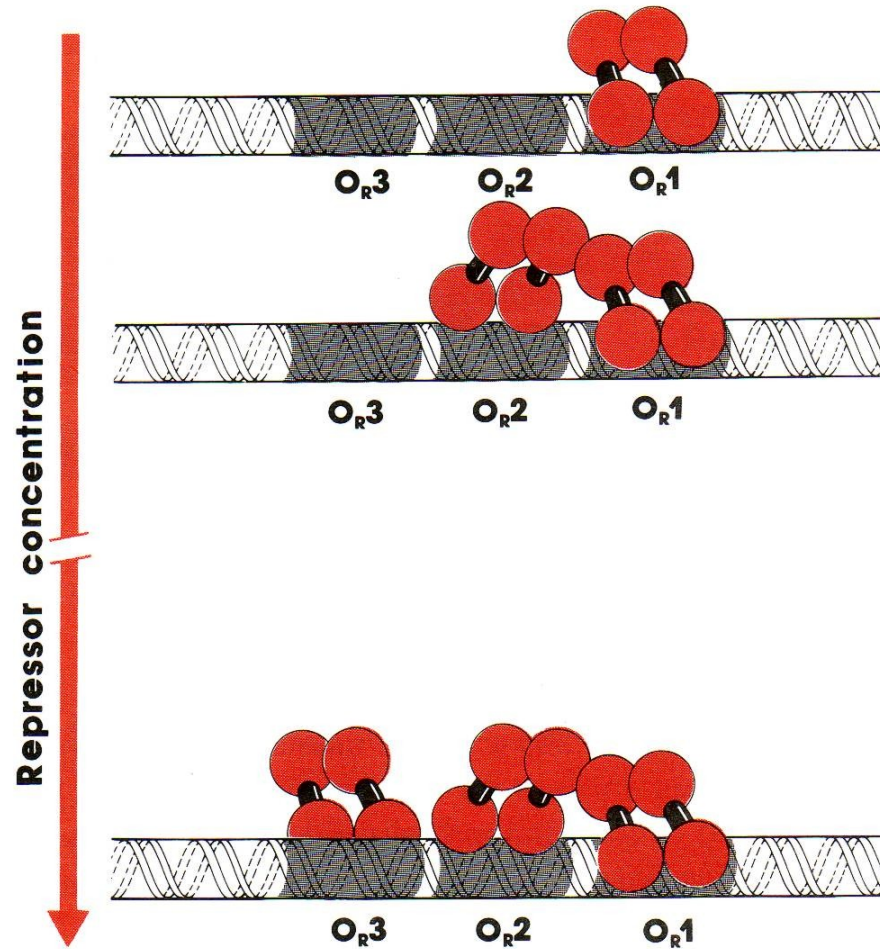


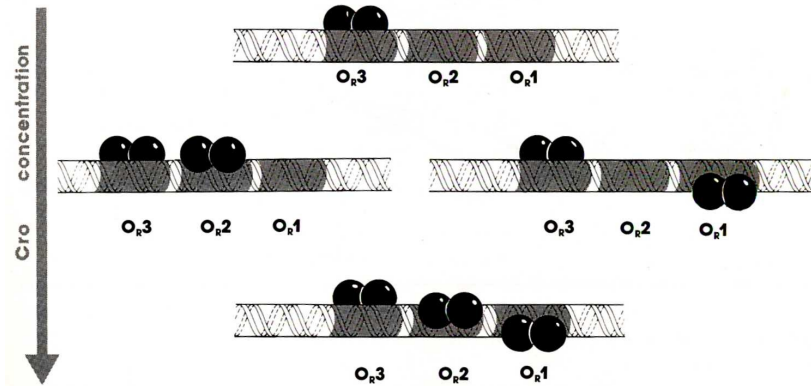
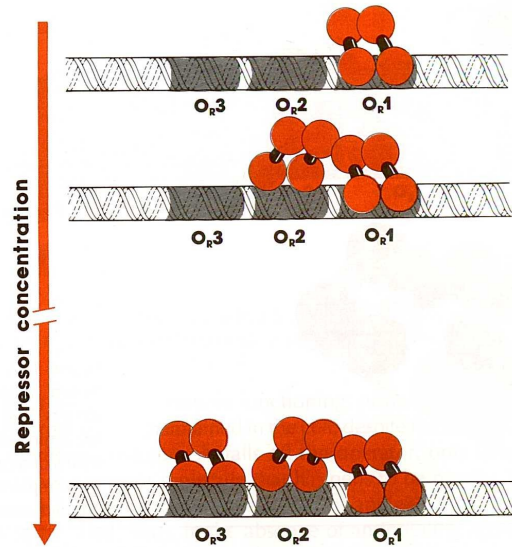
Figure 1.22. Cro bound to  $O_R$ . Cro dimers bind independently to each site in the tripartite operator.



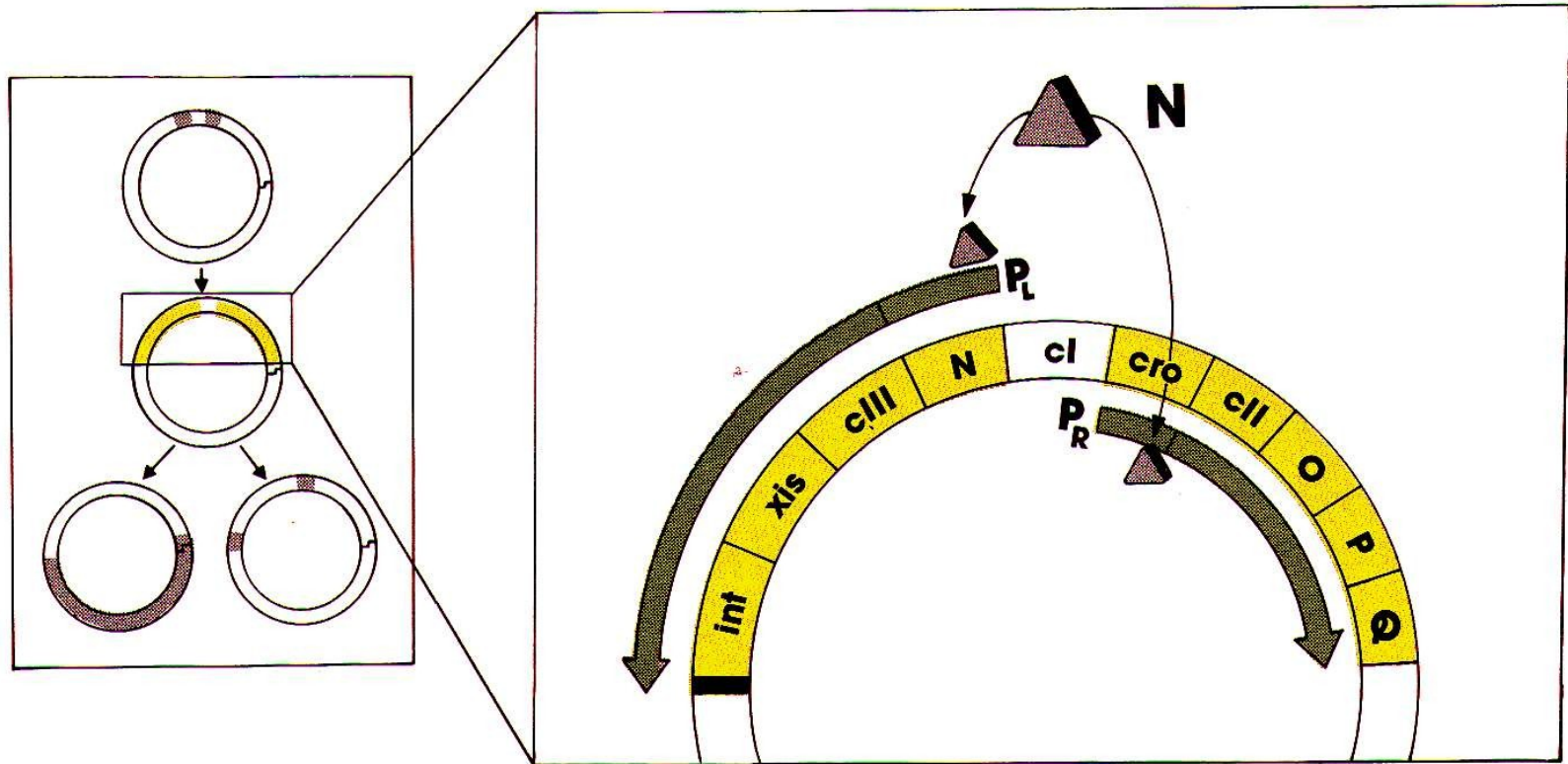
# Liaison de CI sur les opérateurs



# La décision lyse-lysogénie

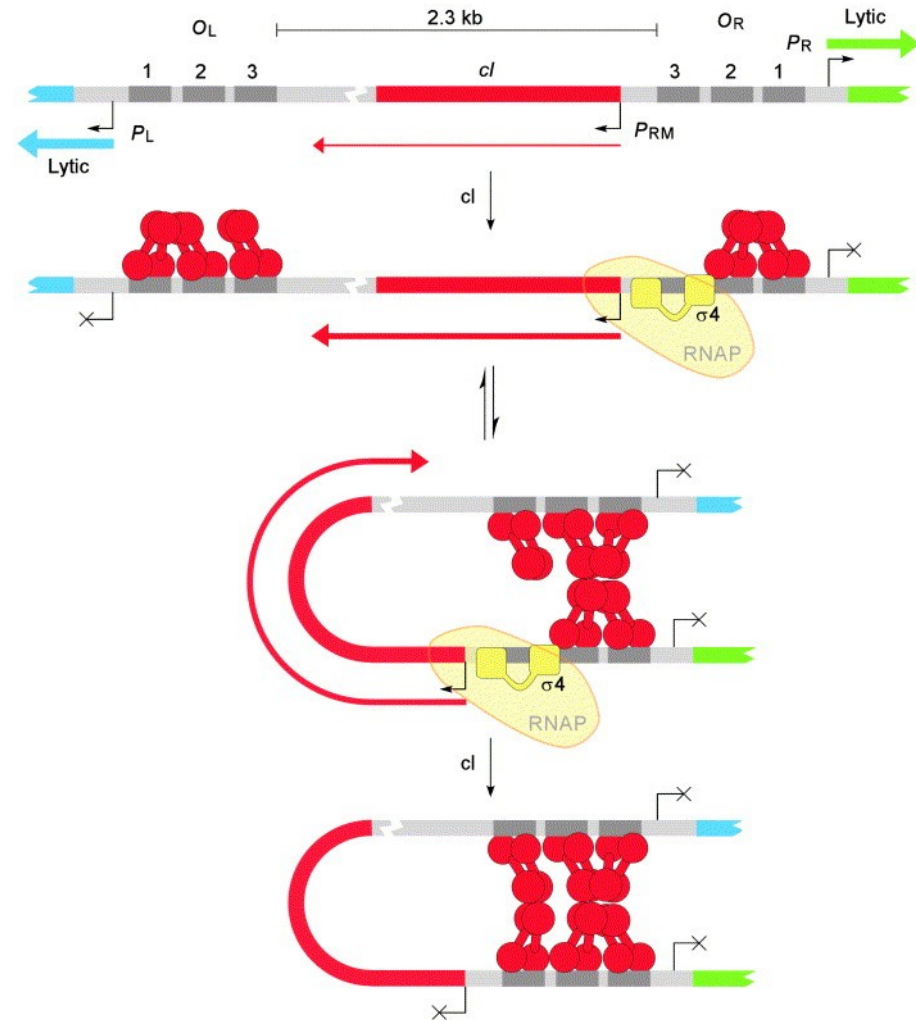


# Evénements précoces après l'infection



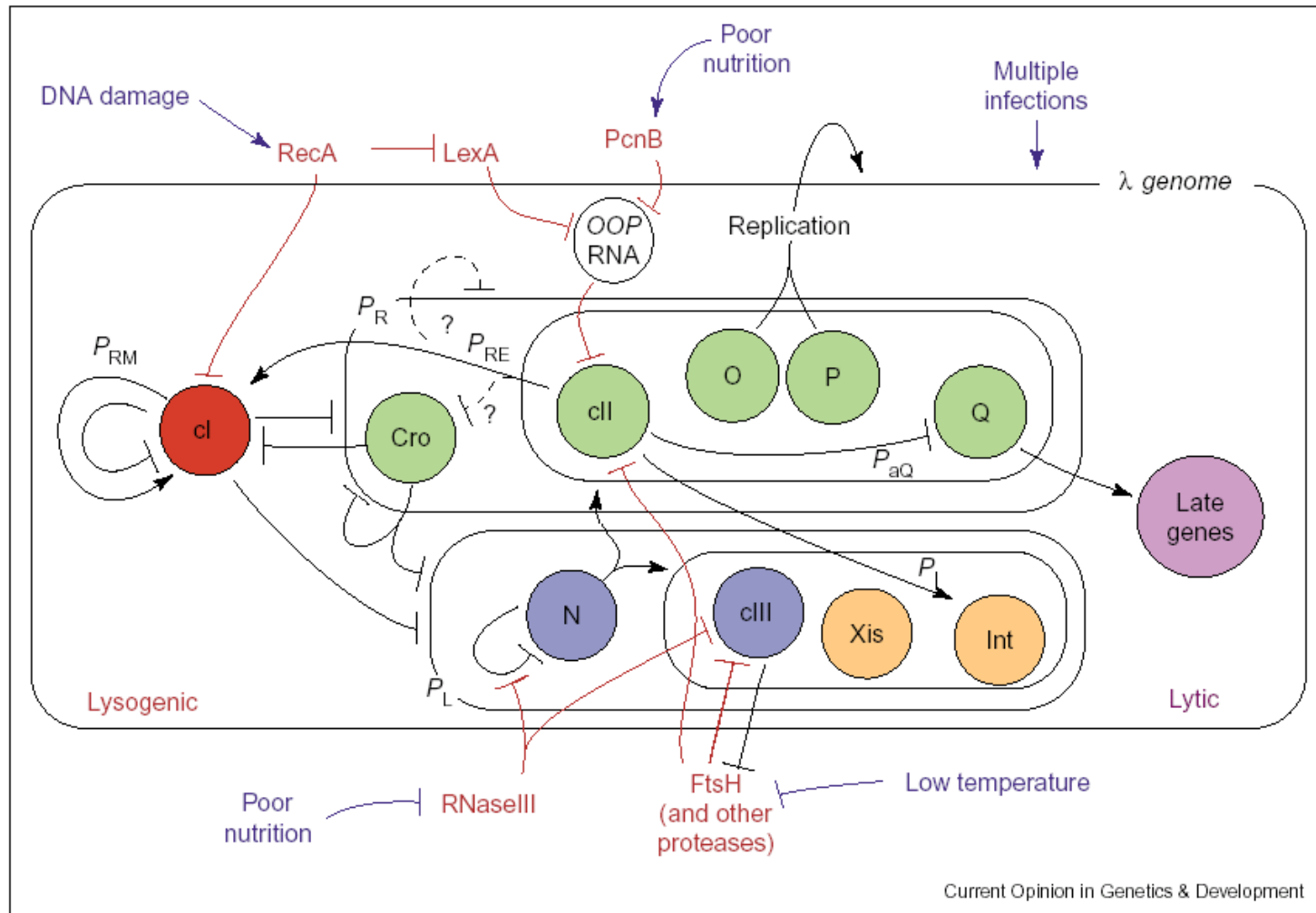
# Régulation de la transcription par CI

- CI participe à la régulation de la transcription aux promoteurs  $p_R$ ,  $p_L$  et  $p_{RM}$ .
- Coopérativité entre les dimères de CI



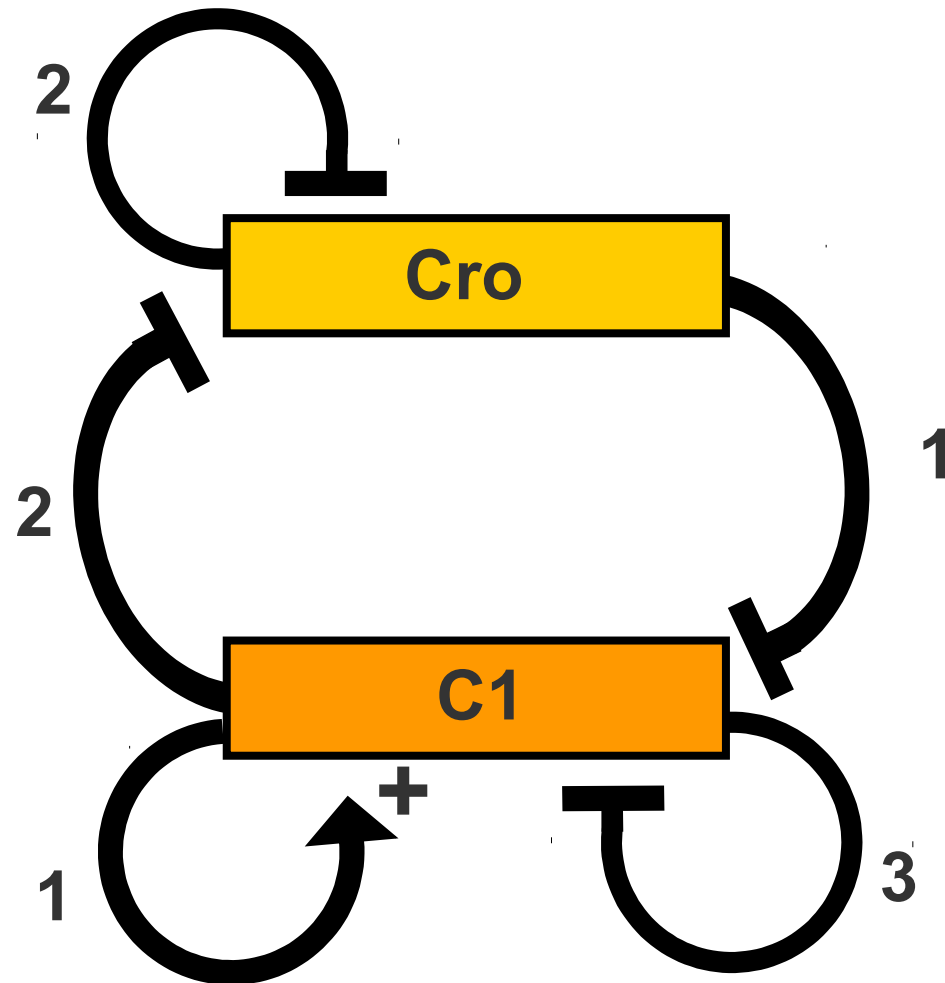
Dodd, I.B. et al. (2005).  
Curr.Op. Gen. Dev., 15, 145-152.

# Complexité du système de régulation du bactériophage $\lambda$



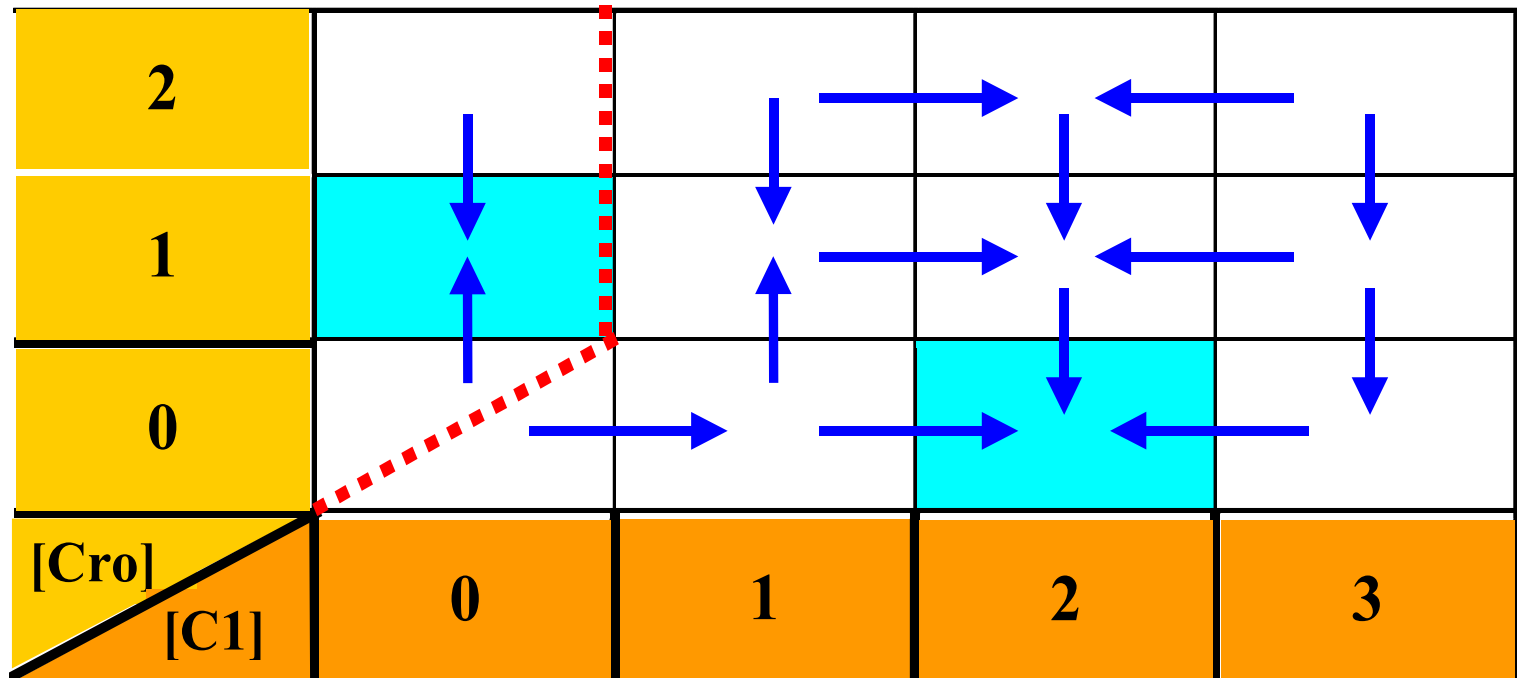
# Modèle simple de la décision lyse-lysogénie

- La connectivité du réseau de régulation



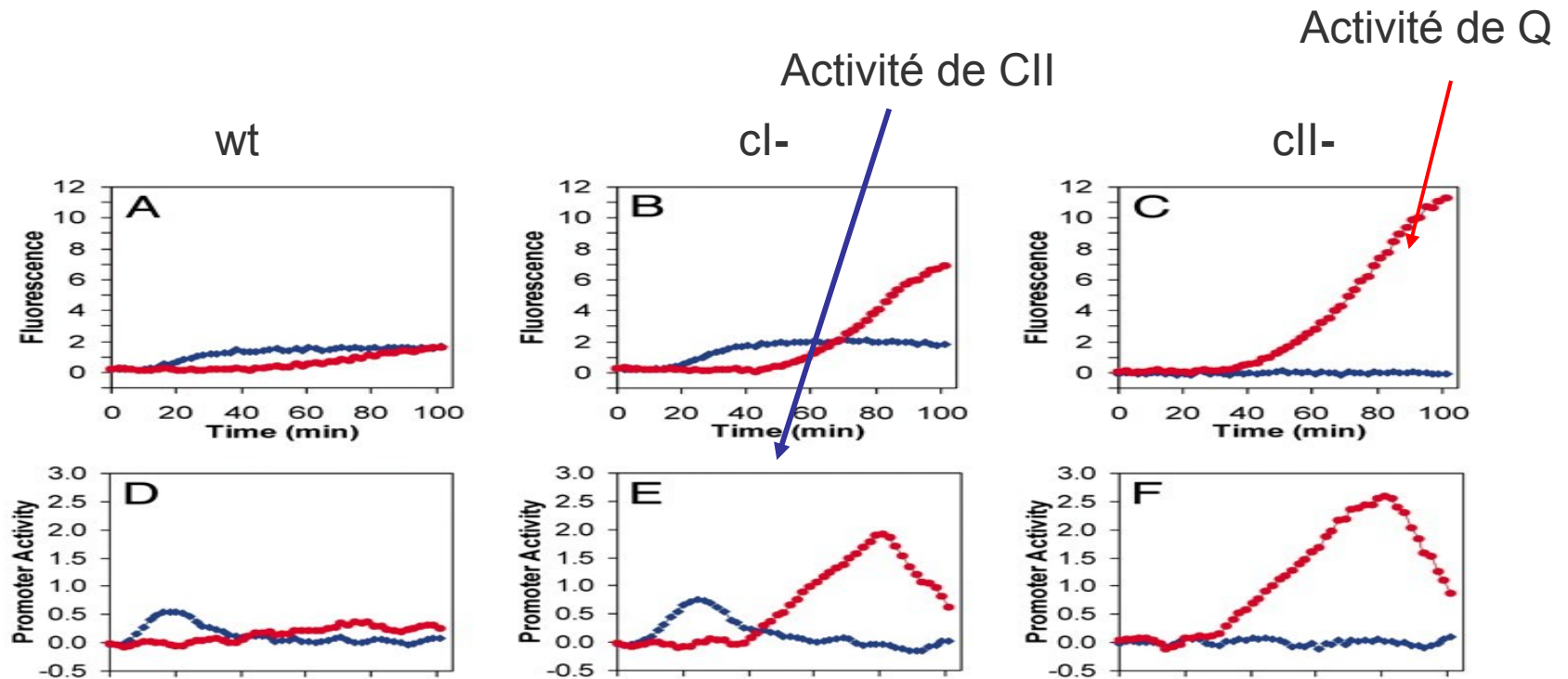


# Dynamique qualitative du réseau de régulation



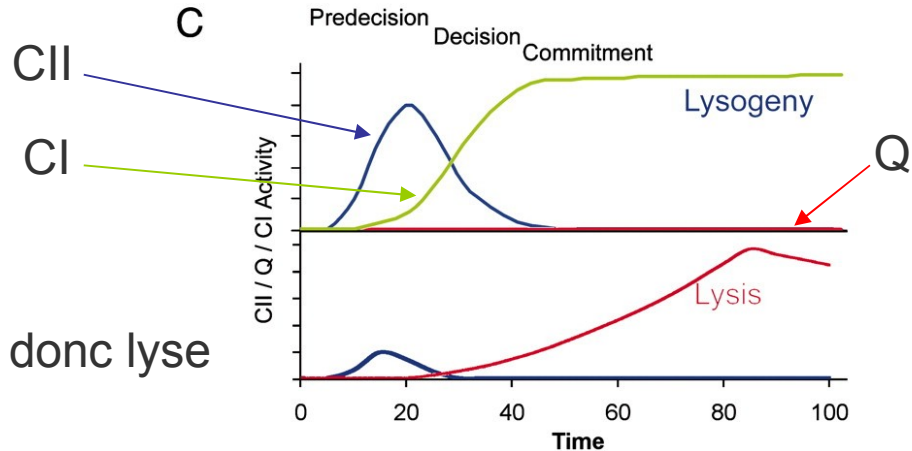
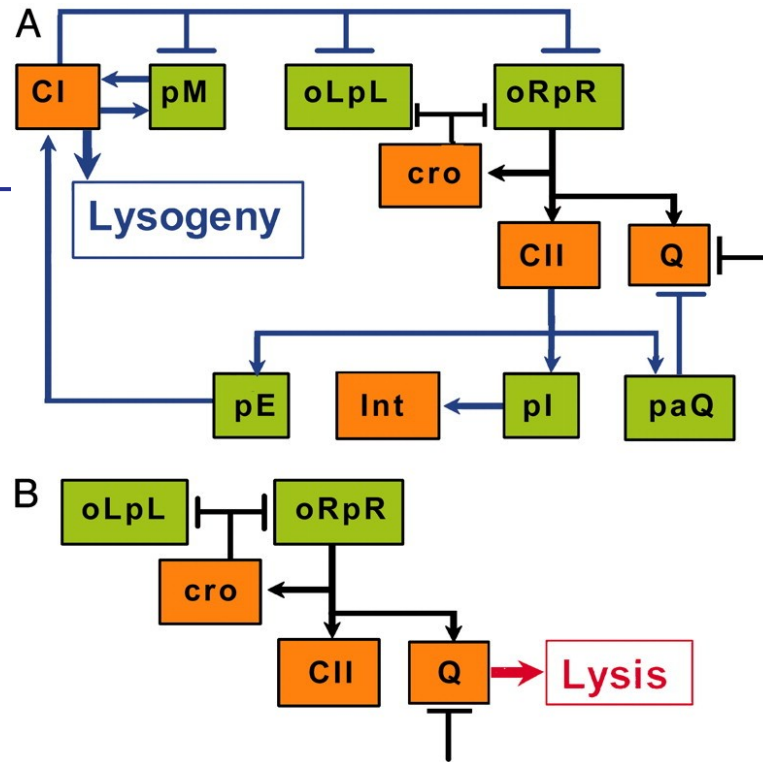
# CII active CI et inhibe Q

- Délai entre activation de CII et de Q



Kobiler, Oren et al. (2005) Proc. Natl. Acad. Sci. USA 102, 4470-4475

# Décision lyse-lysogénie

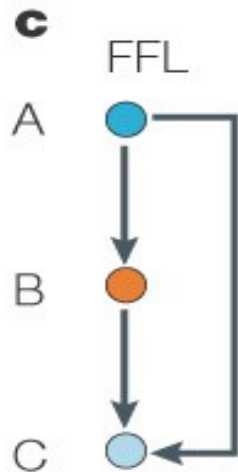


Trop peu de CII, donc lyse

# Caractéristiques de réseaux de régulation transcriptionnelle

## □ Le motifs de connexion

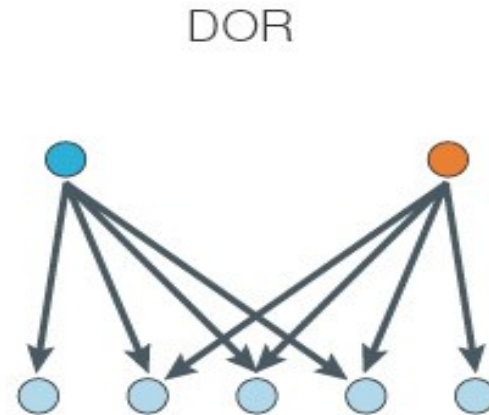
Feed forward loop



Single input module

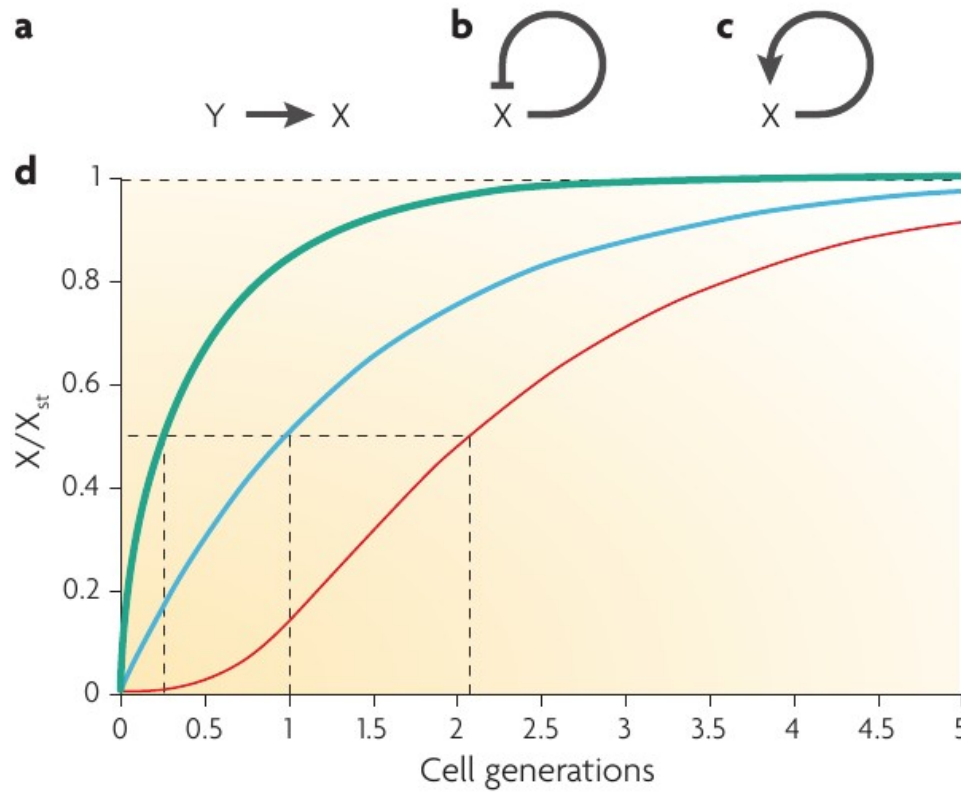


Dense overlapping regulon



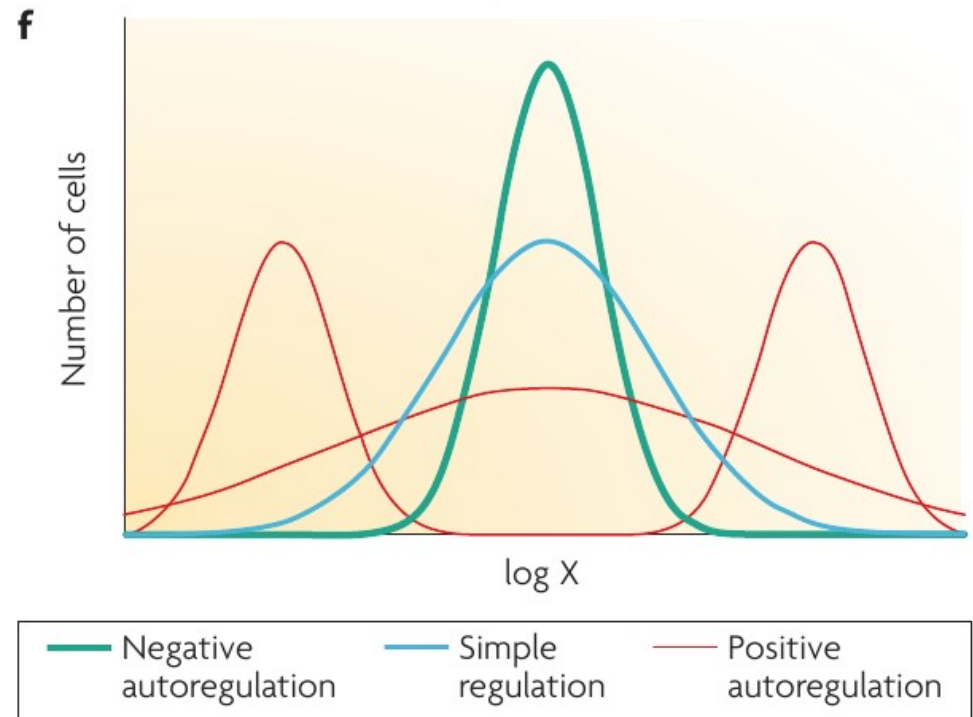
# Motifs de régulation simples

□ La vitesse de réponse dépend de la connectivité



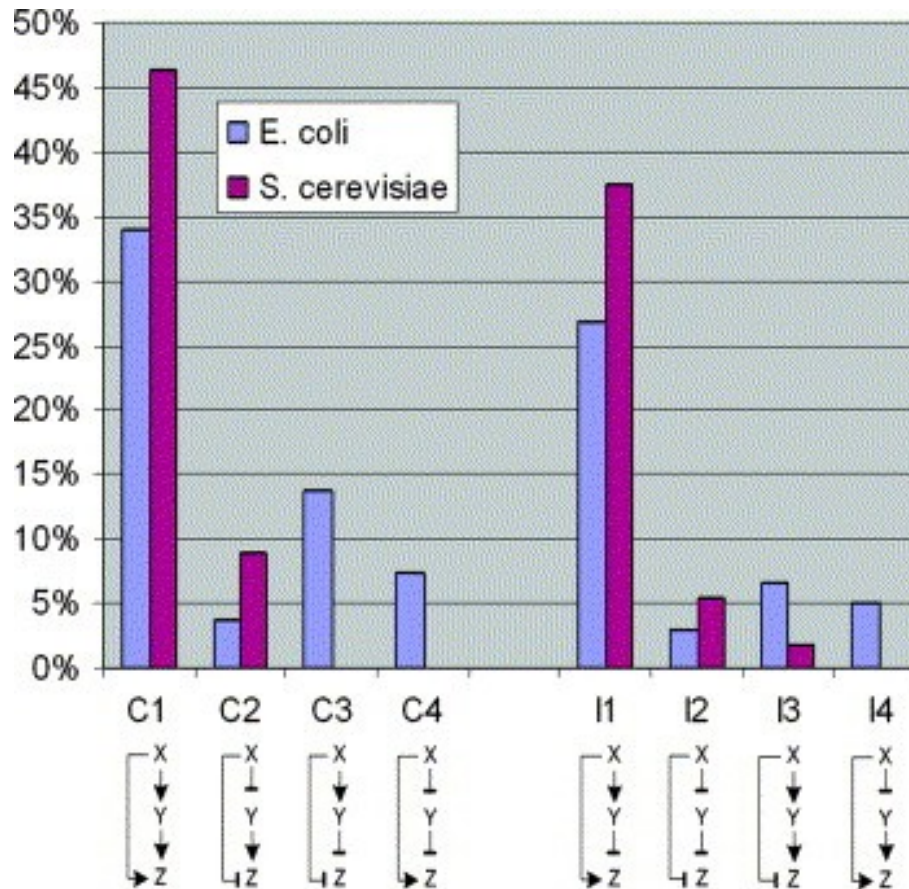
# Distribution des régulateurs

- La régulation négative produit des distributions étroites
- La régulation positive produit des distributions large (même bi-modales)



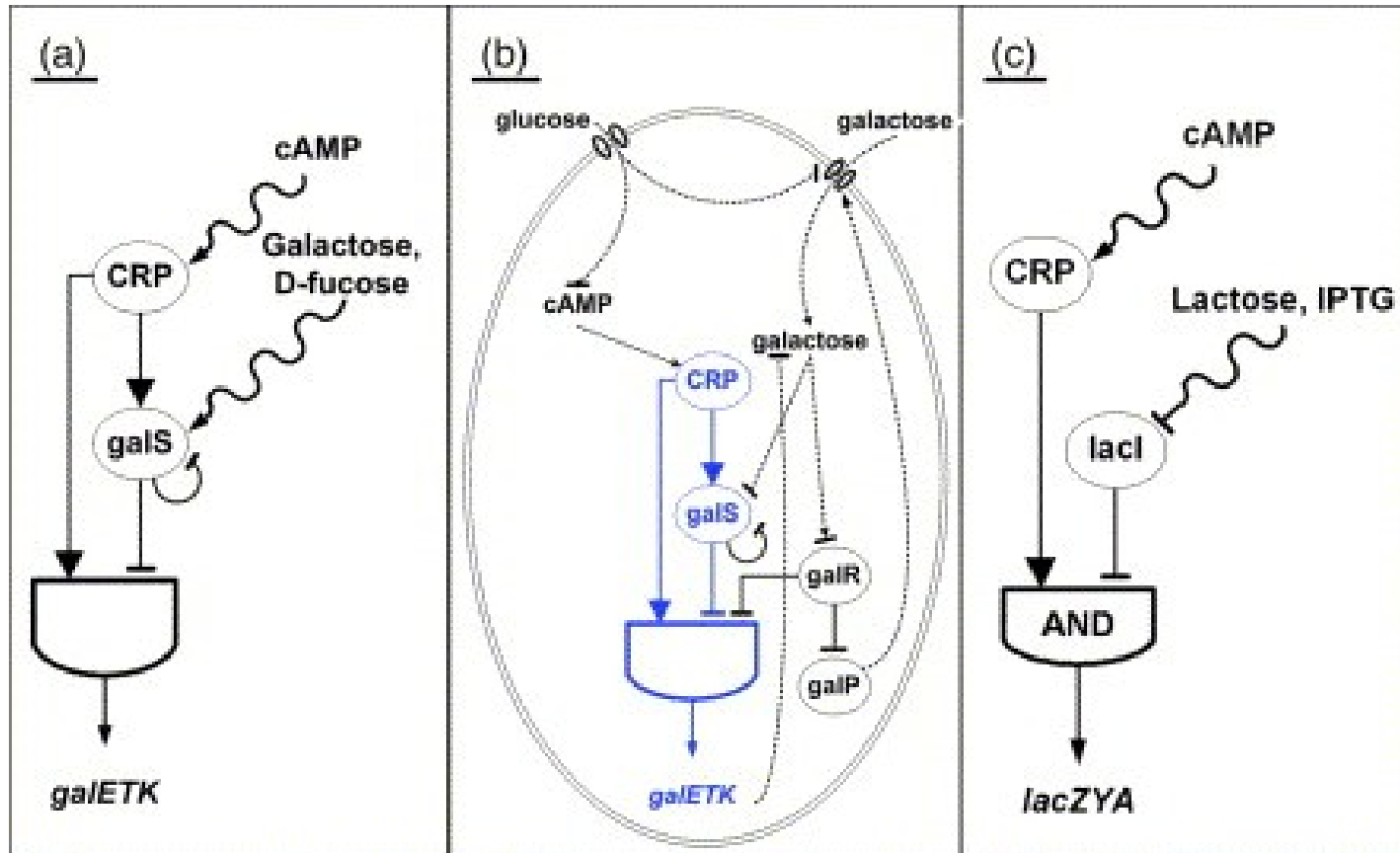
# Les huit types de FFL

□ Distribution similaire chez *E. coli* et *S. cerevisiae*



# The galactose and lactose systems of E. coli

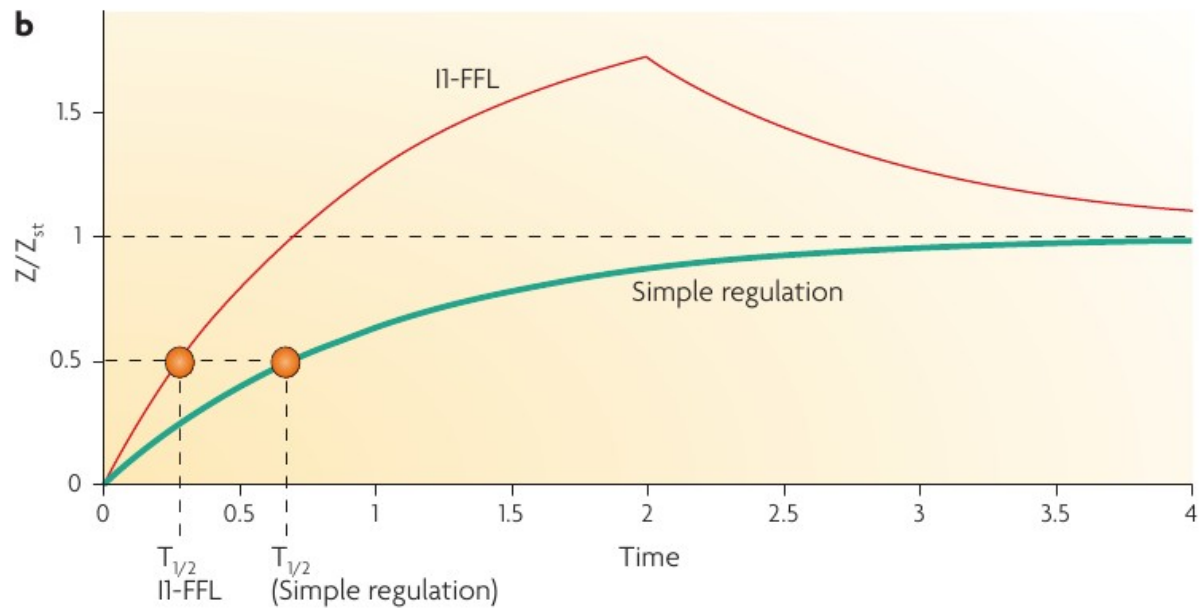
## □ Les FFL





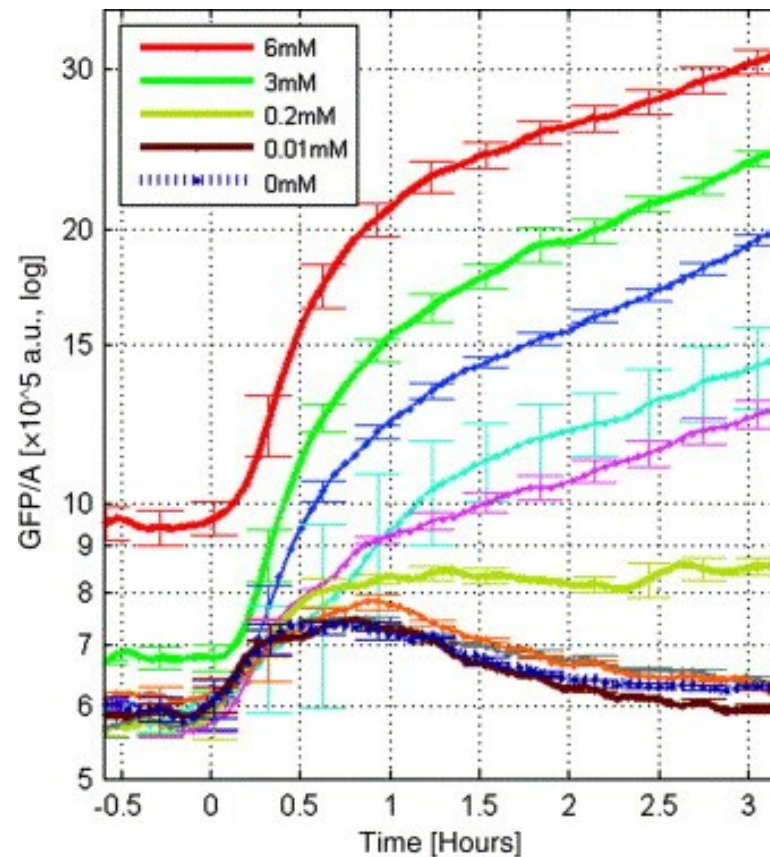
# Feed forward loop

## □ Réponse rapide (théorie)



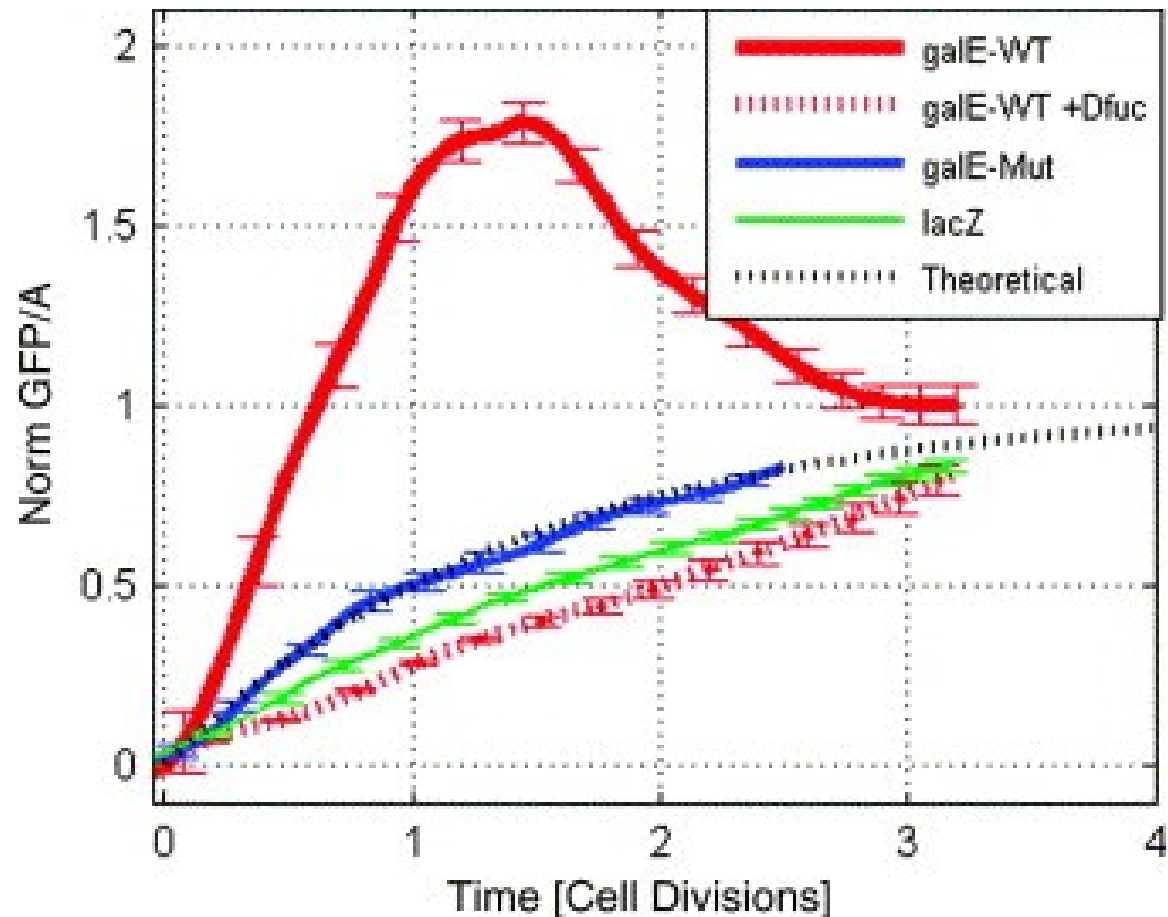
# Dynamique du promoteur *galE*

- Induction du promoteur *galE* par épuisement de glucose à temps zéro
- Différentes concentrations de l'inducteur non-métabolisable, d-fucose



# Expression du promoteur galE par rapport au steady state

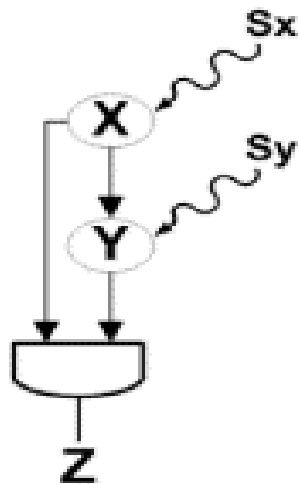
□ Le glucose est épuisé à temps zéro



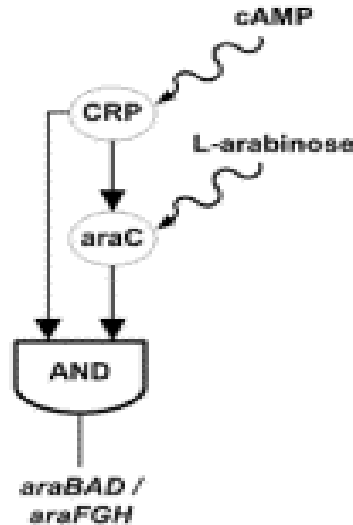
# Les FFL cohérents

□ Le FFL cohérent avec ces inducteurs (ara)

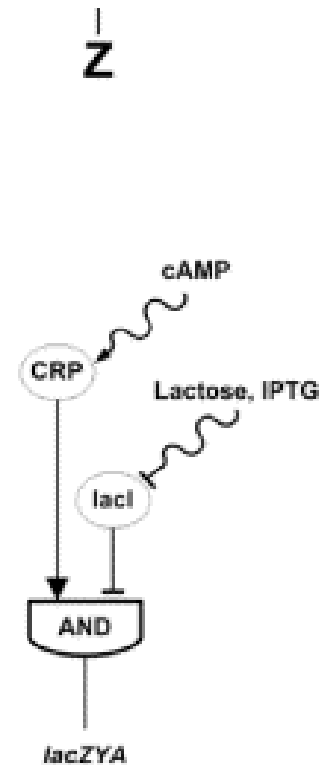
a)



b)



c)



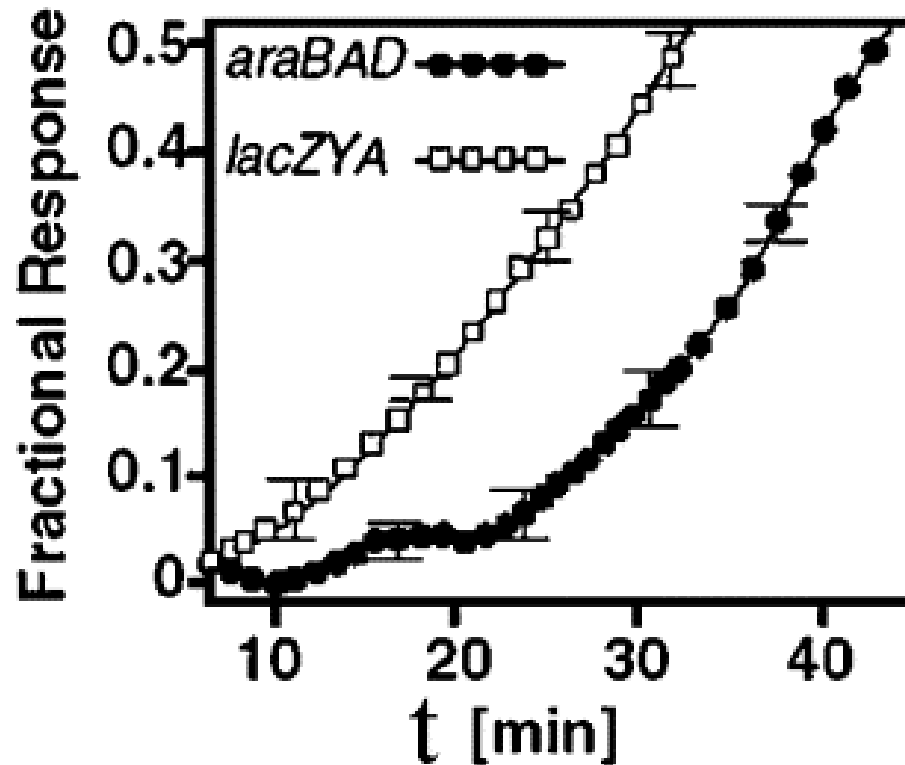
d)

c)

d)

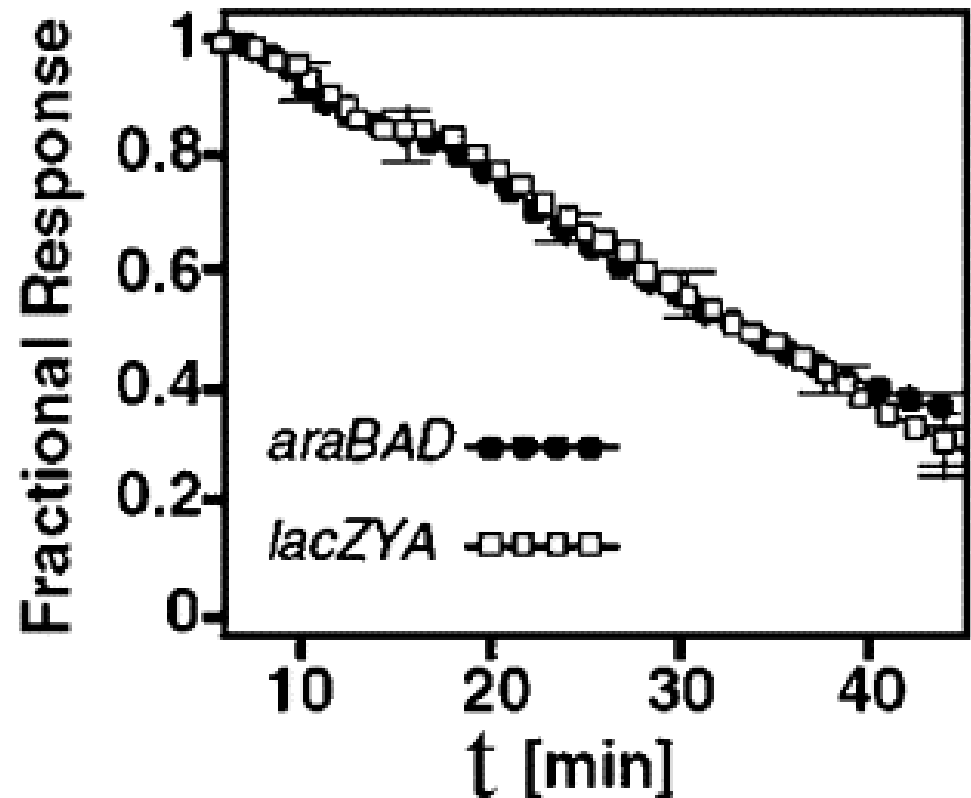
# Mesure de l'activité par la GFP

- 10 mM AMPc sont ajoutés à temps zéro
- Délai d'activation pour le FFL cohérent



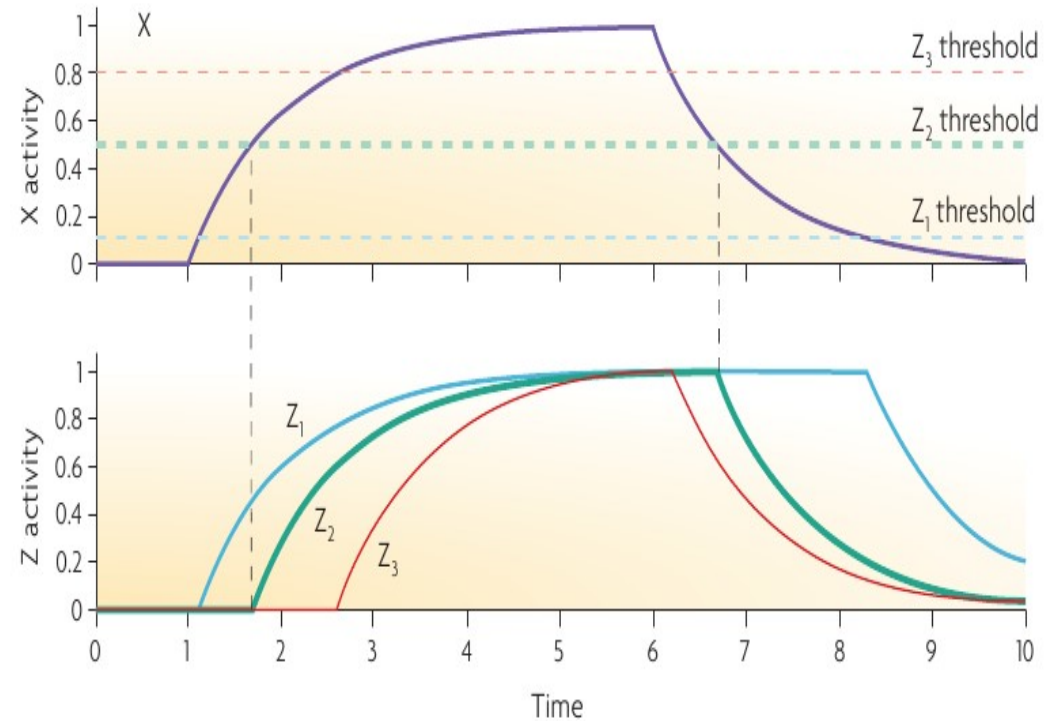
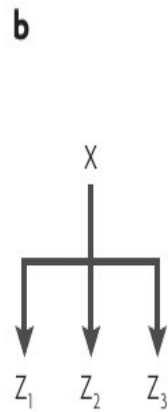
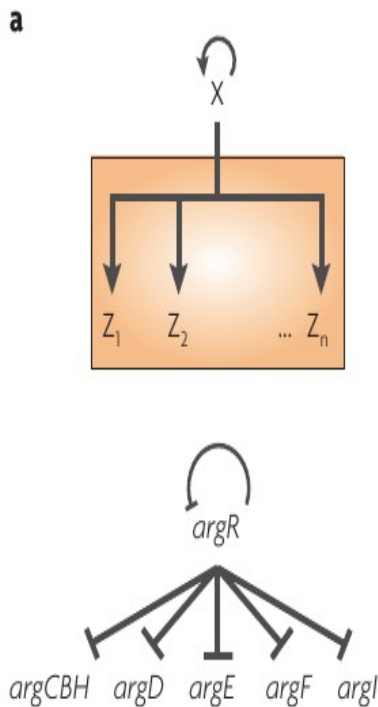
# Enlever l'AMPc

- Les bactéries poussent sur glycérol et glucose est rajouté à temps zéro
- Pas de délai pour arrêt de l'induction



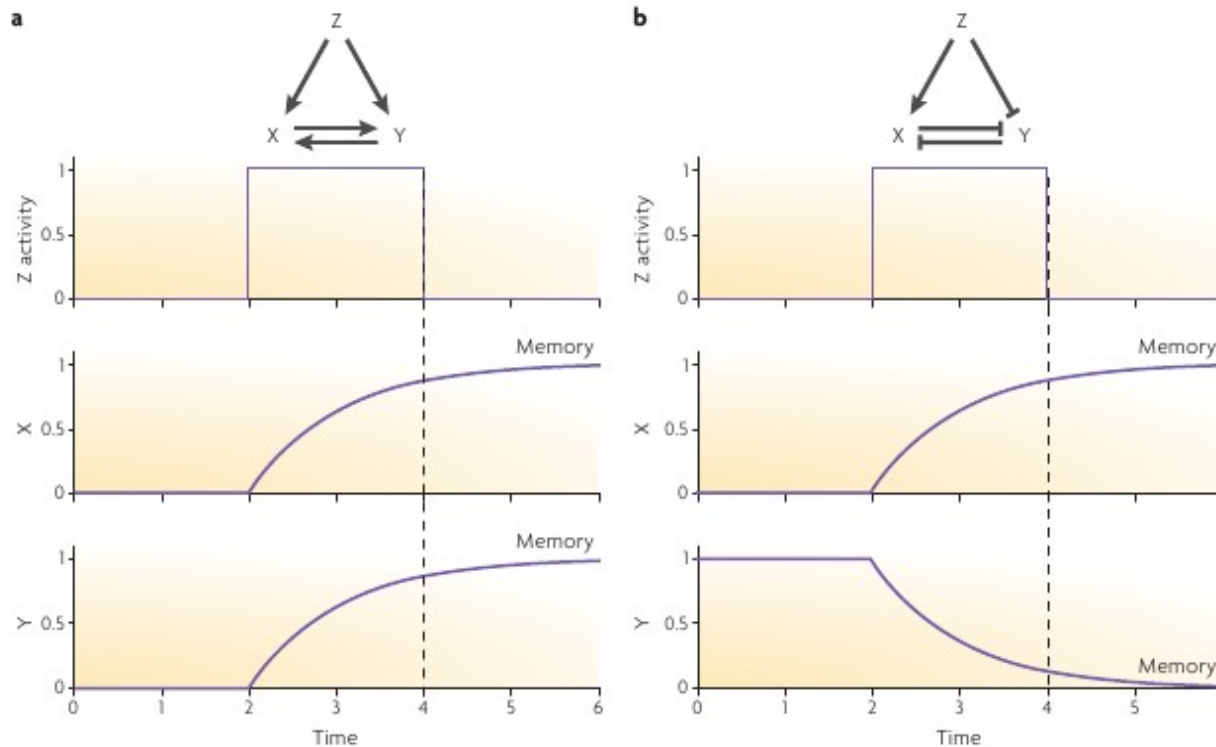
# Single Input Module

## □ Activation séquentielle



# Les réseaux de régulation dans le développement

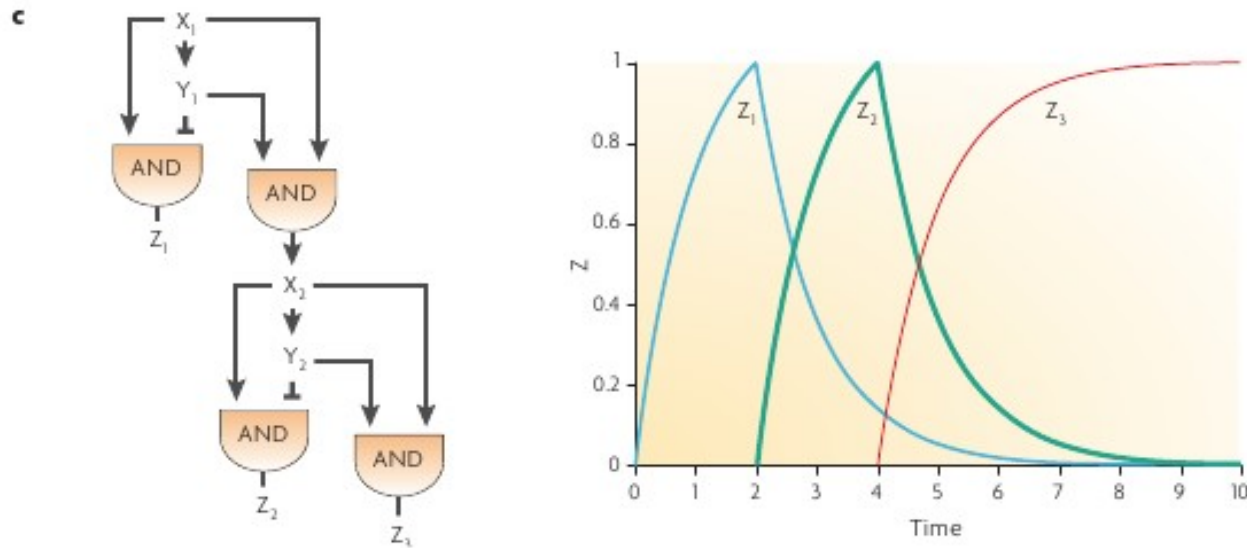
## □ Un signal initial est mémorisé



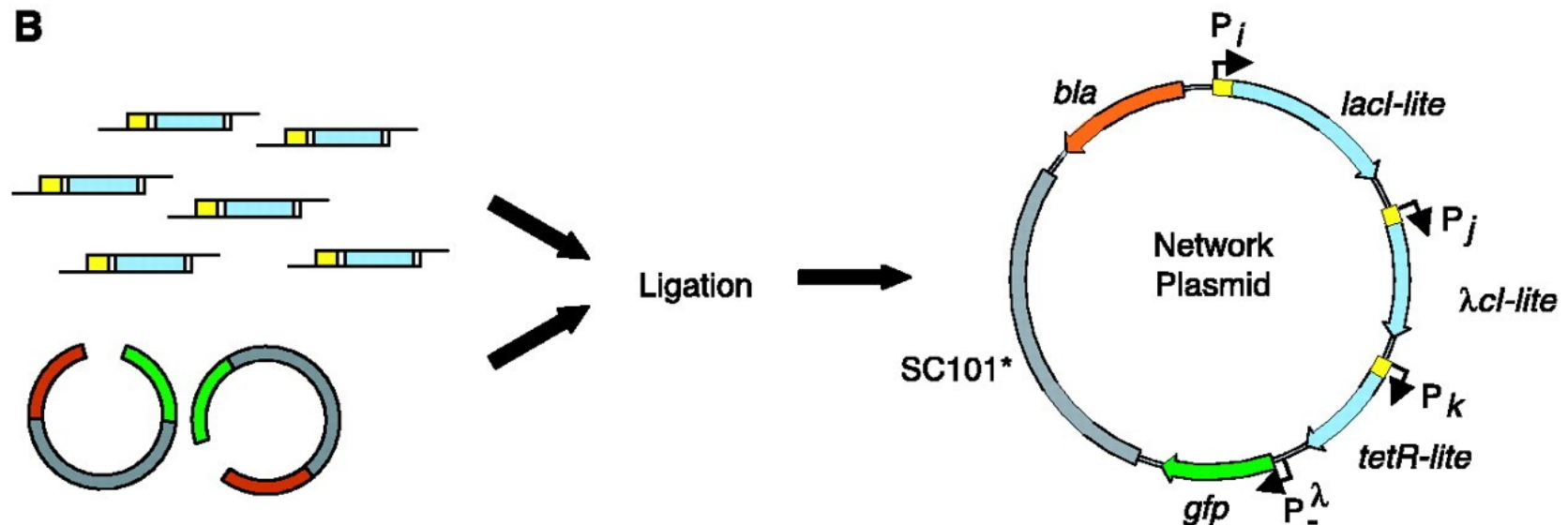
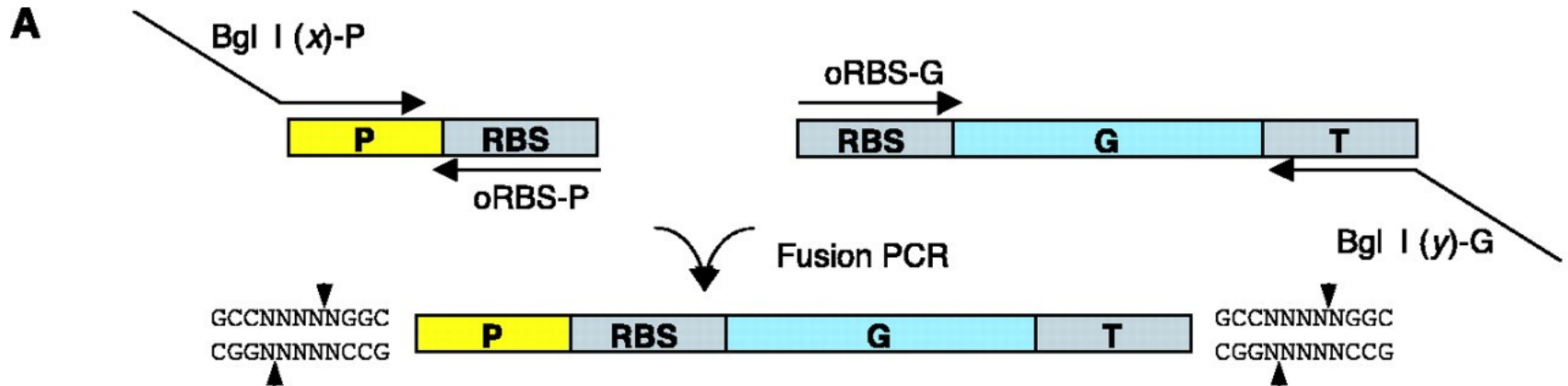


# Les réseaux de régulation dans le développement

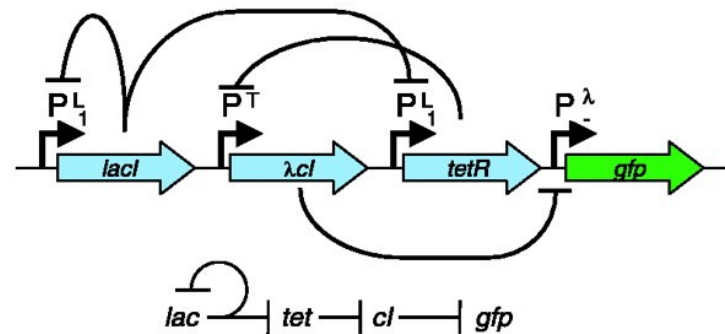
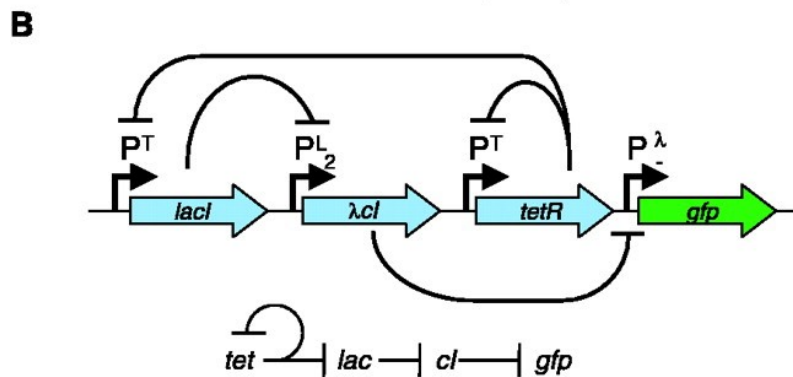
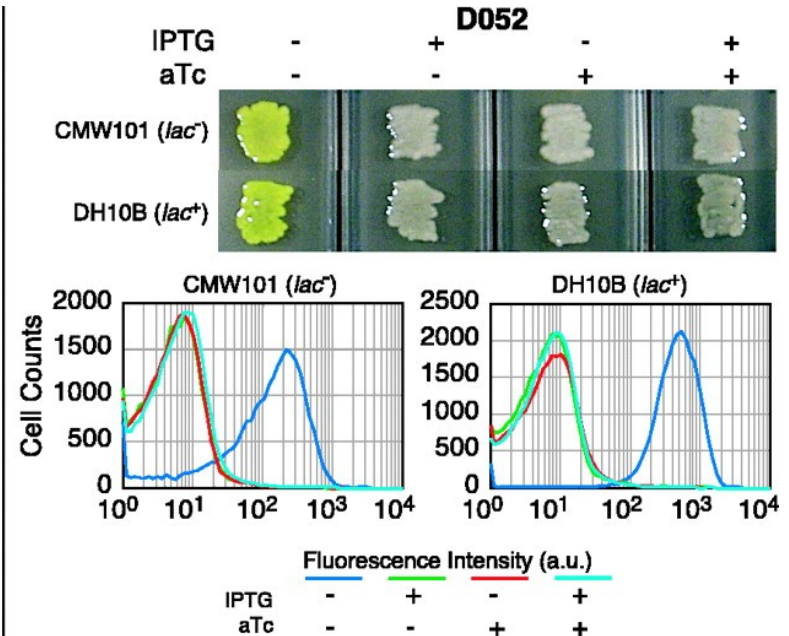
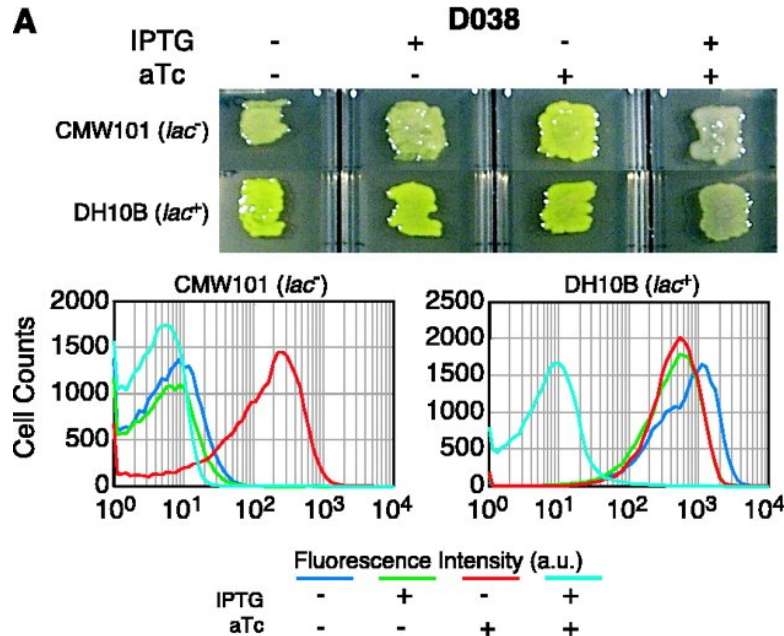
- Les connectivités plus compliquées conduisent à une activation séquentielle



# Combinatorial construction of gene regulatory networks



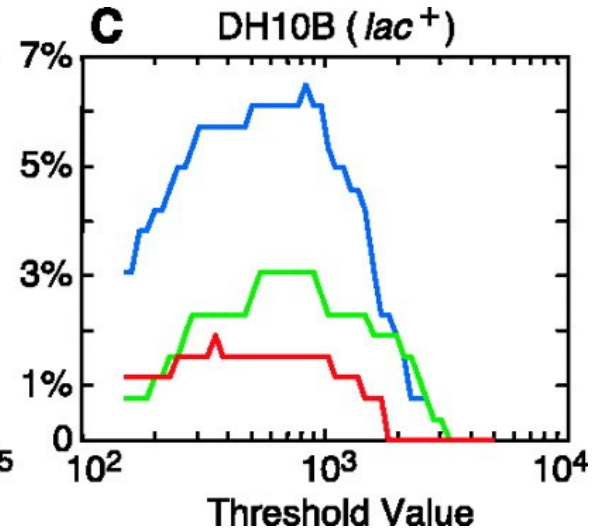
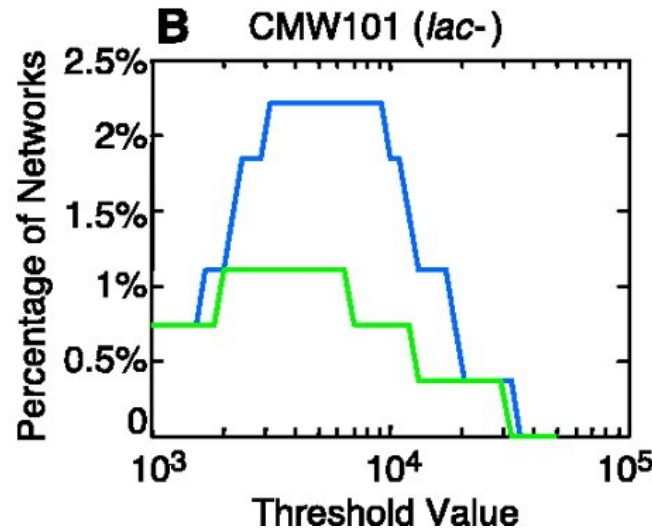
# Two examples of logical circuits: NOR NAND



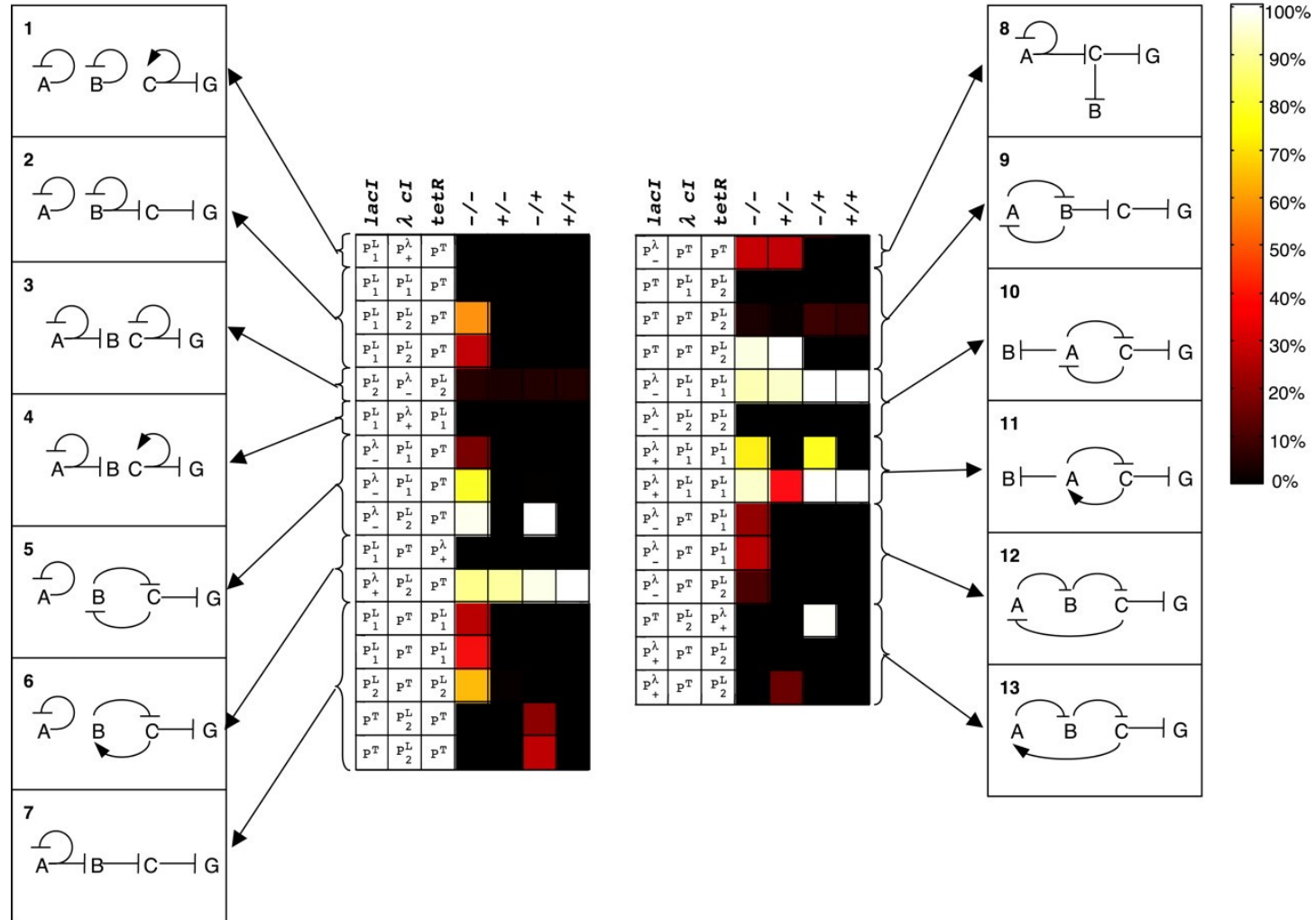
# Proportion of binary logical networks in the two different cell types

**A**

	IPTG	-	+	-	+
	aTc	-	-	+	+
—	NOR	On	Off	Off	Off
—	NOT IF	Off	On	Off	Off
—	NAND	On	On	On	Off

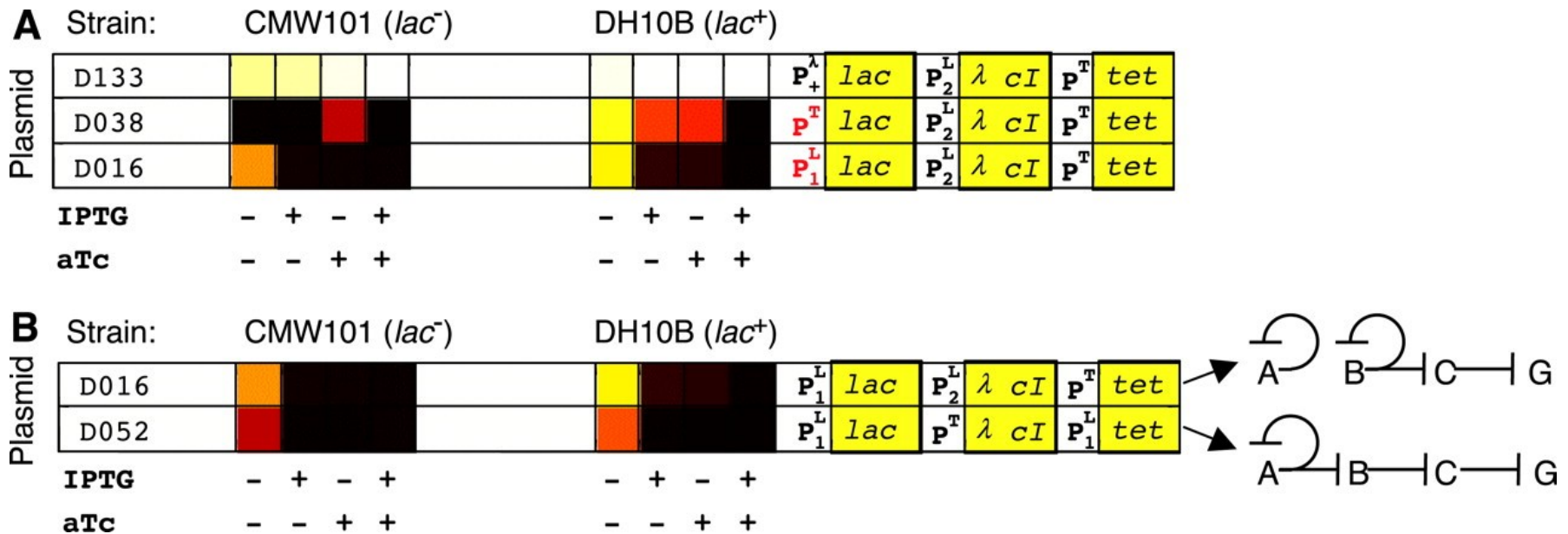


# Dependence of phenotypic behavior on network connectivity

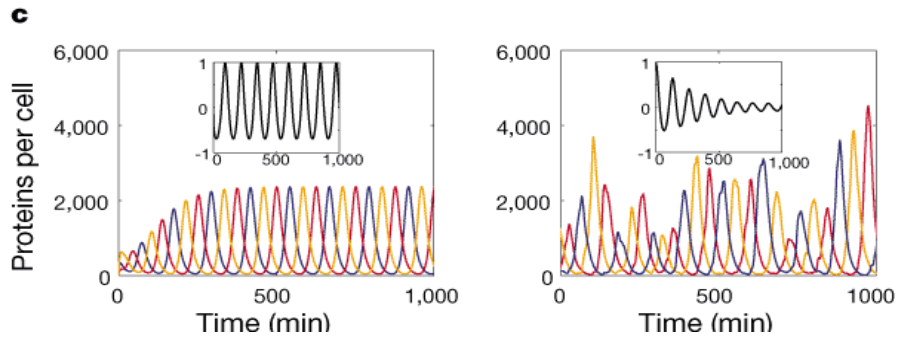
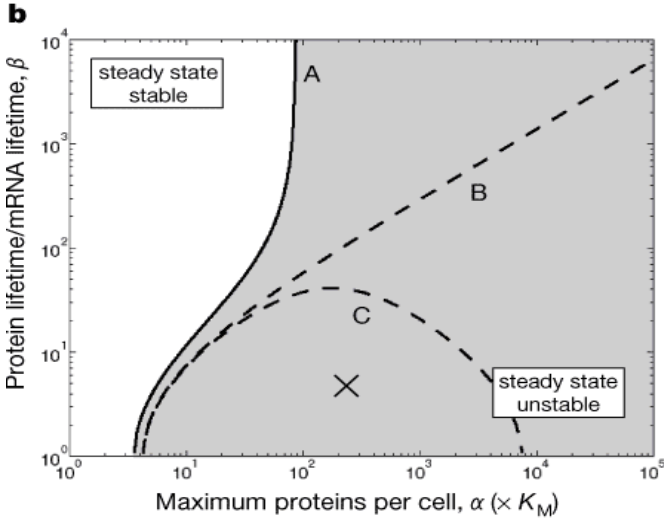
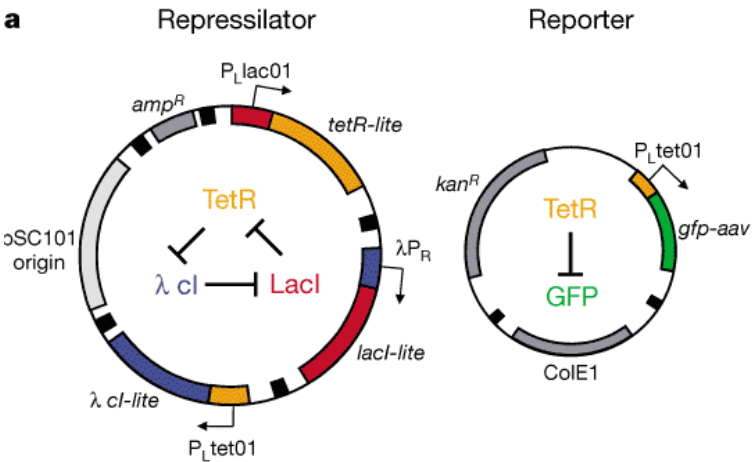


# Comportement dynamique des réseaux

- Small changes in the network lead to very different behaviors
- The same behavior can be achieved by different networks



# A synthetic oscillator



# Synthetic oscillator

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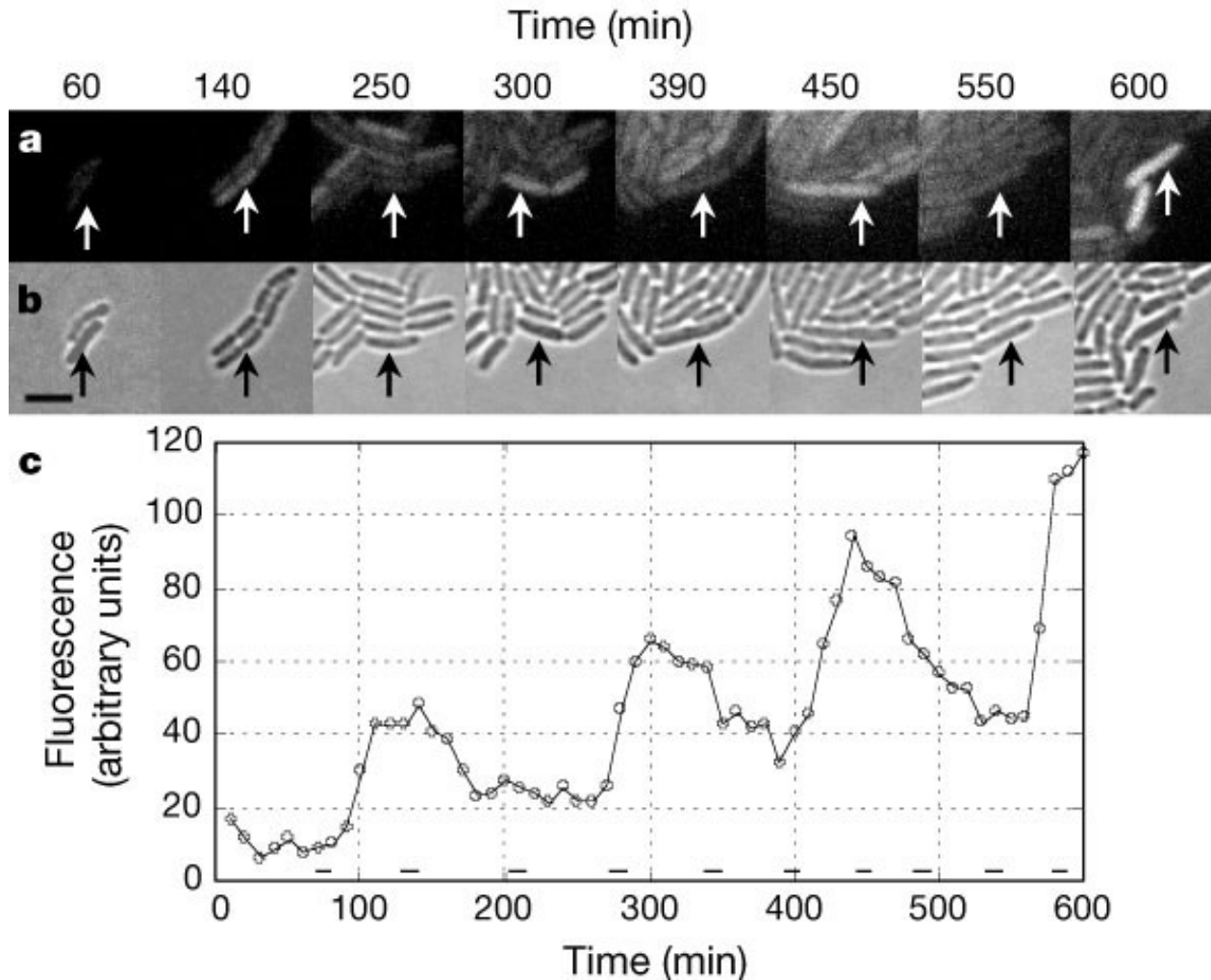
$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{1 + p_j^n} + \alpha_0$$

$$\frac{dp_i}{dt} = -\beta \times (p_i - m_i)$$

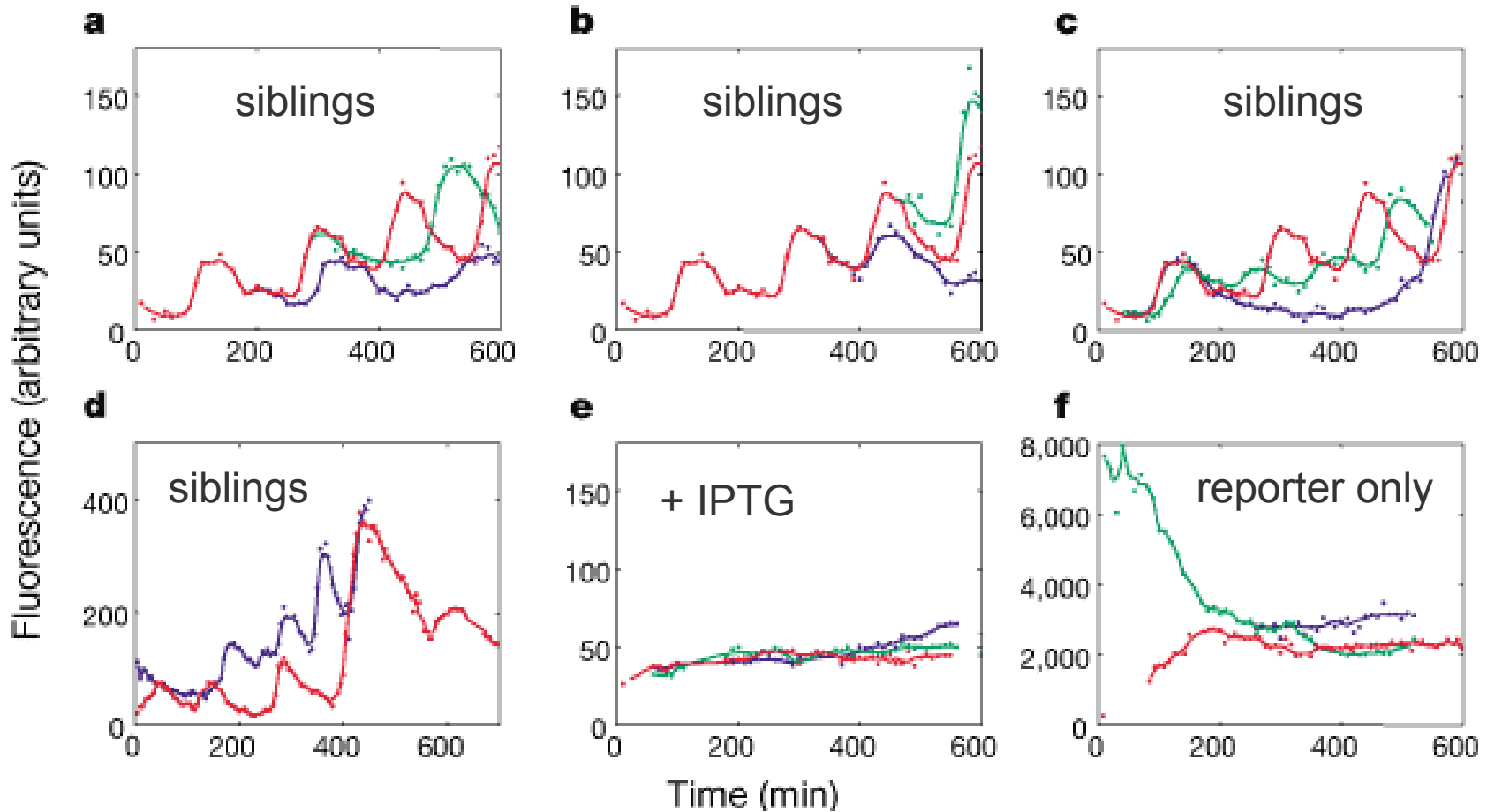
$$\left( \begin{array}{l} i = lacI, tetR, cl \\ j = cl, lacI, tetR \end{array} \right)$$



# Oscillations are maintained through cell division

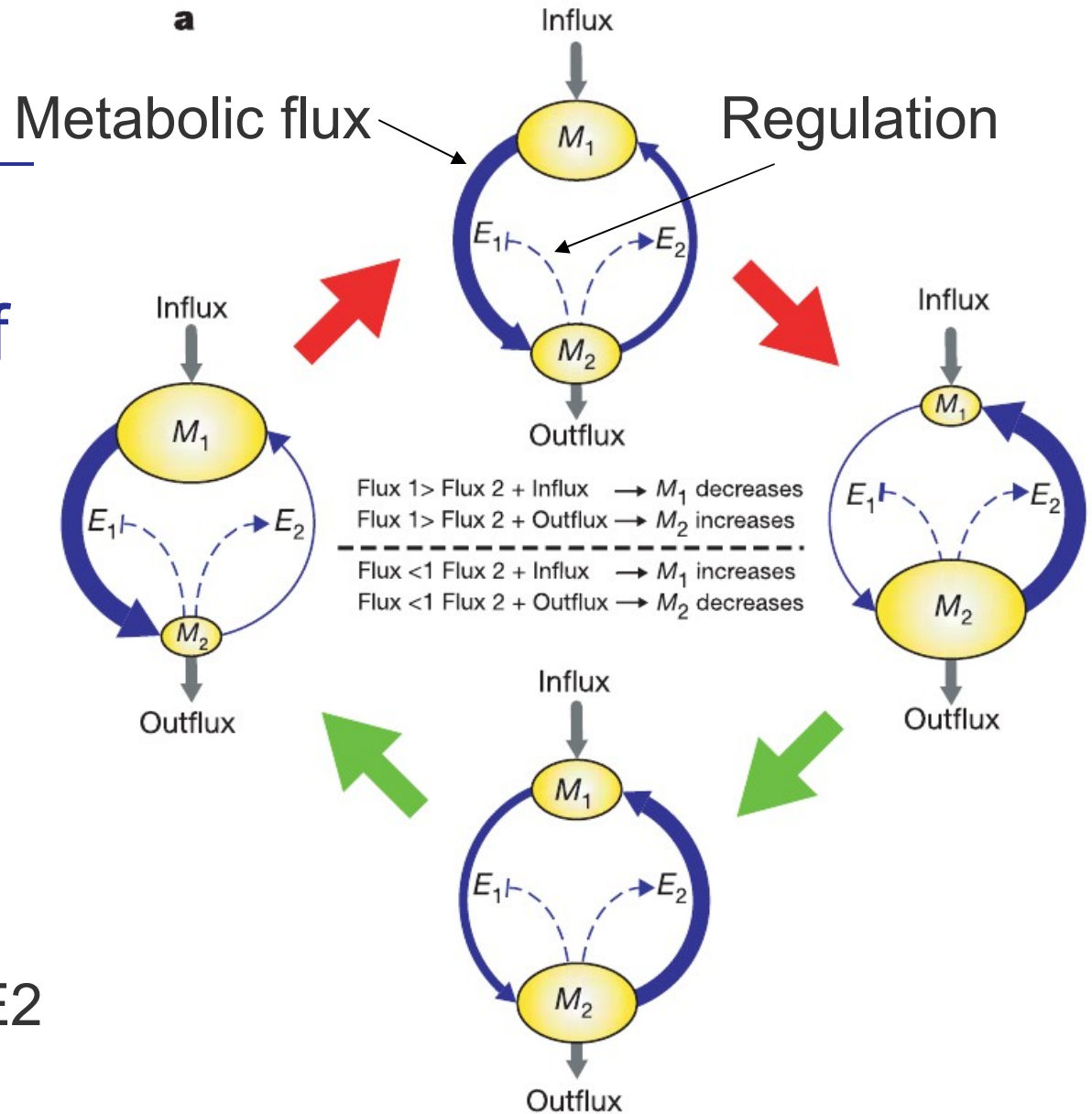


# Examples of oscillatory behavior



# Construction of a metabolic oscillator

Two metabolites, M1 and M2, and their control by enzymes E1 and E2



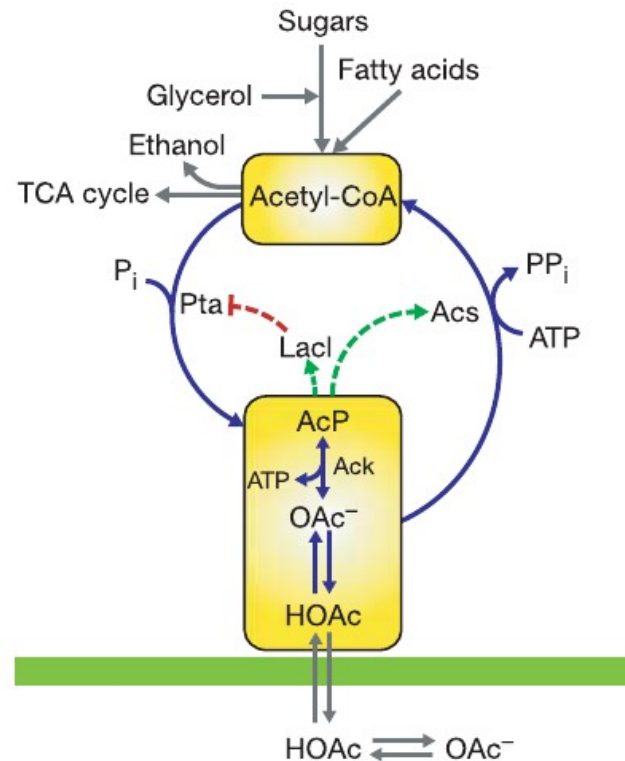
# Construction in Escherichia coli

- Use acetyl-CoA and acetyl-phosphate as metabolites

Pta: phosphate acetyltransferase

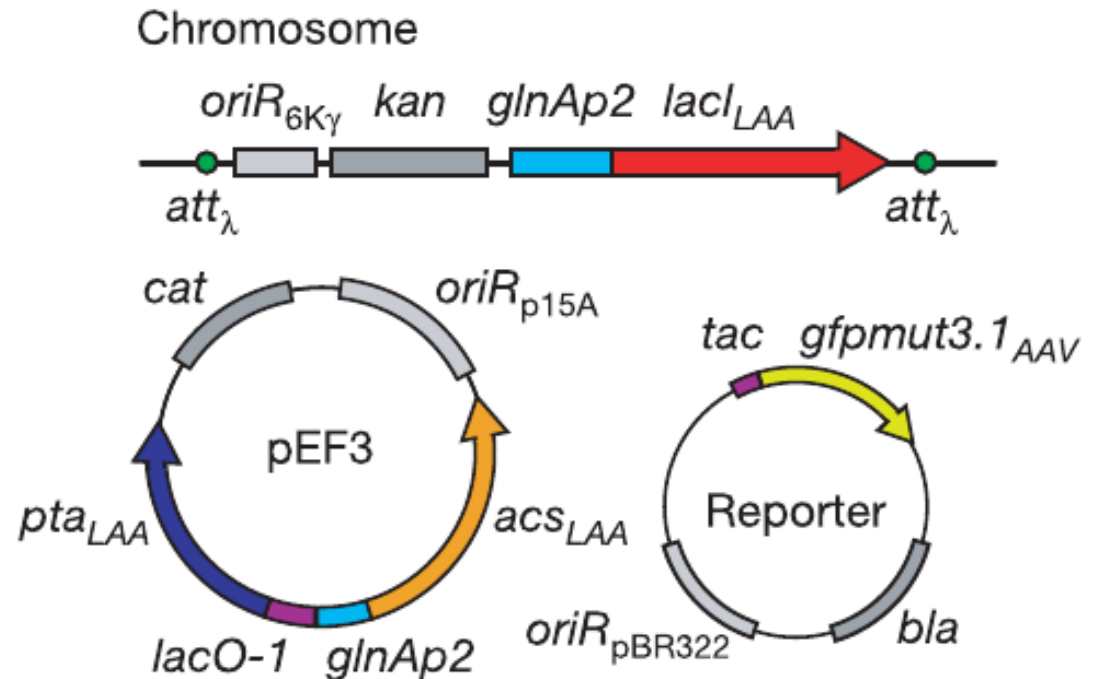
Acs: Acetyl-CoA synthetase

Ack: Acetate kinase



# Change the regulation of the enzymes

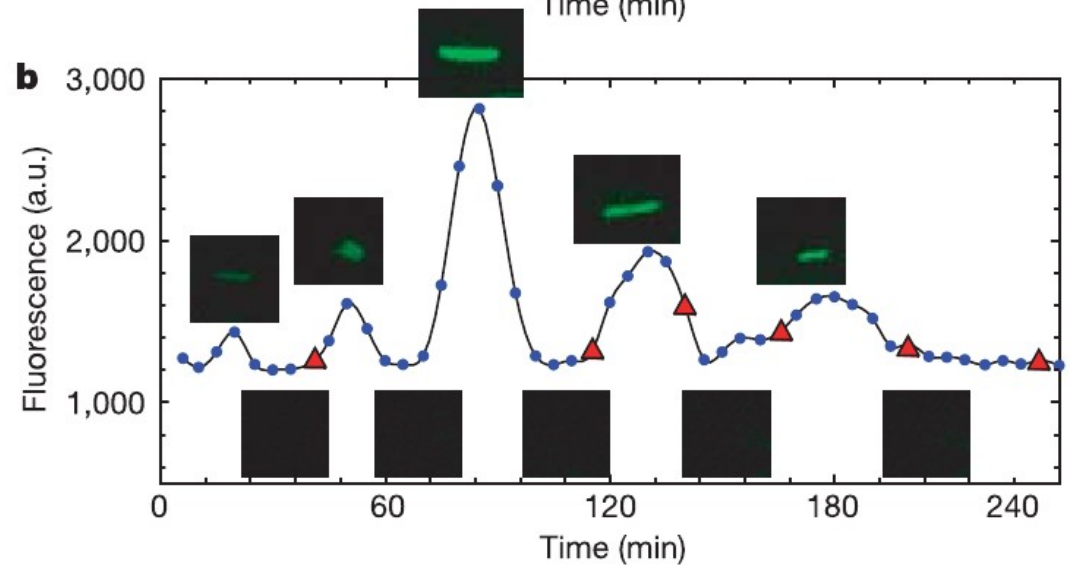
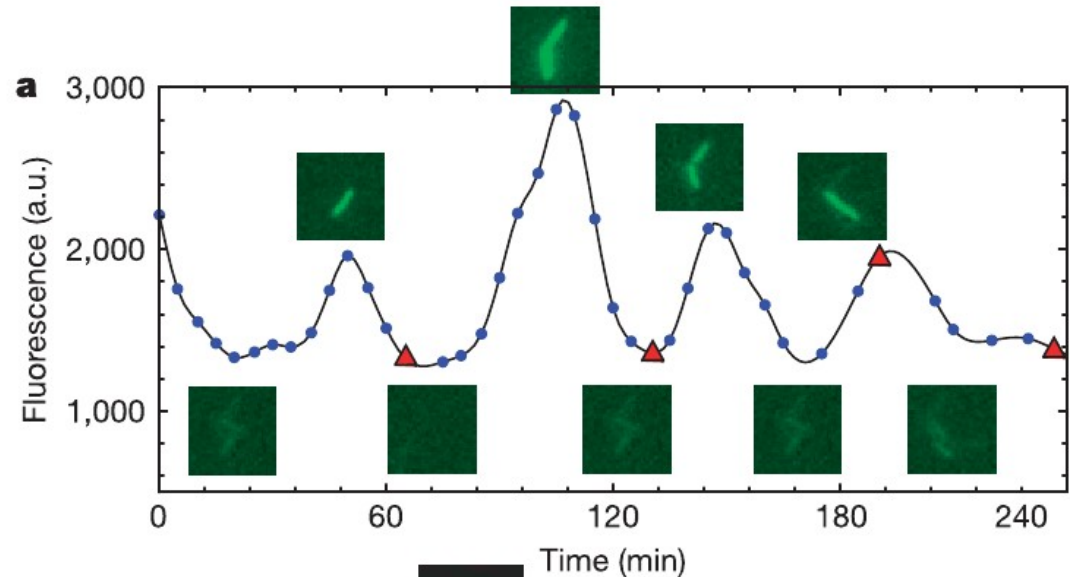
- **Ac-P**  
phosphorylates NRI  
and activates  
*glnAp2*. Put **acs**  
downstream.
- **Pta** is expressed  
from a lac promoter.
- **Lac repressor** is  
under the control of  
*glnAp2*.



Enzyme half-lives are reduced by an *ssrA*-tag

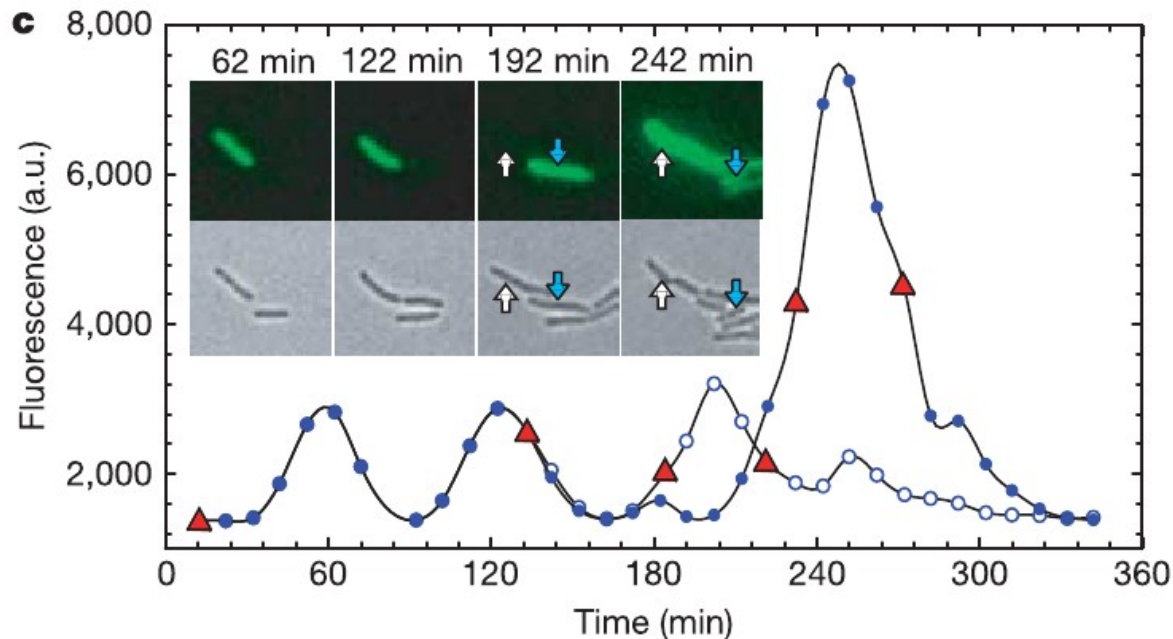
# Oscillations in single cells

- Oscillation in 60% of cells
- Period: 40 min.
- Doubling time: 60 min.



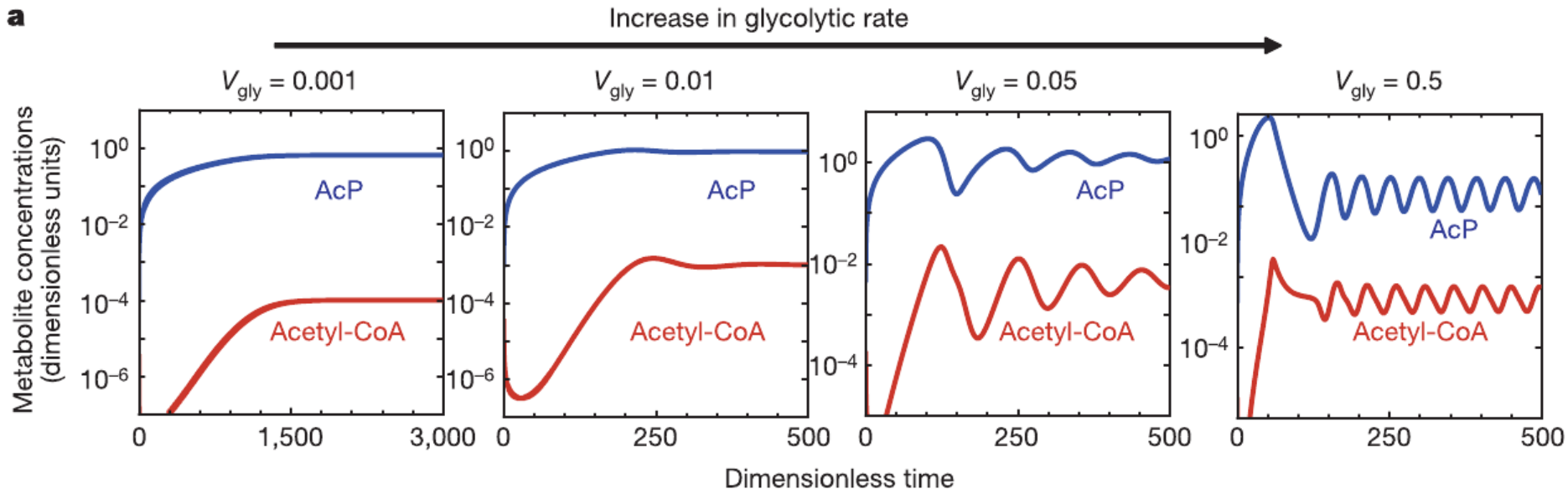
# Oscillations in single cells

- The oscillation is transmitted (more or less faithfully) to daughter cells



# Modeling the oscillator

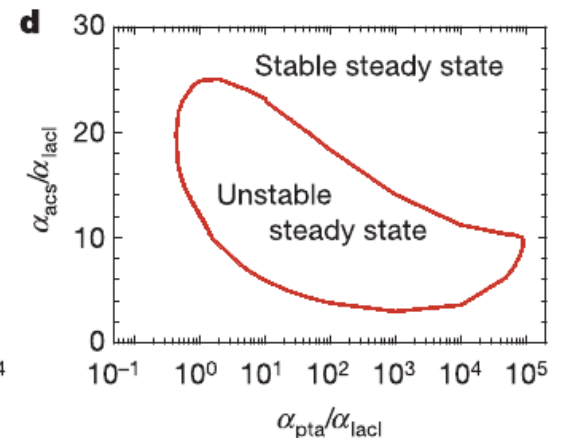
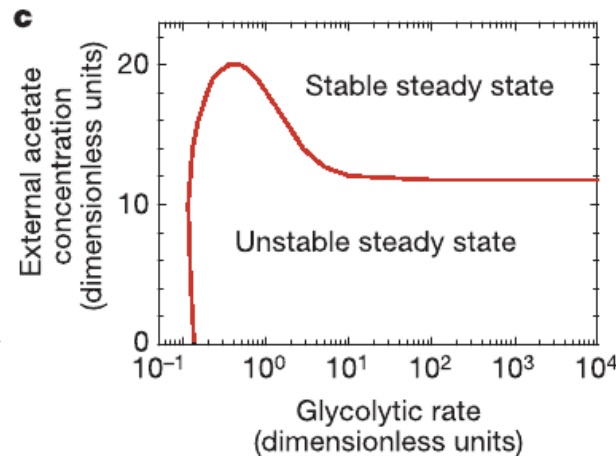
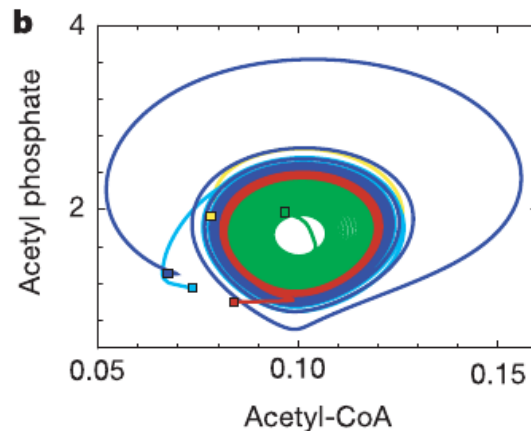
- Use Michaelis-Menten for the enzyme kinetics and Hill functions for the gene regulation
- Oscillations are predicted at high glucose flux





# Modeling the oscillator

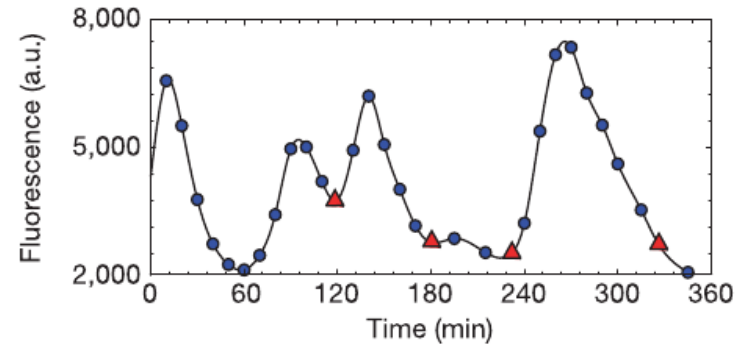
- The oscillator is a limit cycle
- External acetate stabilizes the system
- The enzyme concentrations have to be in the right range



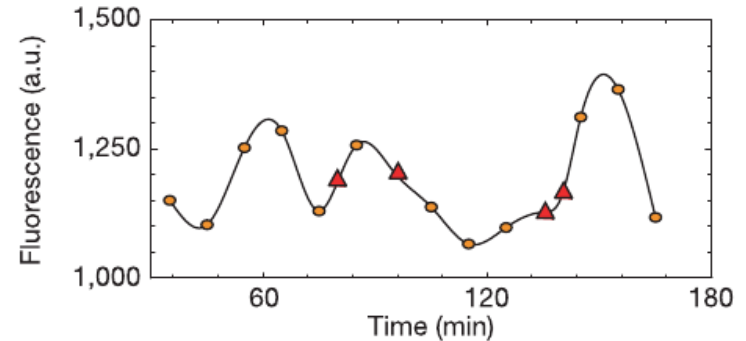
# Test theoretical predictions

- Change the glycolytic flux by changing carbon source

fructose

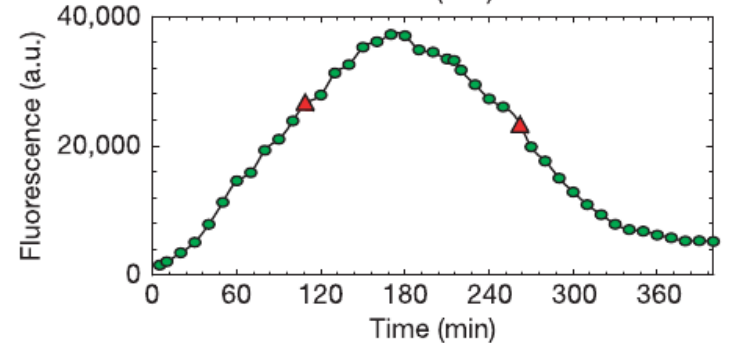


mannose



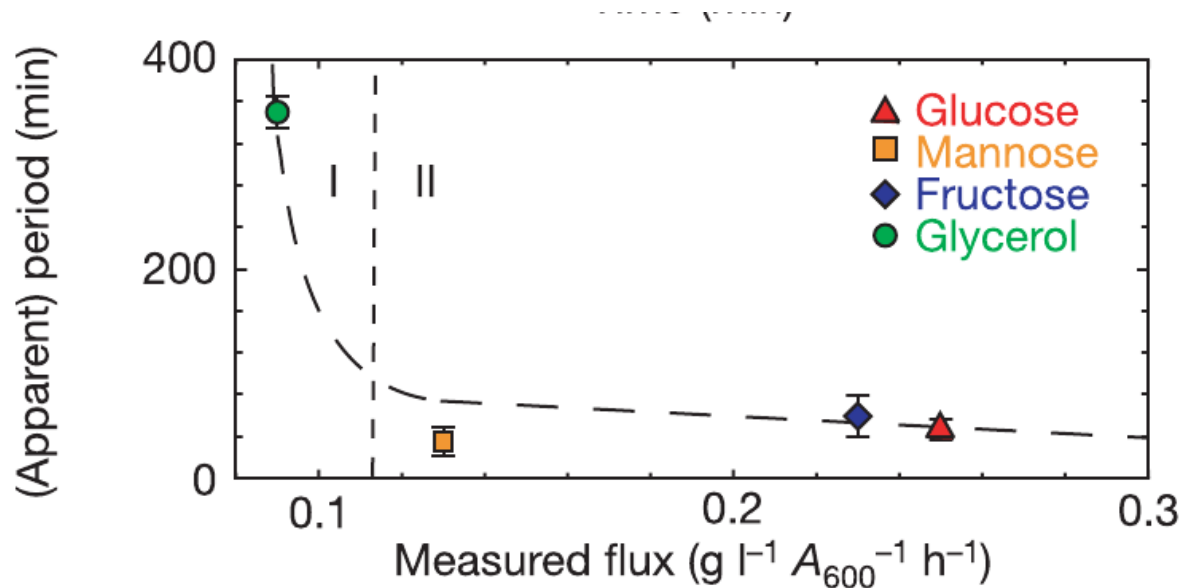
Red triangle:  
cell division

glycerol



# Test theoretical predictions

- Different carbon flux leads to different periods of the oscillation (apparent oscillation for glycerol)



# Test theoretical predictions

- External acetate suppresses the oscillation

