Modeling and simulation of gene regulatory networks 2

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INRIA Grenoble - Rhône-Alpes and IBIS

- IBIS: systems biology group at INRIA/Université Joseph Fourier/CNRS
  - Analysis of bacterial regulatory networks by means of models and experiments
  - Biologists, computer scientists, mathematicians, physicists, …

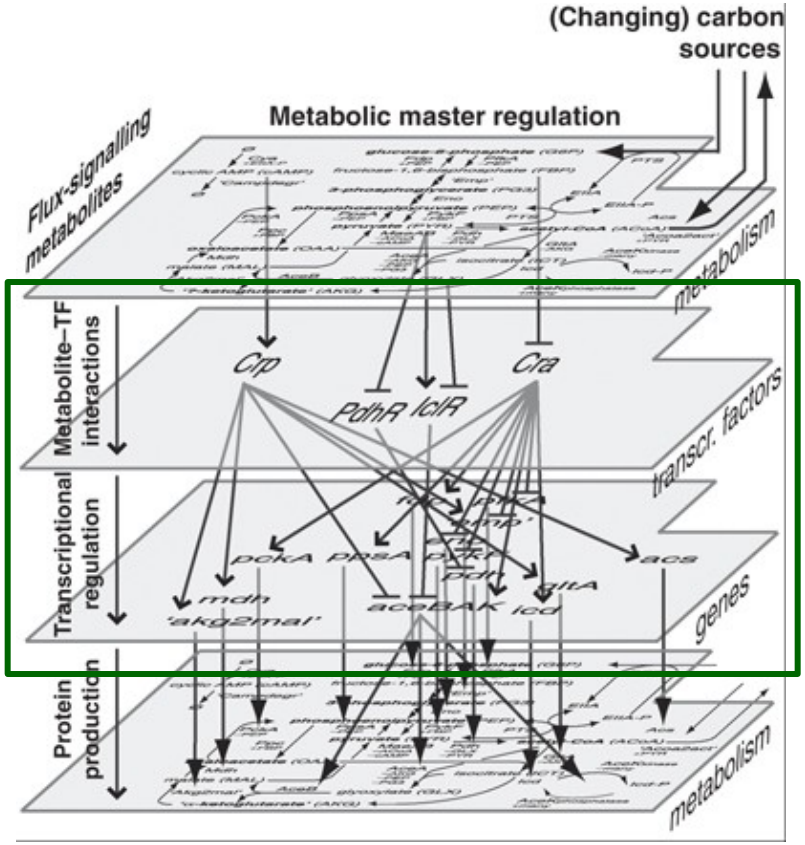
http://ibis.inrialpes.fr

Overview

1. Gene regulatory networks in bacteria
2. **Deterministic modeling of gene regulatory networks**
3. Qualitative modeling of gene regulatory networks
4. Stochastic modeling of gene regulatory networks
5. Some current issues and perspectives

Gene regulatory networks

- **Gene regulatory networks** control changes in gene expression levels in response to environmental perturbations



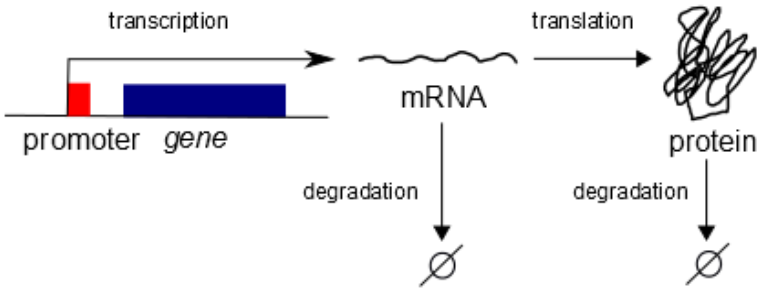
- Gene regulatory networks consist of genes, gene products, signalling metabolites, and their mutual regulatory interactions

  Global regulators of transcription involved in glucose-acetate diauxie in *E. coli*

Kotte *et al.* (2010), *Mol. Syst. Biol.*, 6:355

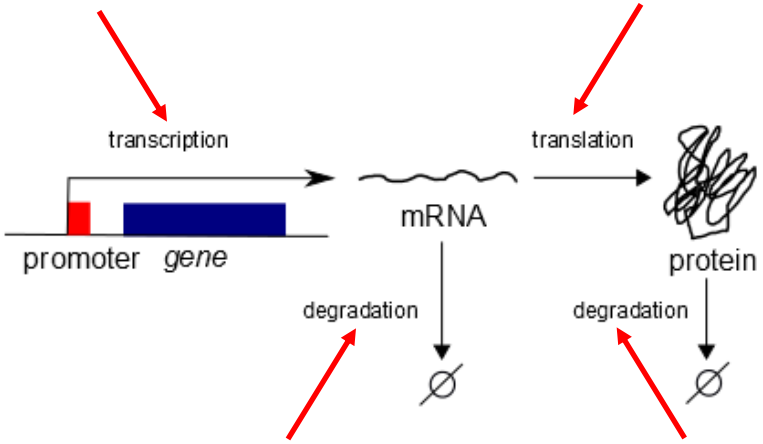
Gene expression

- Typically, and simplifying quite a bit, **gene expression** in bacteria involves:
  - Transcription by RNA polymerase (mRNA)
  - Translation by ribosomes (proteins)
  - Degradation of mRNA and protein



Regulation of gene expression

- Typically, and simplifying quite a bit, **regulation of gene expression** in bacteria involves:
  - Transcription regulation by transcription factors
  - Translation regulation by small RNAs
  - Regulation of degradation by proteases



Modeling of gene regulatory networks

- Different modeling formalisms exist, describing gene expression on different levels of detail

Stochastic master equations

Ordinary differential equations (ODEs)

Boolean networks

Smolen *et al.* (2000), *Bull. Math. Biol.*, 62(2):247-292

Hasty *et al.* (2001), *Nat. Rev. Genet.*, 2(4):268-279

de Jong (2002), *J. Comput. Biol.*, 9(1): 69-105

Szallassi *et al.* (2006), *System Modeling in Cellular Biology*, MIT Press

Bolouri (2008), *Computational Modeling of Gene Regulatory Networks*, Imperial College Press

Karleback and Shamir (2008), *Nat. Rev. Mol. Cell Biol.*, 9(10):770-80

Ordinary differential equation models

- Concentration of proteins, mRNAs, and other molecules at time-point t represented by continuous variable $x_i(t) \in \mathbb{R}_{\geq 0}$

  Concentration on level of individual cell or cell population

- Regulatory interactions, controlling synthesis and degradation, modeled by **ordinary differential equations**

$$\frac{\mathrm{d}\boldsymbol{x}}{\mathrm{d}t} = \dot{\boldsymbol{x}} = \boldsymbol{f}(\boldsymbol{x}),$$

  where $\boldsymbol{x} = [x_1, \ldots, x_n]'$ and $\boldsymbol{f}(\boldsymbol{x})$ is **rate law**

- Kinetic theory of biochemical reactions provides well-established framework for specification of rate laws

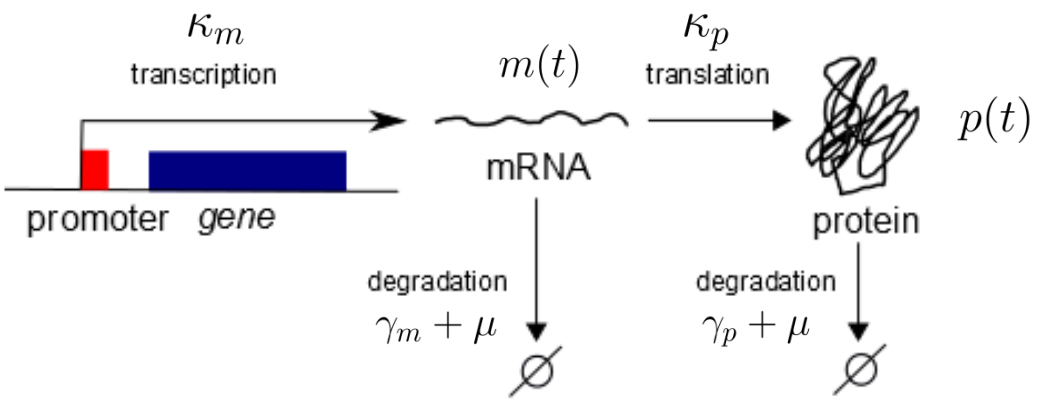
Heinrich and Schuster (1996), *The Regulation of Cellular Systems*, Chapman & Hall
Cornish-Bowden (1995), *Fundamentals of Enzyme Kinetics*, Portland Press

Modeling of gene regulatory networks

- ODE model of gene expression, distinguishing **transcription** and **translation**

$$\dot{m} = \kappa_m - (\gamma_m + \mu)\, m$$

$$\dot{p} = \kappa_p\, m - (\gamma_p + \mu)\, p$$



$m(t) \geq 0$, concentration mRNA

$p(t) \geq 0$, concentration protein

$\kappa_m, \kappa_p > 0$, synthesis rate constants

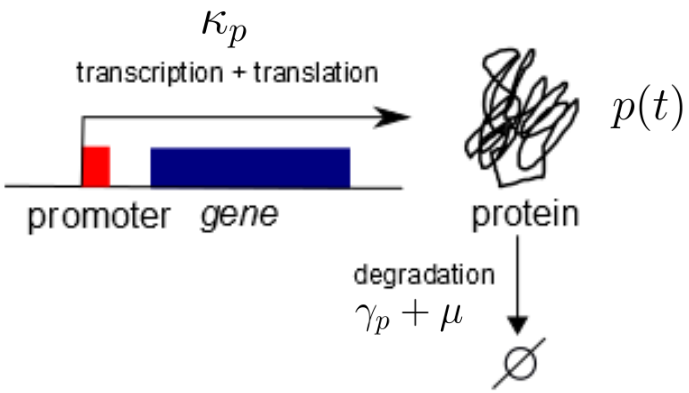
$\gamma_m, \gamma_p > 0$, degradation rate constants

$\mu \geq 0$, growth rate

Modeling of gene regulatory networks

- ODE model of gene expression, collapsing **transcription** and **translation**

$$\dot{p} = \kappa_p - (\gamma_p + \mu)\, p$$



$p(t) \geq 0$, concentration protein

$\kappa_p > 0$, synthesis rate constant

$\gamma_p > 0$, degradation rate constant

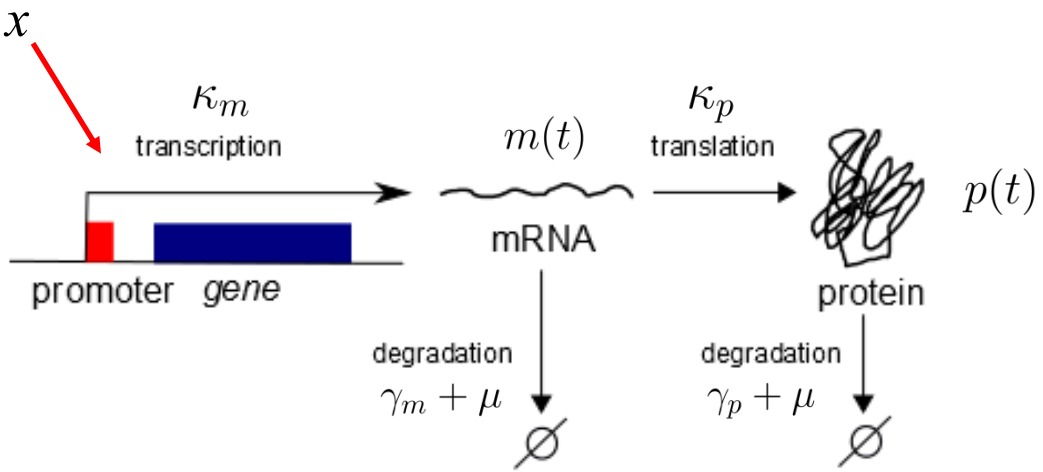
$\mu \geq 0$, growth rate

Modeling of gene regulatory networks

- ODE model of gene expression, taking into account **regulation** of transcription

$$\dot{m} = \kappa_m f(x) - (\gamma_m + \mu)\, m$$

$$\dot{p} = \kappa_p\, m - (\gamma_p + \mu)\, p$$



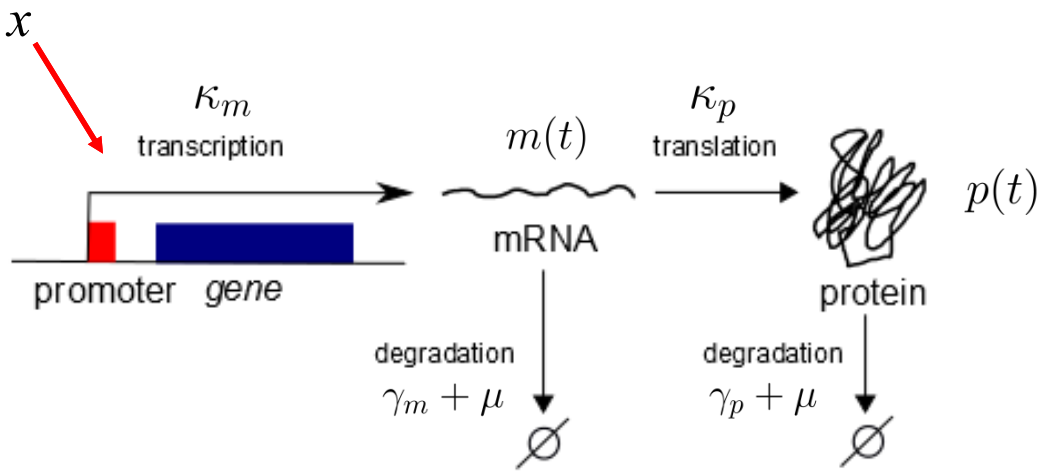
- Regulation function $f(x)$ describes modulation of synthesis rate by transcription factor
  - Generalization to regulation on translational and proteolytic level

Modeling of gene regulatory networks

- ODE model of gene expression, taking into account **regulation** of transcription

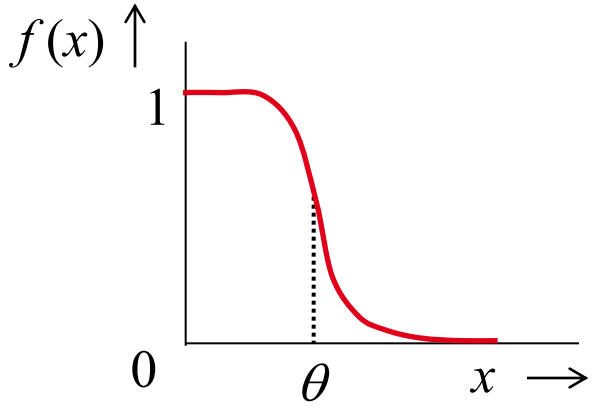
$$\dot{m} = \kappa_m f(x) - (\gamma_m + \mu)\, m$$

$$\dot{p} = \kappa_p\, m - (\gamma_p + \mu)\, p$$



- Regulation function $f(x)$ typically has **sigmoidal** form, due to cooperative nature of regulation

$$f(x) = \frac{\theta^n}{\theta^n + x^n}\,, \quad \theta > 0 \text{ threshold}, \quad n > 1 \text{ cooperativity}$$

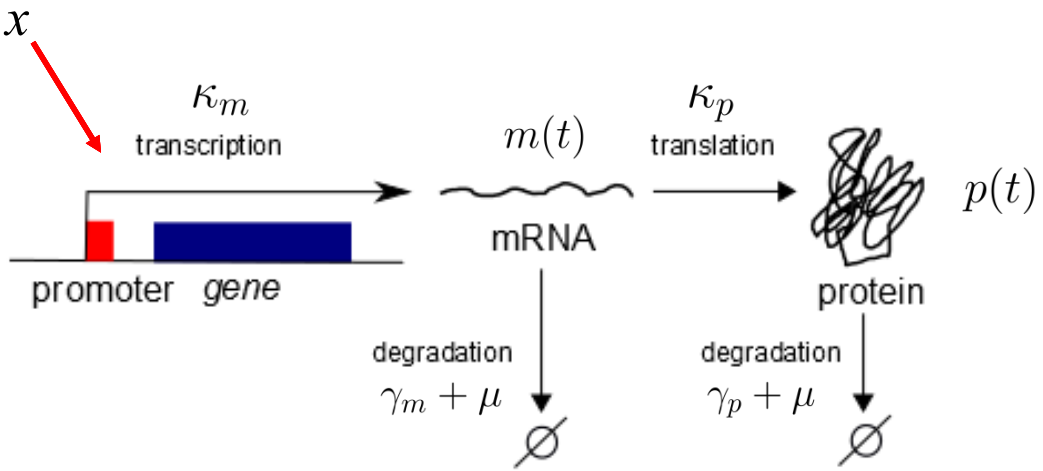


Modeling of gene regulatory networks

- ODE model of gene expression, taking into account **regulation** of transcription

$$\dot{m} = \kappa_m f(x) - (\gamma_m + \mu)\, m$$

$$\dot{p} = \kappa_p\, m - (\gamma_p + \mu)\, p$$



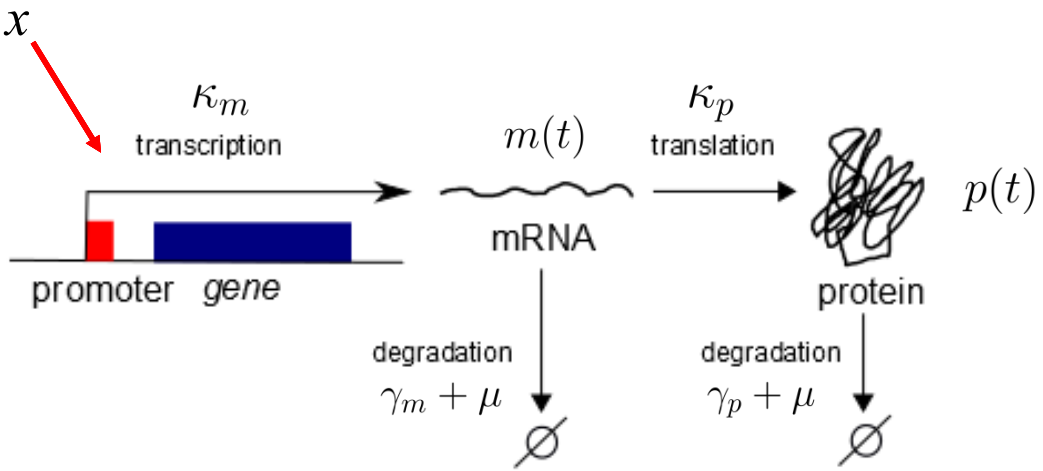
- Regulation function $f(x)$ typically has **sigmoidal** form, accounting for cooperative nature of regulation
- Implicit modeling assumptions:
  - Ignore gene expression machinery (RNA polymerase, ribosome)
  - Simplification of complex protein-DNA interactions to response function

Modeling of gene regulatory networks

- ODE model of gene expression, taking into account **regulation** of transcription

$$\dot{m} = \kappa_m f(x) - (\gamma_m + \mu)\, m$$

$$\dot{p} = \kappa_p\, m - (\gamma_p + \mu)\, p$$



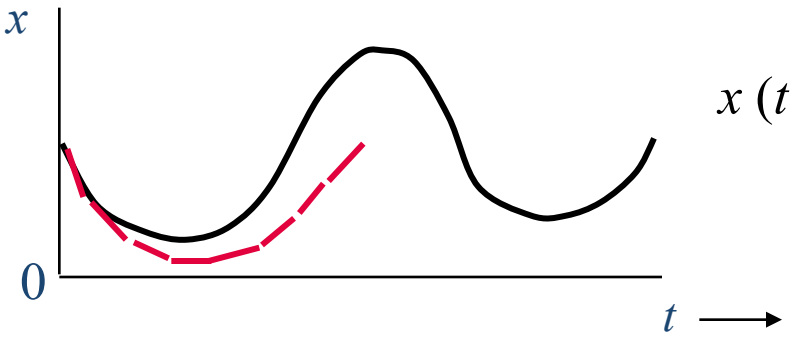
- Gene regulatory network has many genes with mutual regulatory interactions: model of coupled ODEs

Analysis and numerical simulation

- No analytical solution for most nonlinear differential equations
- **Dynamic systems theory** provides techniques for analysis of nonlinear differential equations, but usually not scalable
  - Phase portrait
  - Bifurcation analysis

Kaplan and Glass (1995), *Understanding Nonlinear Dynamics*, New York

- Approximation of solution obtained by **numerical simulation**, given parameter values and initial conditions $\boldsymbol{x}(0) = \boldsymbol{x}^0$

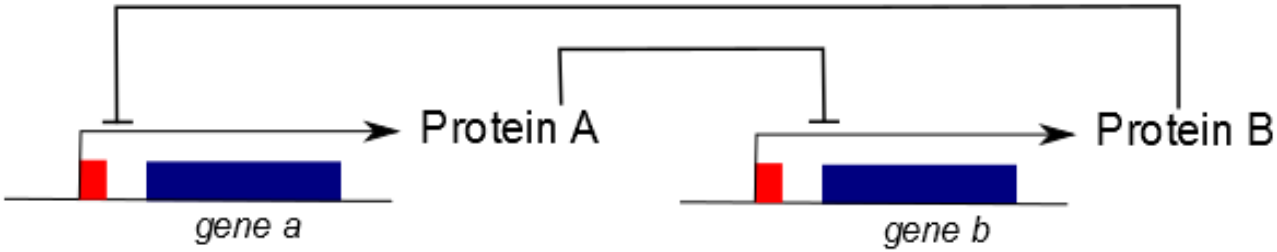


$$x(t+\Delta t) = x(t) + \int_t^{t+\Delta t} f(x)\,\mathrm{d}t \approx x(t) + f(x)\,\Delta t$$

Lambert (1991), *Numerical Methods for Ordinary Differential Equations*, Wiley

Cross-inhibition network

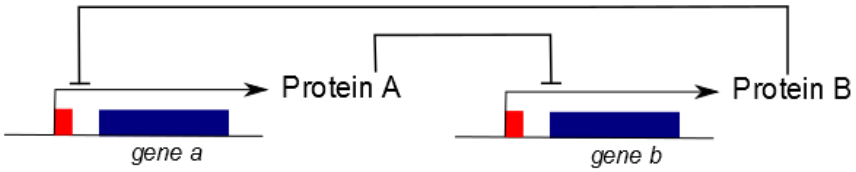
- **Cross-inhibition** network consists of two genes, each coding for transcription regulator inhibiting expression of other gene



- Cross-inhibition network is example of **positive feedback**, important for phenotypic differentiation (multi-stability)

Thomas and d'Ari (1990), *Biological Feedback*, CRC Press

ODE model of cross-inhibition network



$$\dot{x}_a = \kappa_a f(x_b) - \gamma_a x_a$$

$$\dot{x}_b = \kappa_b f(x_a) - \gamma_b x_b$$

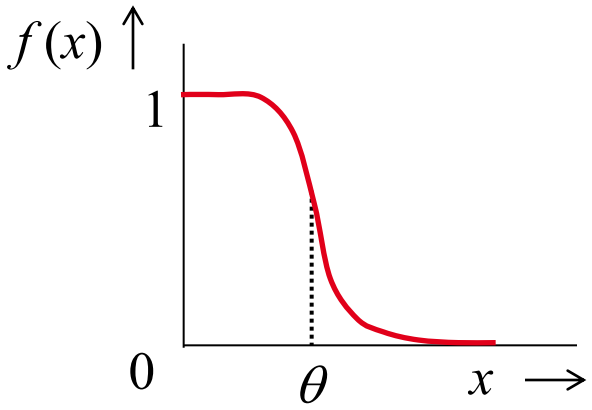
$x_a(t) \geq 0$, concentration protein A

$x_b(t) \geq 0$, concentration protein B

$\kappa_a , \kappa_b > 0$, synthesis rate constants

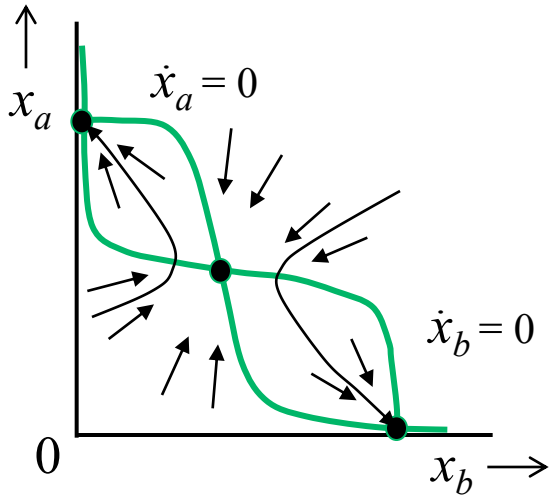
$\gamma_a , \gamma_b > 0$, degradation rate constants

$$f(x) = \frac{\theta^n}{\theta^n + x^n} \quad , \quad \theta > 0 \text{ threshold,} \quad n > 1 \text{ cooperativity}$$



Bistability of cross-inhibition network

- Analysis of **steady states** in phase plane



$$\dot{x}_a = 0 \Rightarrow x_a = (\kappa_a / \gamma_a) f(x_b)$$
$$\dot{x}_b = 0 \Rightarrow x_b = (\kappa_b / \gamma_b) f(x_a)$$

- System is **bistable**: two stable and one unstable steady state.
- For almost all initial conditions, system will converge to one of two stable steady states (**differentiation**)
- System returns to steady state after small perturbation

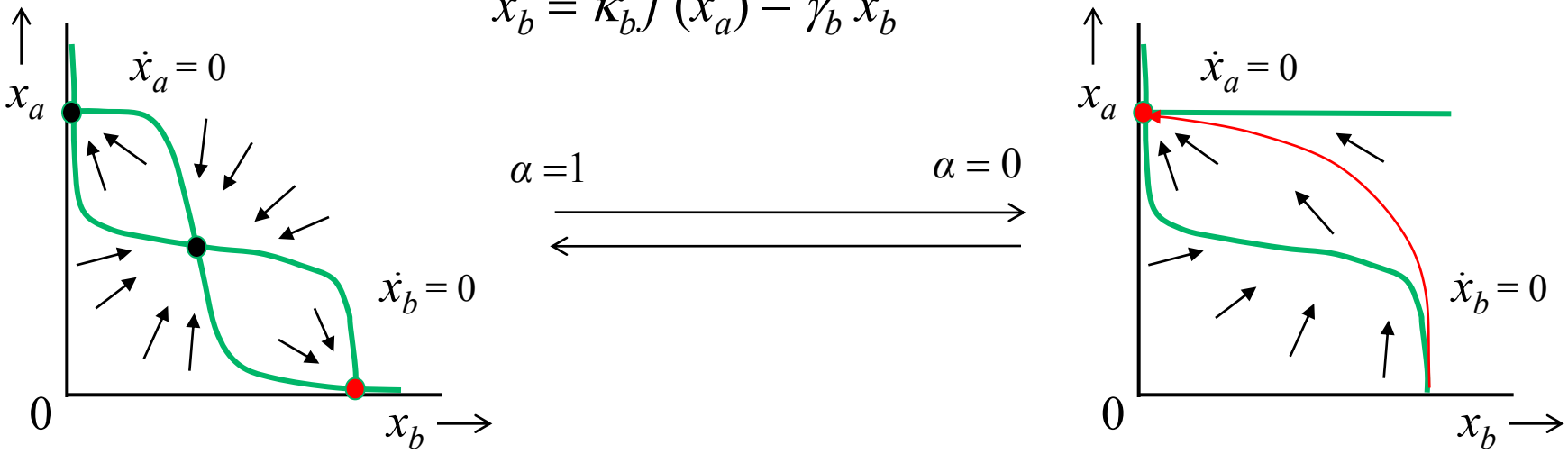
Hysteresis in cross-inhibition network

- Transient perturbation may cause irreversible switch from one steady state to another (**hysteresis**)

  Modulation of regulatory effect of one of regulators (α)

$$\dot{x}_a = \kappa_a f(\alpha x_b) - \gamma_a x_a$$

$$\dot{x}_b = \kappa_b f(x_a) - \gamma_b x_b$$

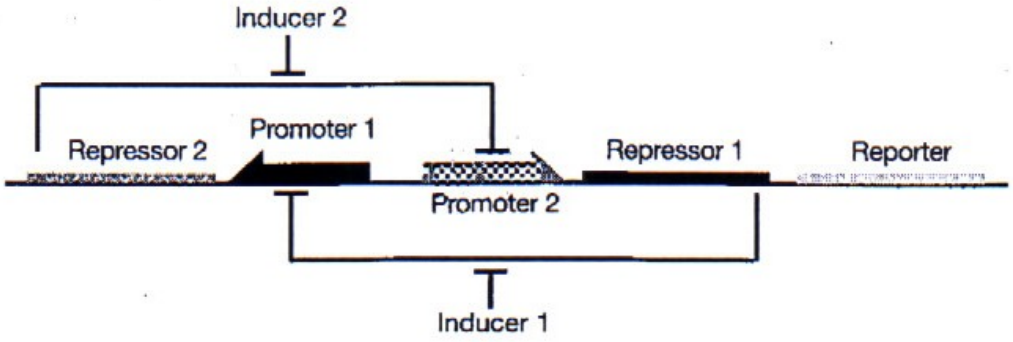


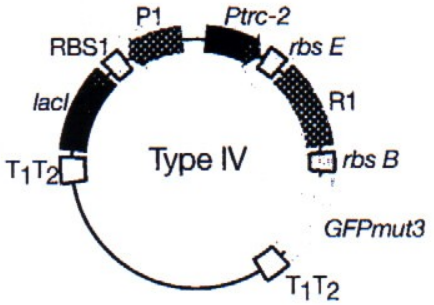
- Change in parameter causes saddle-note **bifurcation**

Construction of cross inhibition network

- Construction of cross inhibition network *in vivo*

Gardner *et al.* (2000), *Nature*, 403(6786): 339-42





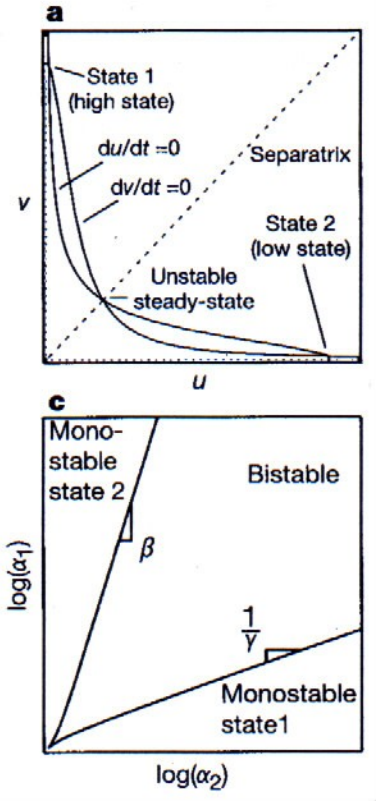
- ODE model of network

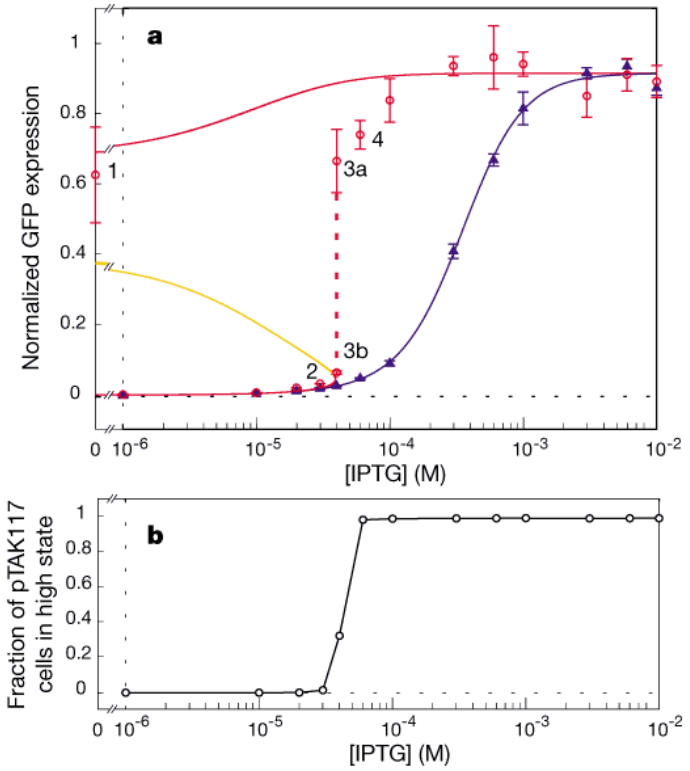
$$\dot{u} = \frac{\alpha_1}{1 + v^{\beta}} - u \qquad \dot{v} = \frac{\alpha_2}{1 + u^{\gamma}} - v$$

Experimental test of model

- Experimental test of mathematical model (bistability and hysteresis)

Gardner *et al.* (2000), *Nature*, 403(6786): 339-42

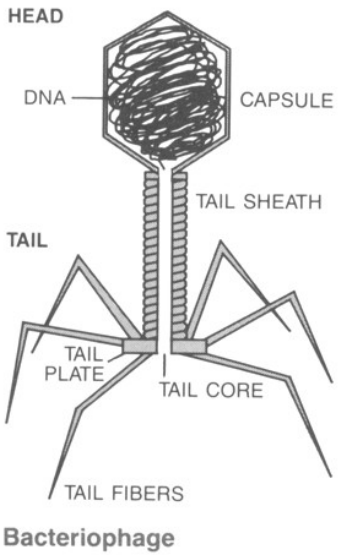


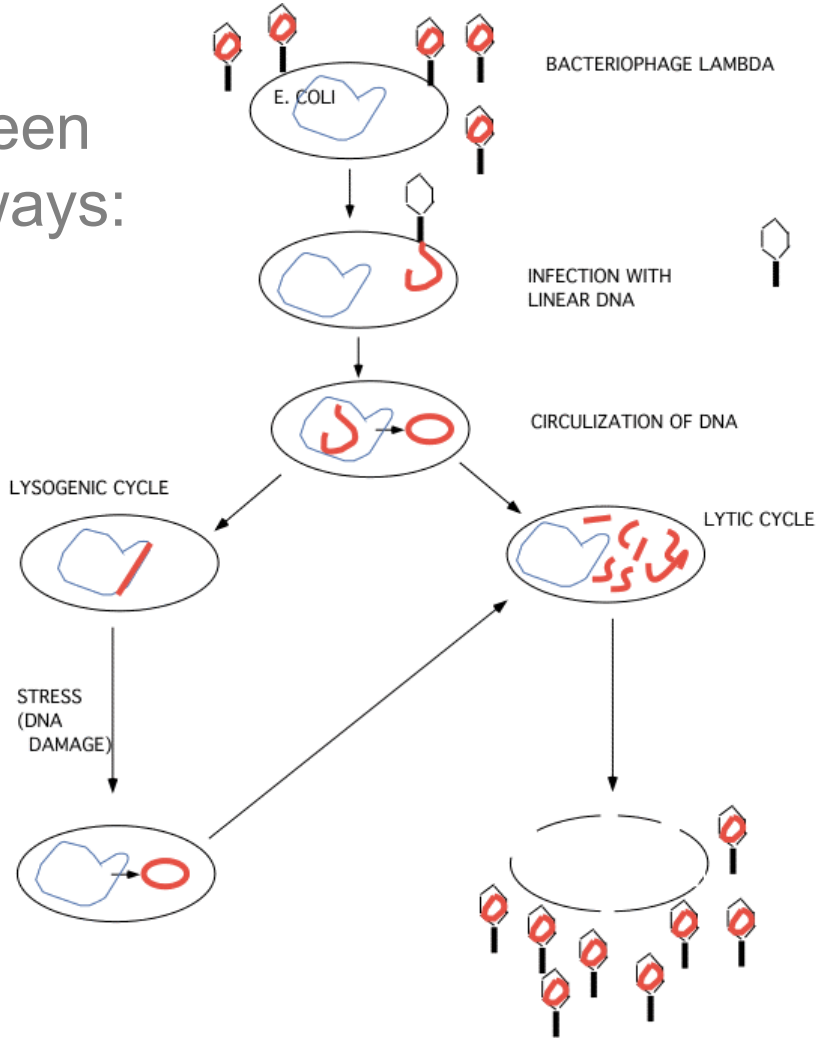


Bacteriophage λ infection of *E. coli*

- Response of *E. coli* to phage λ infection involves decision between alternative developmental pathways: **lysis** and **lysogeny**

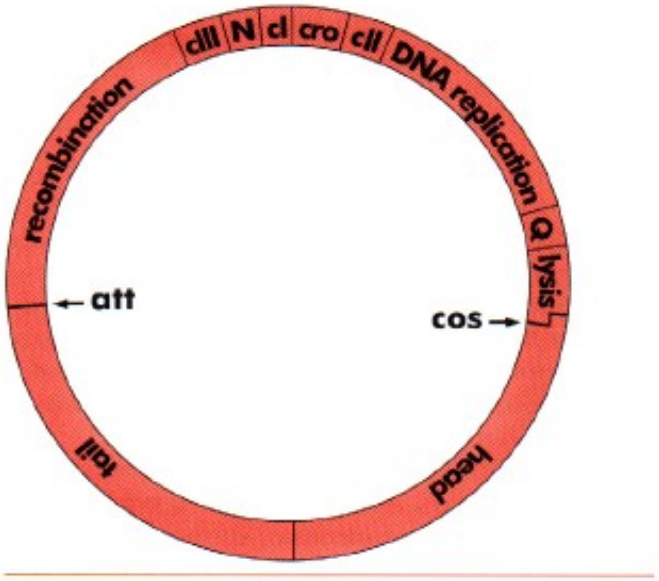
  Ptashne, *A Genetic Switch*, Cell Press,1992

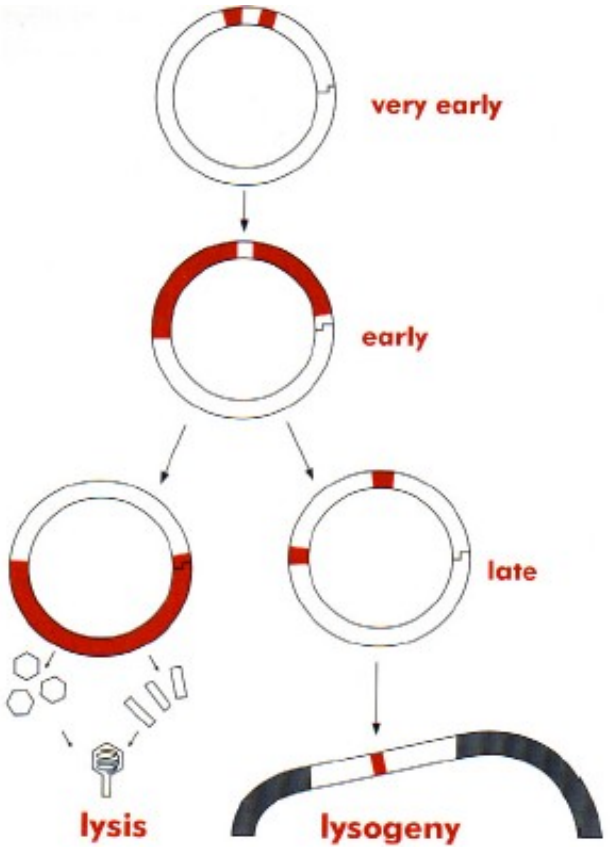




Bistability in phage λ

- Lytic and lysogenic pathways involve different patterns of gene expression

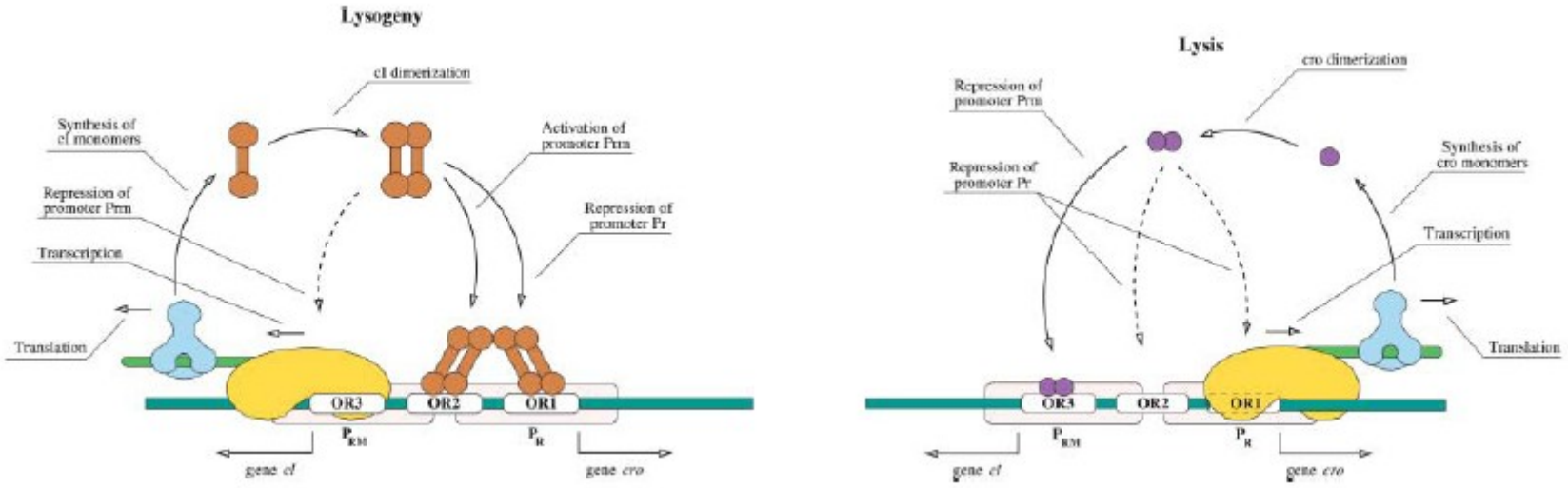




Ptashne, *A Genetic Switch*, Cell Press,1992

Control of phage λ fate decision

- Cross-inhibition feedback plays key role in establishment of lysis or lysogeny, as well as in induction of lysis after DNA damage



Santillán and Mackey (2004), *Biophys. J.*, 86(1):75-84

Simple model of phage λ fate decision

- Differential equation model of cross-inhibition feedback network involved in phage λ fate decision
  - mRNA and protein, delays, thermodynamic description of gene regulation

$$\frac{d[M_{cI}]}{dt} = k^{q}_{cI}[O_R] f^{q}_{RM}([CI_2]_{\tau_M}, [CI_2]_{\tau_M}) + k^{s}_{cI}[O_R] f^{s}_{RM}([CI_2]_{\tau_M}, [Cro_2]_{\tau_M}) - (\gamma_M + \mu)[M_{cI}],$$

$$\frac{d[M_{cro}]}{dt} = k_{cro}[O_R] f_R([CI_2]_{\tau_M}) - (\gamma_M + \mu)[M_{cro}],$$

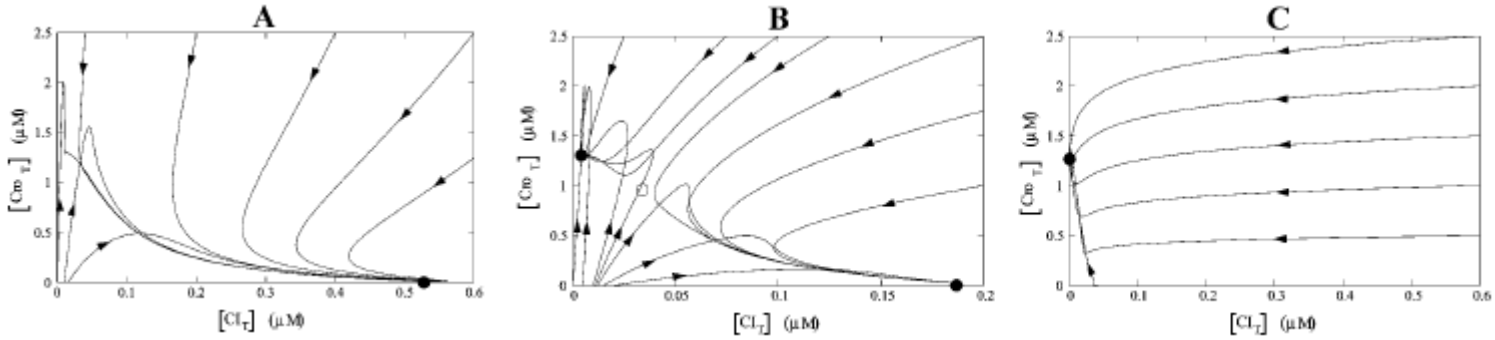
$$\frac{d[CI_T]}{dt} = \upsilon_{cI}[M_{cI}]_{\tau_{cI}} - (\gamma_{cI} + \mu)[CI_T],$$

$$\frac{d[Cro_T]}{dt} = \upsilon_{cro}[M_{cro}]_{\tau_{cro}} - (\gamma_{cro} + \mu)[Cro_T].$$

Santillán and Mackey (2004), *Biophys. J.*, 86(1):75-84

Analysis of phage λ model

- Bistability (lysis and lysogeny) only occurs for certain parameter values
- Switch from lysogeny to lysis involves bifurcation from one monostable regime to another, due to change in degradation constant



Santillán and Mackey (2004), *Biophys. J.*, 86(1):75-84

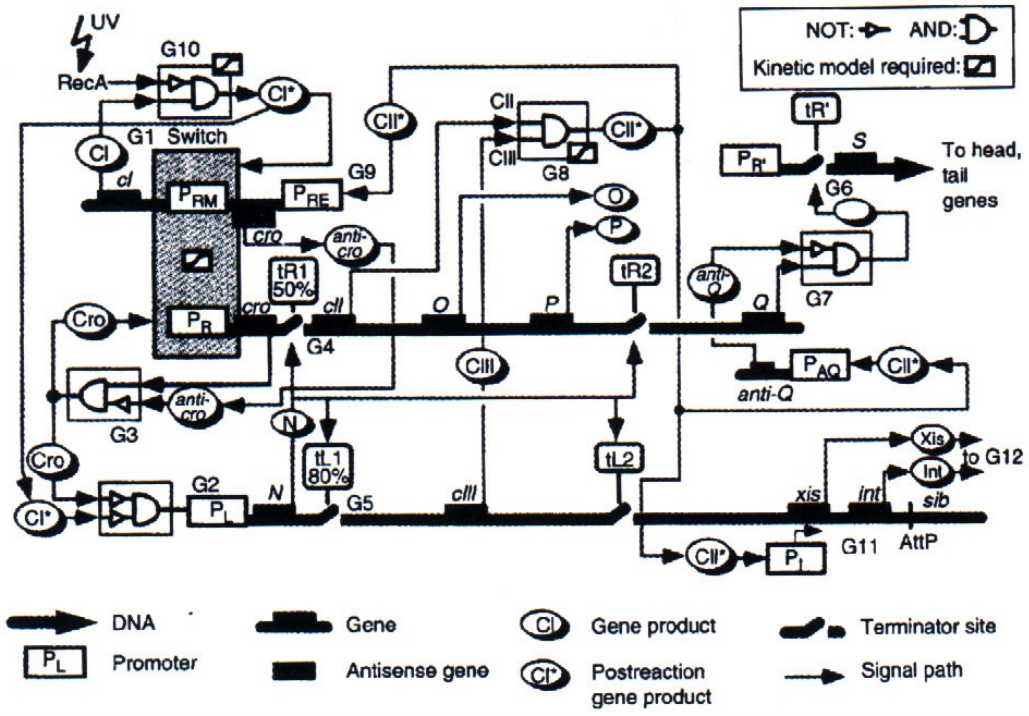
Extended model of phage λ infection

- ODE model of the **extended network** underlying decision between lysis and lysogeny
  - Role of other regulatory proteins (CII, N, Q, …)

McAdams and Shapiro (1995), *Science*, 269(5524):650-6

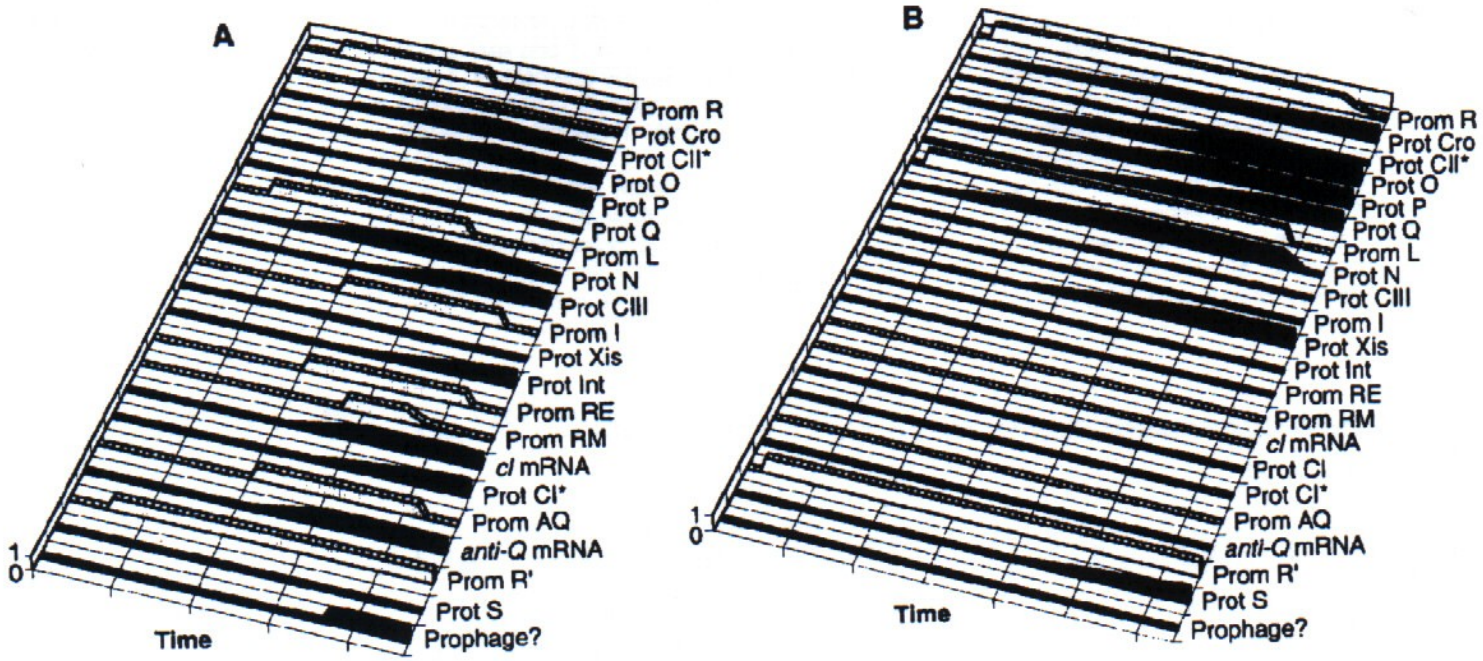
- Recent experimental work downplays importance of mutual inhibition of CI and Cro in lysis-lysogeny decision

Oppenheim *et al.* (2005), *Annu. Rev. Genet.*, 39:409–29



Simulation of phage λ infection

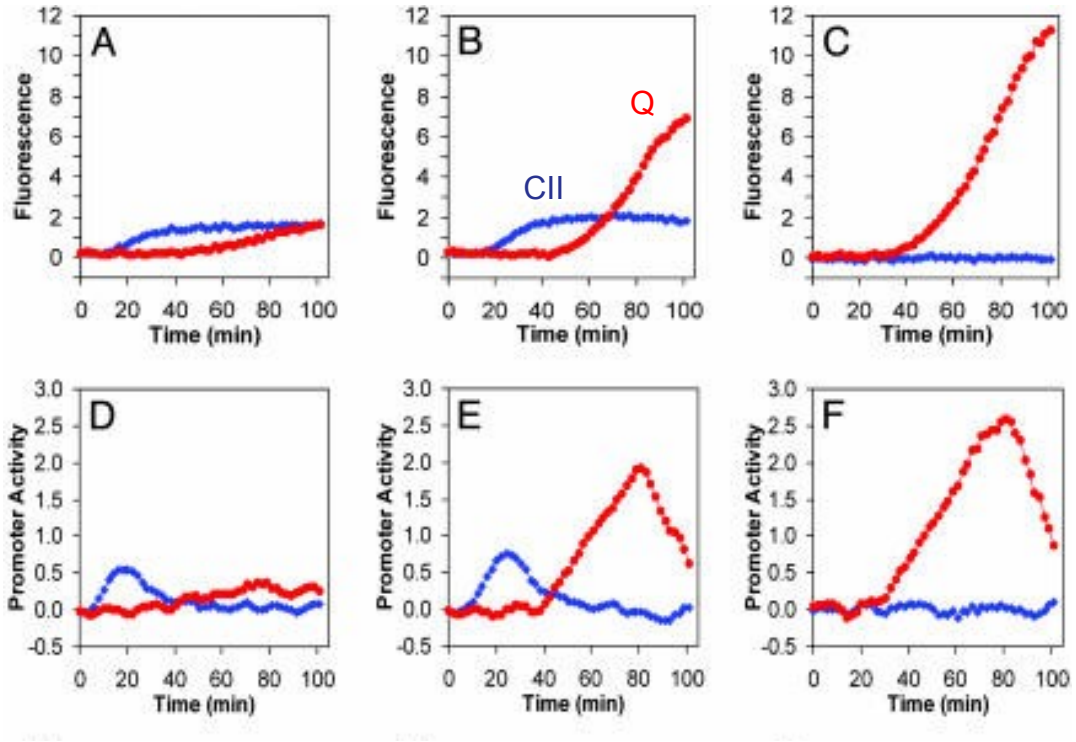
- Numerical simulation of promoter activity and protein concentrations in (a) lysogenic and (b) lytic pathways



- Cell follows one of two pathways for different initial conditions

Real-time monitoring of phage λ infection

- New measurement techniques allow real-time and *in-vivo* monitoring of the execution of lytic and lysogenic pathways
  - Use of fluorescent reporter genes in combination with automated plate readers



Kobiler *et al.* (2005), *Proc. Natl. Acad. Sci. USA*, 102(12): 4470-5

Other examples of bistability

- Many other examples of bistability exist in bacteria
  - Lactose utilization in *E. coli*
  - Persister cells and antibiotic resistance in *E. coli*
  - Genetic competence in *B. subtilis*
  - …

Dubnau and Losick (2006), *Mol. Microbiol.*, 61 (3):564–72

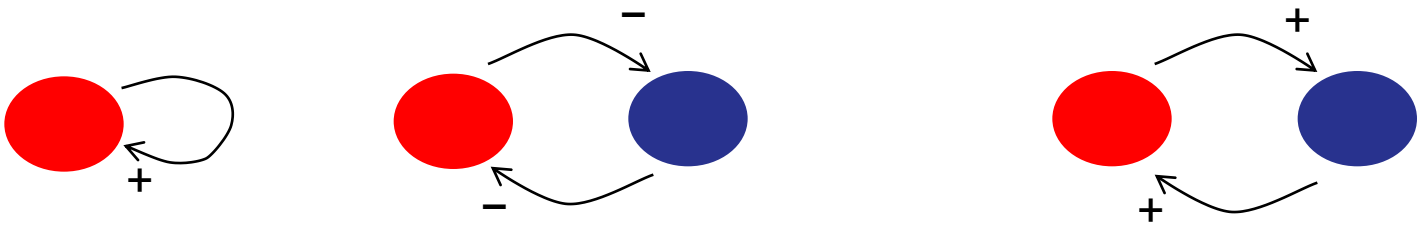
- Can we find general **design principles**, relating network structure to bistability and other properties of network dynamics?

Alon (2007), *An Introduction to Systems Biology*, Chapmann&Hall/CRC

Necessary condition for bistability

- **Necessary condition** for bistability, or multistability, is the occurrence of **positive feedback** loops in the regulatory network

Thomas and d'Ari (1990), *Biological Feedback*, CRC Press



- Increasingly general mathematical proofs of necessary condition for bistability, or multistability, in regulatory networks

  Regulatory interactions (activation/inhibition) lead to non-zero signs (+/-) in Jacobian matrix

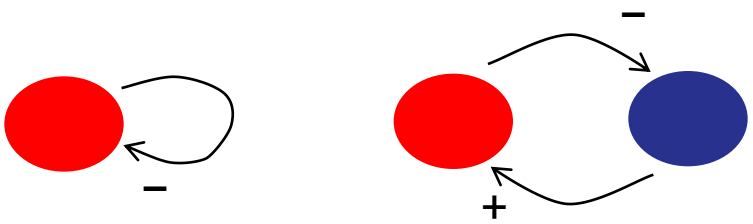
  Soulé (2003), *ComPlexUs*, 1:123-33

- **Condition is not sufficient**, as the actual occurrence of bistability depends on parameter values

Necessary condition for oscillations

- **Necessary condition** for oscillations is the occurrence of **negative feedback** loops in the regulatory network

Thomas and d'Ari (1990), *Biological Feedback*, CRC Press

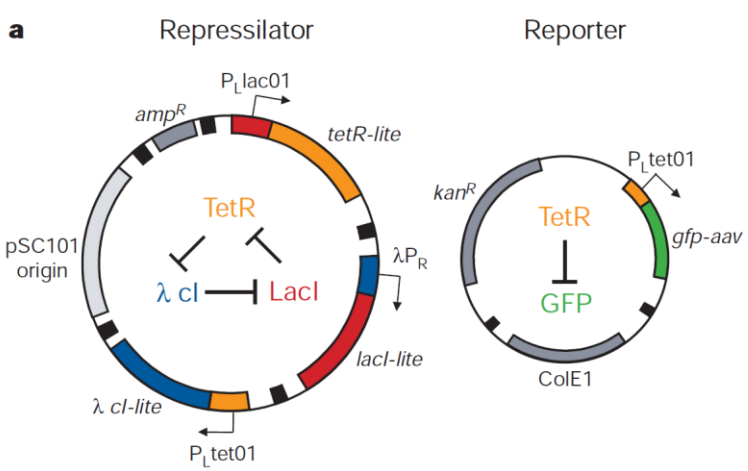


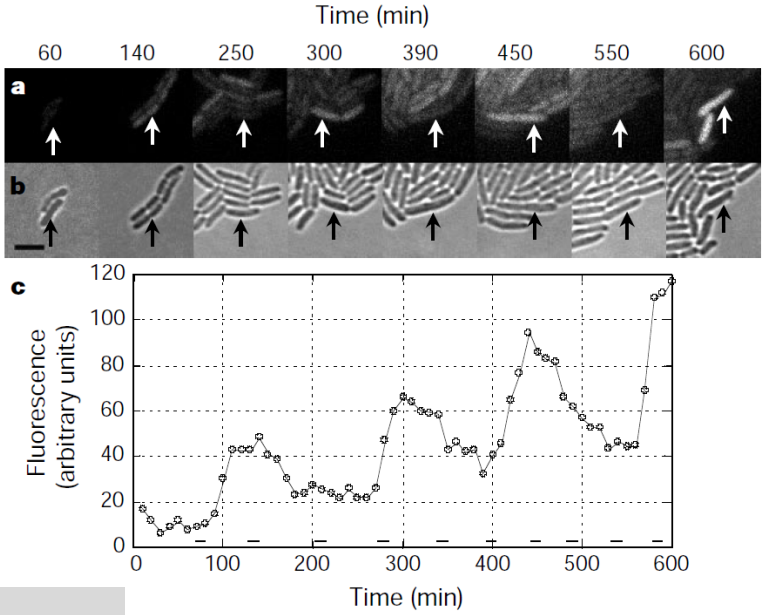
- **Condition is not sufficient**, as the actual occurrence of (stable) oscillations depends on: parameter values, nonlinearities, number of genes, …

Purcell *et al.* (2010), *J. R. Soc. Interface*, 7(52):1503-24

Construction of oscillator network

- Construction of oscillator *in vivo*: **repressilator**





- ODE model of oscillator

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{(1+p_j^n)} + \alpha_0 \qquad \begin{pmatrix} i = lacI, tetR, cI \\ j = cI, lacI, tetR \end{pmatrix}$$

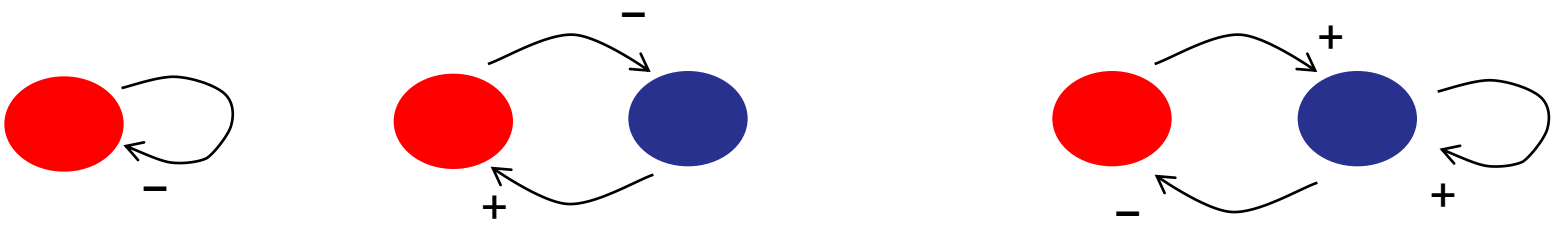
$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$

Elowitz and Leibler (2000), *Nature*, 403(6767):335-8

Necessary condition for oscillations

- **Necessary condition** for oscillations is the occurrence of **negative feedback** loops in the regulatory network

Thomas and d'Ari (1990), *Biological Feedback*, CRC Press



- **Condition is not sufficient**, as the actual occurrence of (stable) oscillations depends on: parameter values, nonlinearities, number of genes, …
- Combination of negative with positive feedback tends to stabilize oscillations

Purcell *et al.* (2010), *J. R. Soc. Interface*, 7(52):1503-24

Conclusions

- Ordinary differential equation (ODE) models describe dynamics of gene regulatory networks in deterministic way
- ODE models provide general formalism for which powerful analysis and simulation techniques exist
- ODE models are based on well-developed theoretical framework and have been applied to many gene regulatory networks
- Difficulties with ODE models:
  - Numerical techniques are often difficult to apply due to lack of quantitative data on model parameters
  - Assumptions of continuous and deterministic change of concentrations may not be valid on molecular level

Merci !

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