



# Quantitative modeling of gene regulatory networks

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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, …

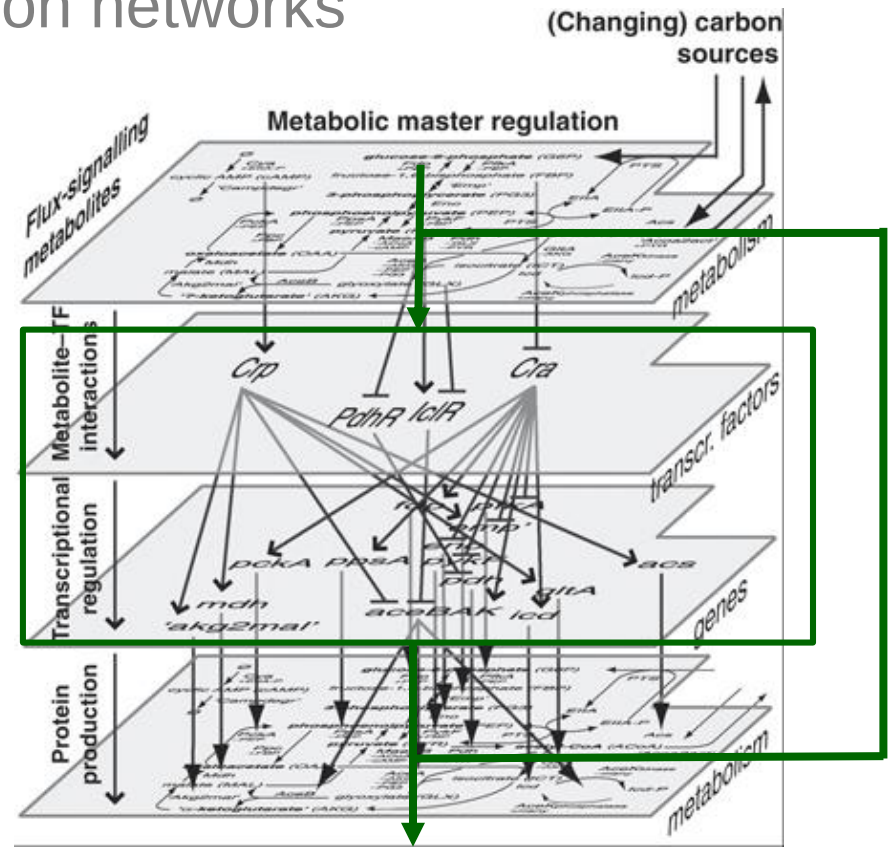
http://ibis.inrialpes.fr

# Overview

- Part 1. Systems biology and kinetic modeling
- Part 2. Metabolic network modeling
- Part 3. Gene regulatory network modeling
  - **Quantitative modeling of gene regulatory networks**
  - Qualitative modeling of gene regulatory networks
  - Stochastic modeling of gene regulatory networks
  - Practical on integrated models of bacterial growth (Matlab)
- Part 4. Models and data

# Gene regulatory networks

- Focus on **subsystems** that can be studied in isolation due to **modular structure** of reaction networks
  - Time-scale hierarchies
  - Connectivity structure
- **Gene regulatory networks**
  - Genes, proteins, and regulatory interactions
  - Reactions involved in transcription and translation and their regulation
  - Time-scale: min (mRNA) to h (proteins)

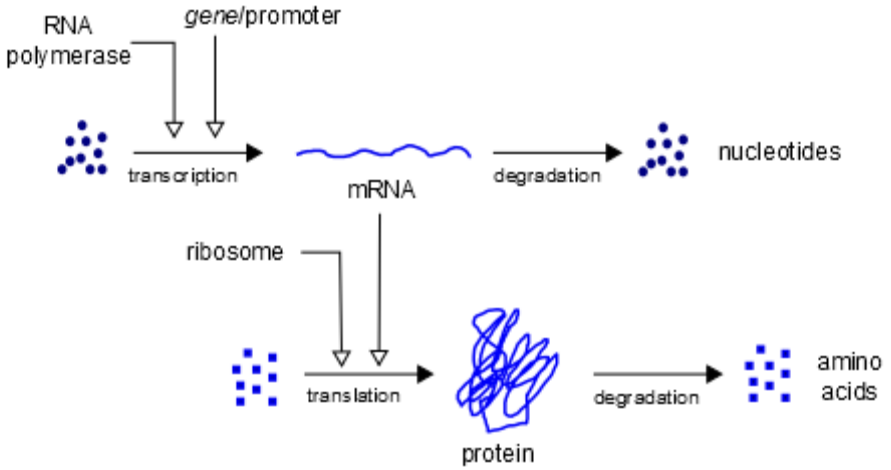



Kotte *et al.* (2010), *Mol. Syst. Biol.*, 6: 355

# 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

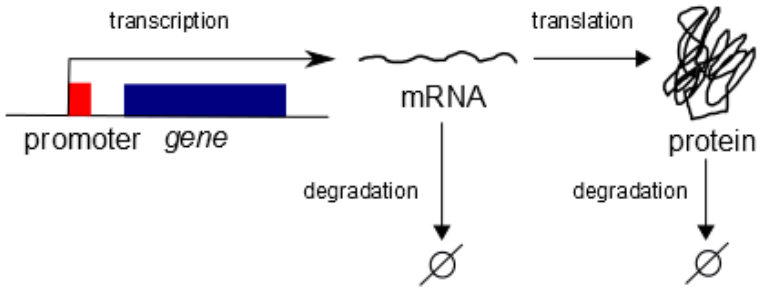
**Biochemical view:**



# Gene expression

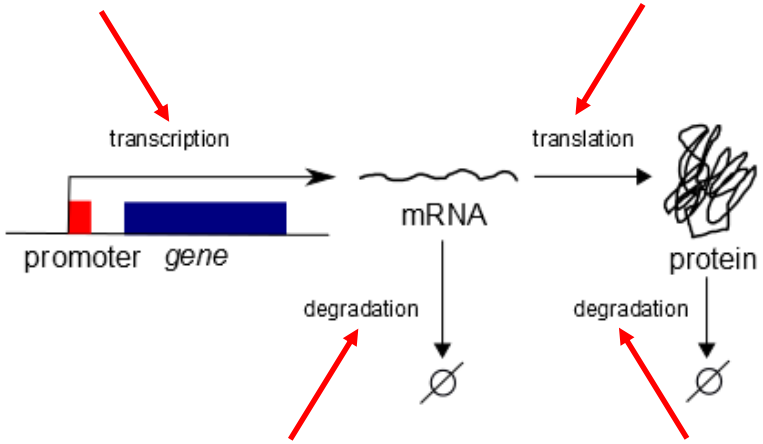
- Typically, and simplifying quite a bit, **gene expression** in bacteria involves:
  - Transcription by RNA polymerase (mRNA)
  - Translation by ribosomes (proteins)
  - Degradation of mRNA and protein

**Simplified view:**



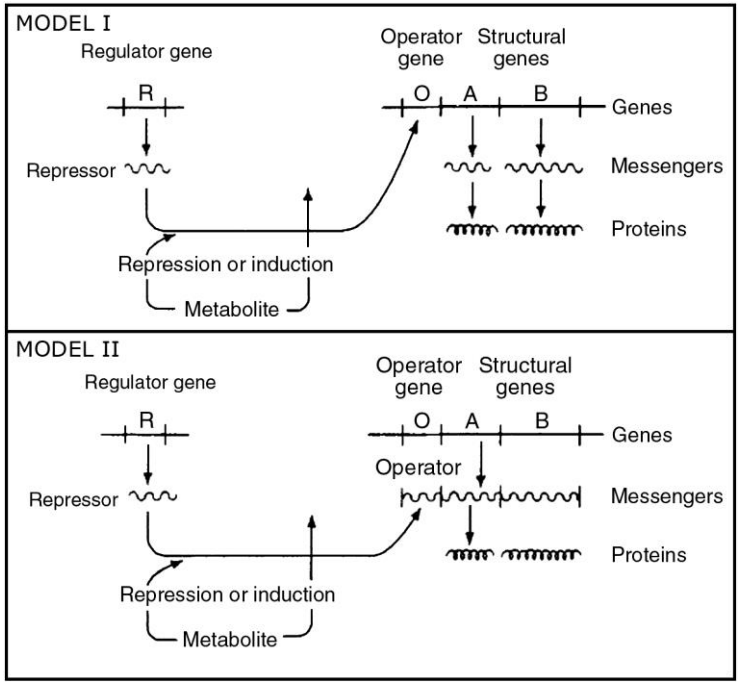
# Regulation of gene expression

- 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



# Gene regulatory networks

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




Original *lac* operon model

Jacob and Monod (1961), *J. Mol. Biol.*, 3(3):318-56

# Gene regulatory networks

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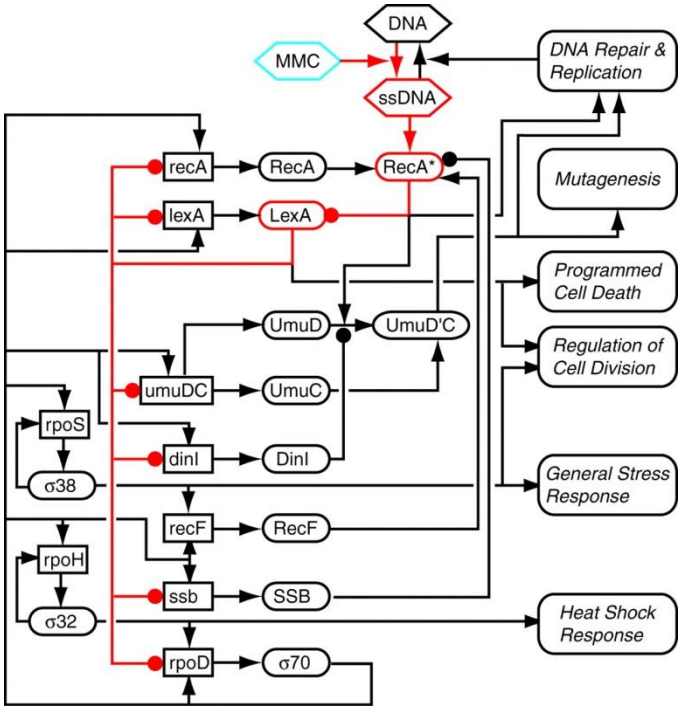


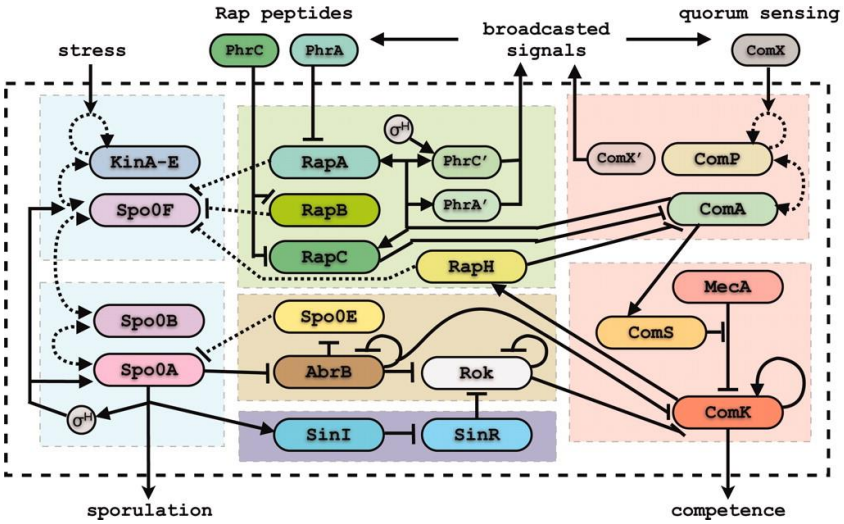

SOS response network in *E. coli*

Gardner *et al.* (2011), *Science*, 301(5629):102-5

# Gene regulatory networks

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

Sporulation and competence network in *B. subtilis*

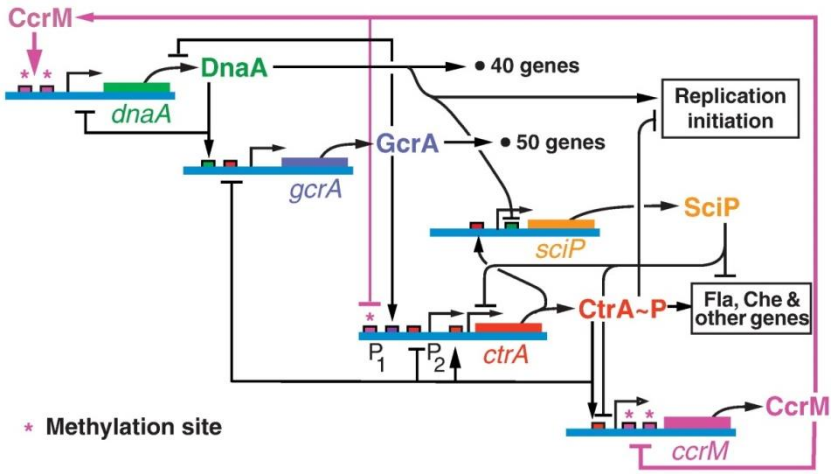



Schultz *et al.* (1961), *Proc. Natl. Acad. Sci. USA*, 106(50):21027-34

# Gene regulatory networks

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

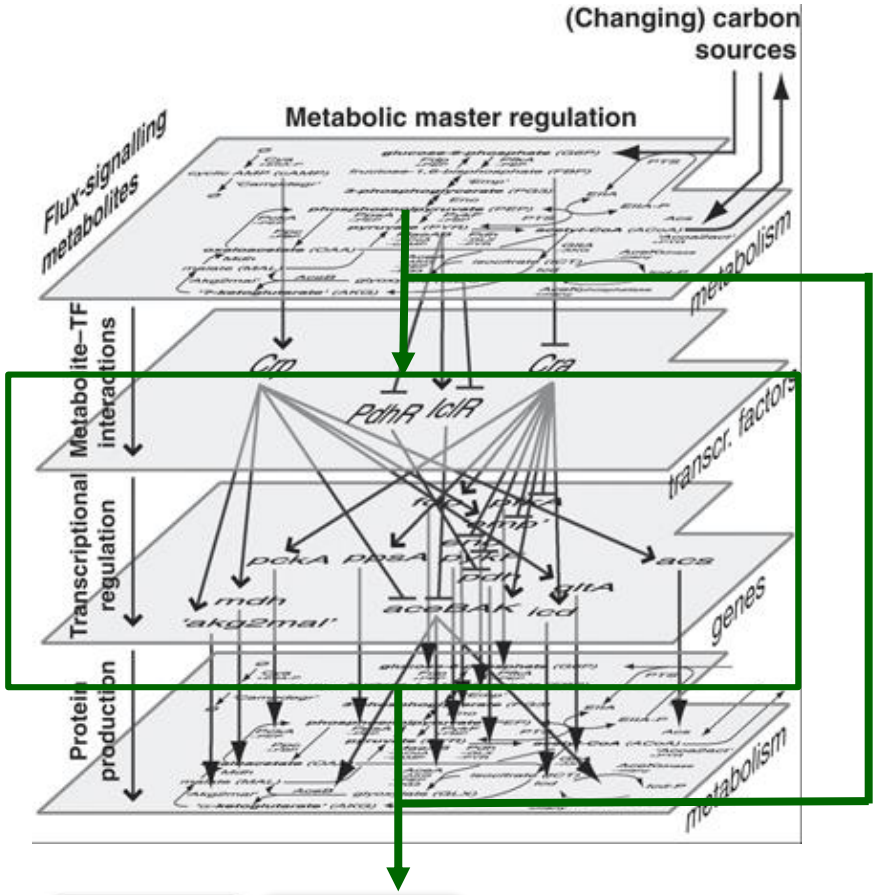
*Cauleobacter* cell cycle network




McAdams and Shapiro (2011), *J. Mol. Biol.*, 409(1):28-35

# Broader view on gene regulatory networks

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

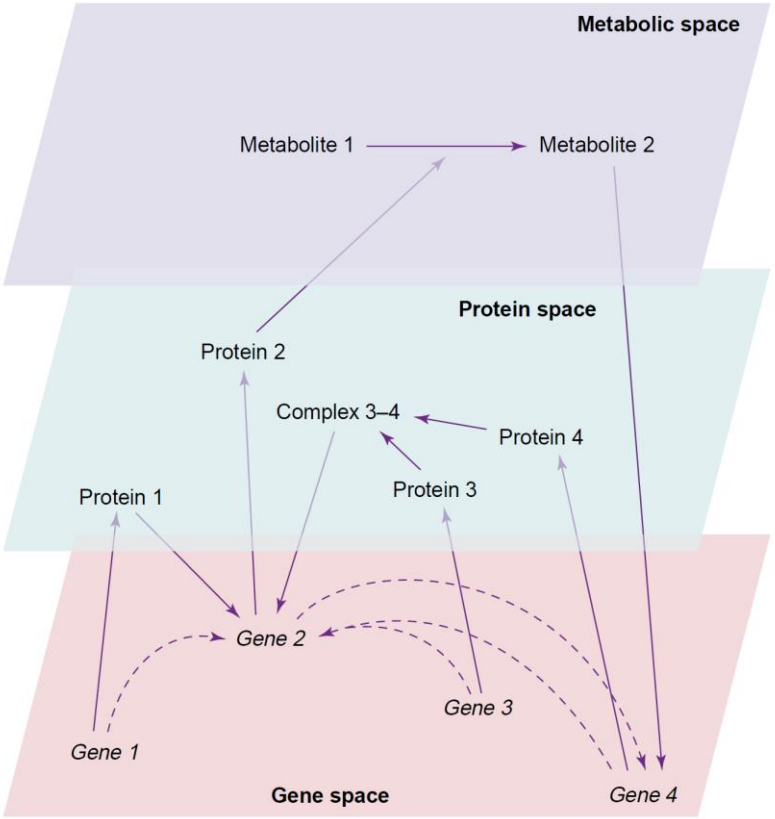



- **But**: adaptation of gene expression leads to changes in metabolism which feed back into regulatory network
- Indirect regulatory interactions: **metabolic coupling**

Baldazzi *et al.* (2010), *PLoS Comput. Biol.*, 6(6):e1000812

# Broader view on gene regulatory networks

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




- **But**: adaptation of gene expression leads to changes in metabolism which feed back into regulatory network
- Indirect regulatory interactions: **metabolic coupling**

Braznik *et al.* (2002), *Trends Biotechnol.*, 20(11):467-71

# Modeling of gene regulatory networks

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


Stochastic master equations

Ordinary differential equations (ODEs)

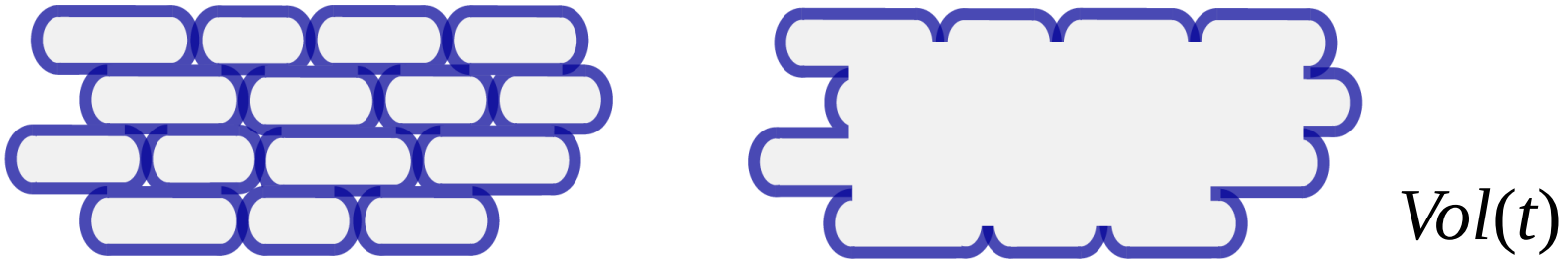
Boolean networks

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 (growing) cell population




- Concentration variable defined by dividing amount of molecules by volume $x_i(t) = X_i(t)/Vol(t)$

de Jong *et al.* (2017), *J. Roy. Soc. Interface*, 14(136):20170502

# Ordinary differential equation models

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

  Concentration on level of (growing) cell population

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

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

  where $\boldsymbol{x} = [x_1, \ldots, x_n]'$ and $\boldsymbol{v}(\boldsymbol{x})$ 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

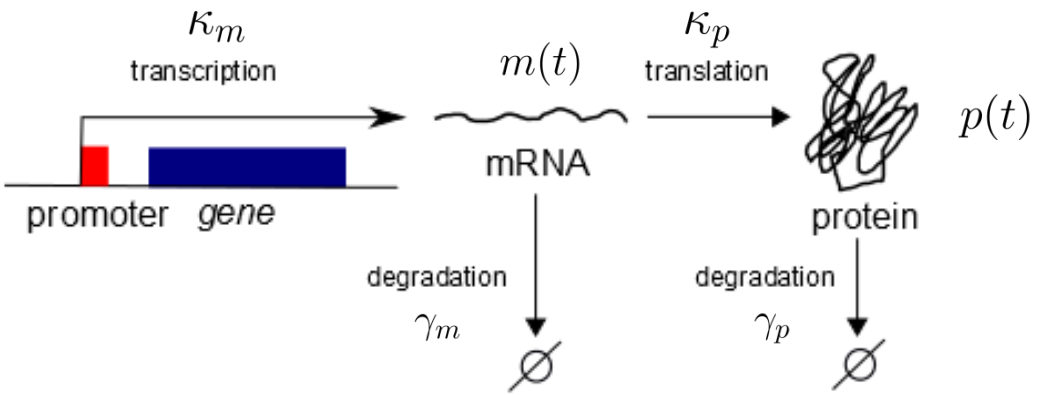
Cornish-Bowden (1995), *Fundamentals of Enzyme Kinetics*, Portland Press

# Modeling of gene regulatory networks

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

$$\dot{m} = \kappa_m - \gamma_m m$$

$$\dot{p} = \kappa_p m - \gamma_p p$$




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

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

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

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

# Modeling of gene regulatory networks

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

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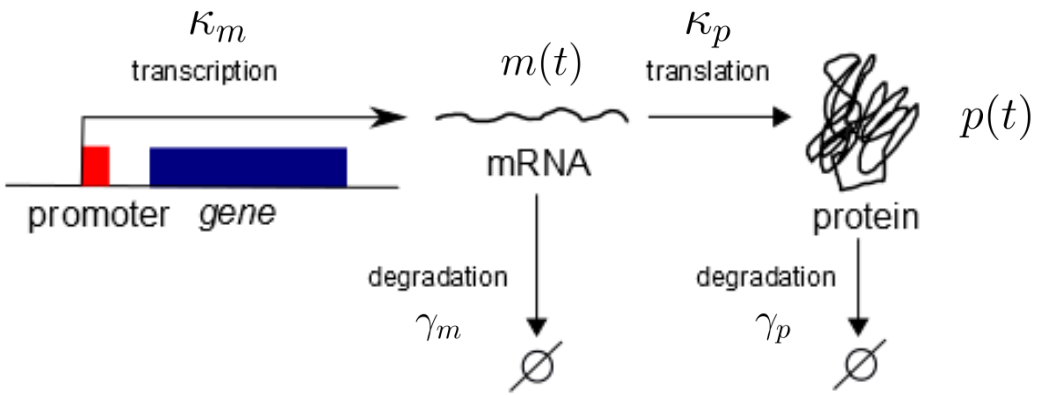
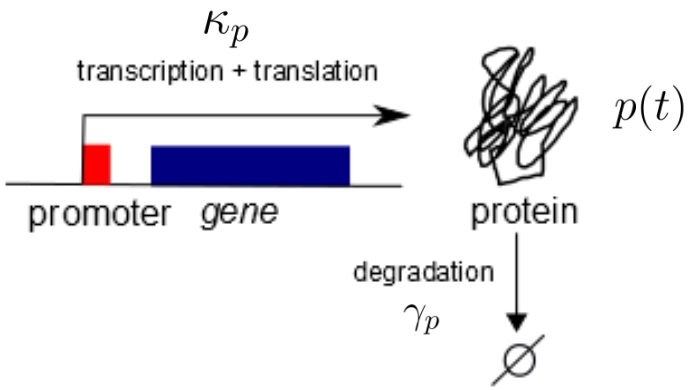



- **Question:** write down gene expression model in stoichiometric form

# Modeling of gene regulatory networks

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

$$\dot{p} = \kappa_p - \gamma_p\, p$$




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

$\kappa_p > 0$, synthesis rate constant

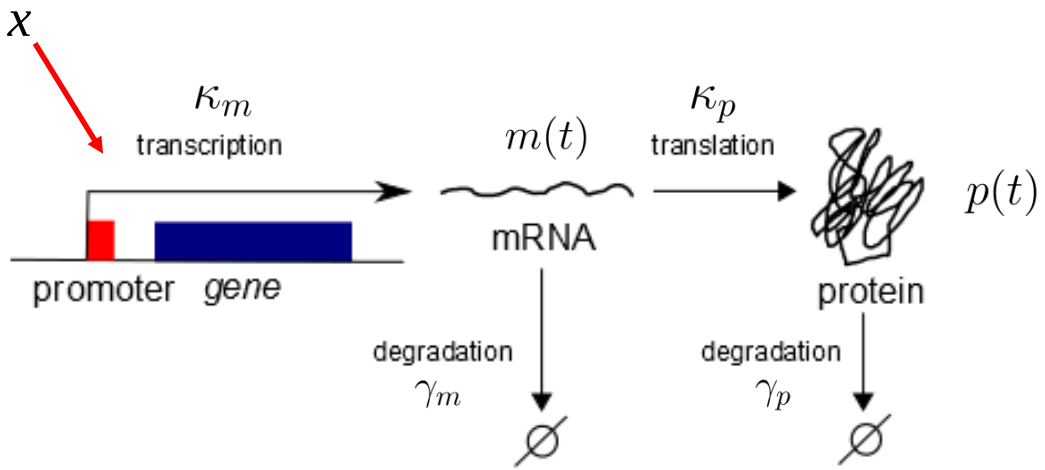
$\gamma_p > 0$, degradation rate constant

# Modeling of gene regulatory networks

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

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

$$\dot{p} = \kappa_p m - \gamma_p p$$



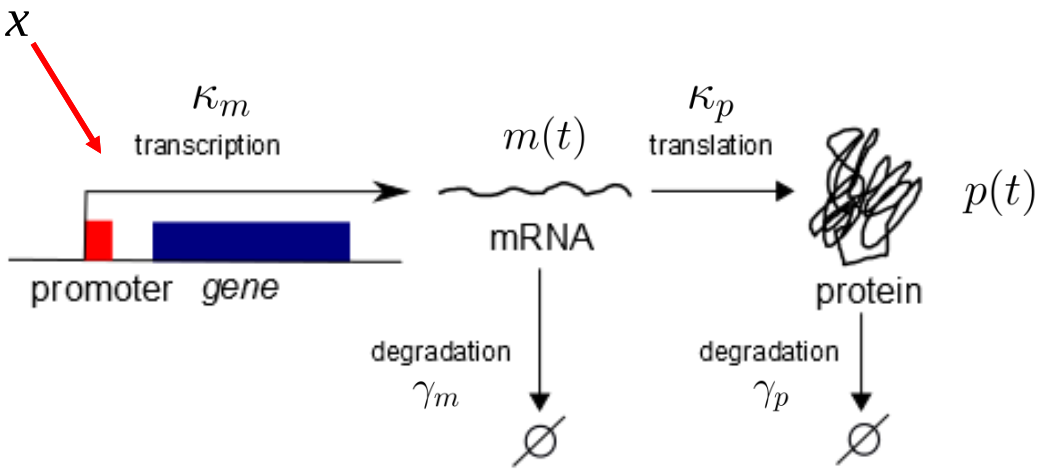

- Regulation function $f(x)$ describes modulation of synthesis rate by transcription factor
  - Generalization to regulation on translational and proteolytic level

# Modeling of gene regulatory networks

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

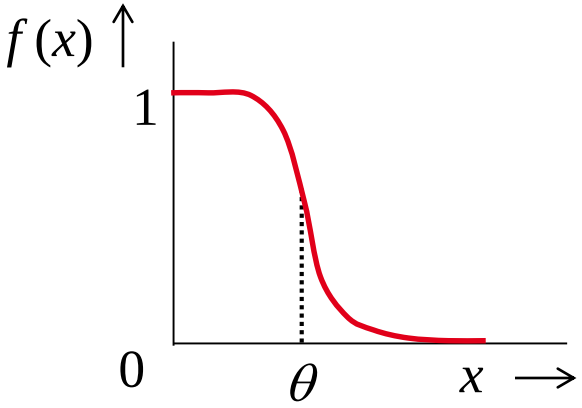
$$\dot{m} = \kappa_m f(x) - \gamma_m m$$

$$\dot{p} = \kappa_p m - \gamma_p p$$




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

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

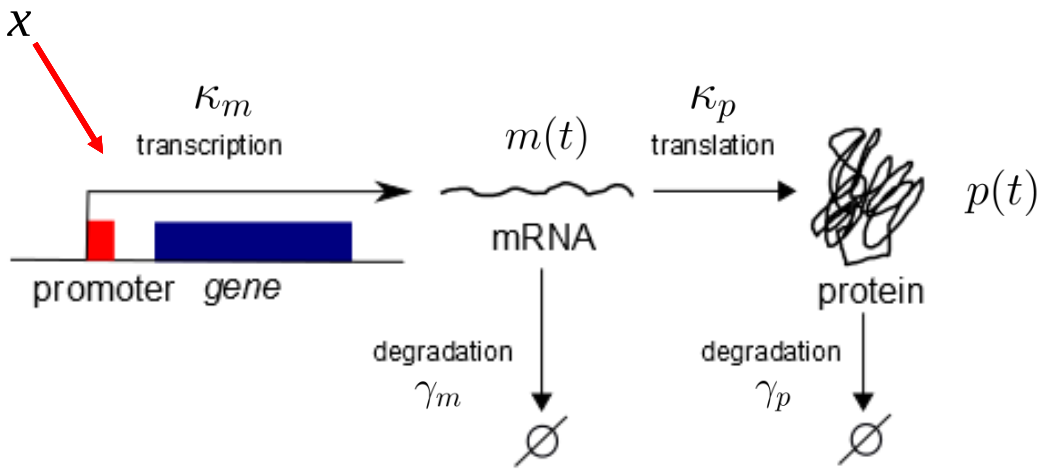


# Modeling of gene regulatory networks

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

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

$$\dot{p} = \kappa_p m - \gamma_p p$$



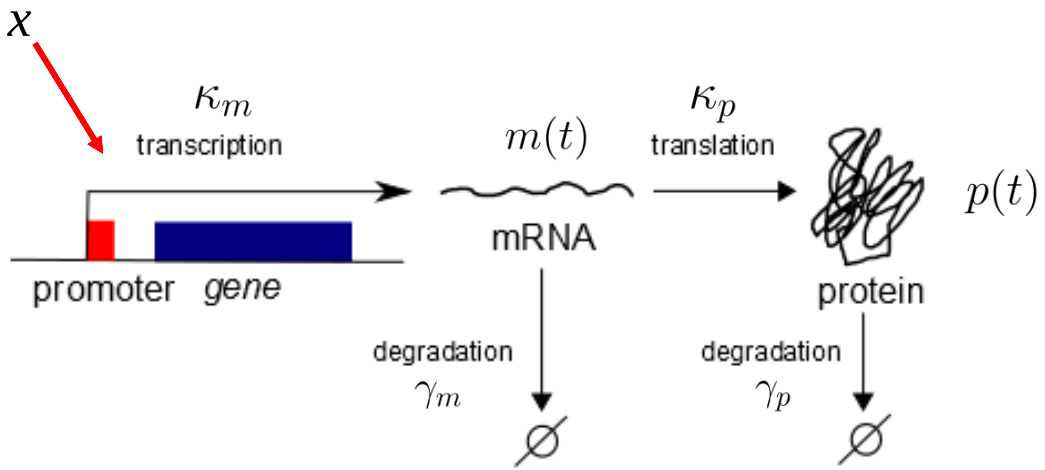

- Regulation function $f(x)$ typically has **sigmoidal** form, accounting for cooperative nature of regulation
- Implicit modeling assumptions:
  - Ignore gene expression machinery (RNA polymerase, ribosome)
  - Simplification of complex protein-DNA interactions to response function
  - No effect of growth dilution

# Modeling of gene regulatory networks

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

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

$$\dot{p} = \kappa_p m - \gamma_p p$$



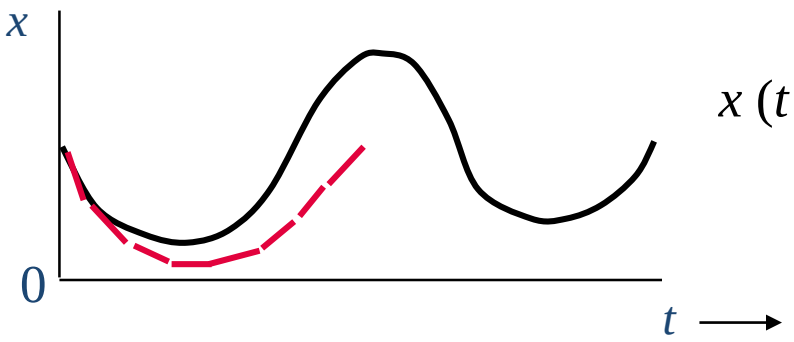

- Gene regulatory network has many genes with mutual regulatory interactions: model of coupled ODEs

# 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 $\boldsymbol{x}(0) = \boldsymbol{x}^0$

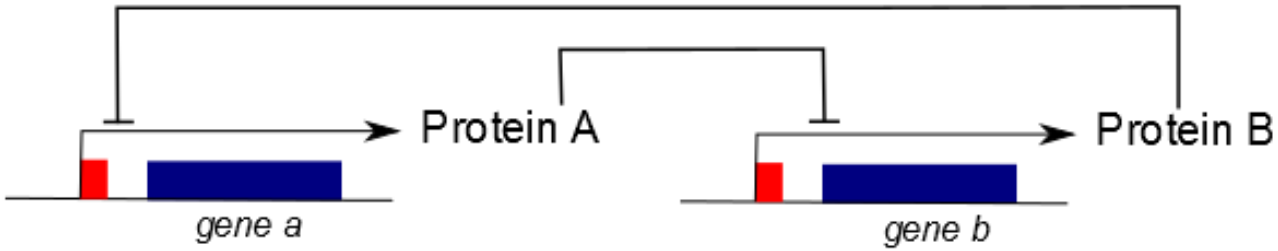



$$x(t + \Delta t) = x(t) + \int_{t}^{t+\Delta t} f(x)\,\mathrm{d}t \approx x(t) + f(x)\,\Delta t$$

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

# Cross-inhibition network

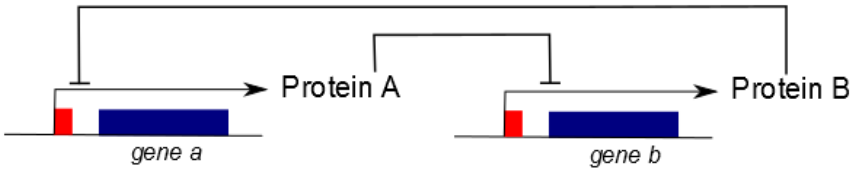
- **Cross-inhibition** network consists of two genes, each coding for transcription regulator inhibiting expression of other gene




- 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




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

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

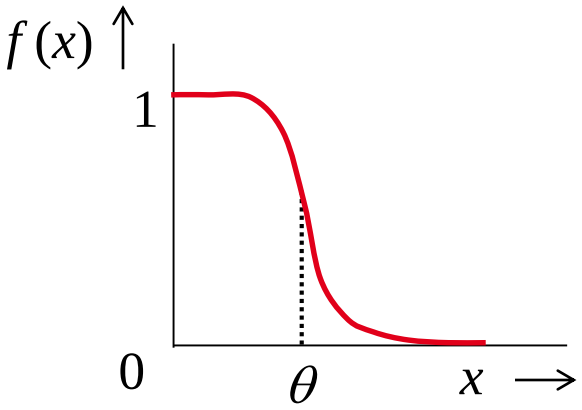
$x_a(t) \geq 0$, concentration protein A

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

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

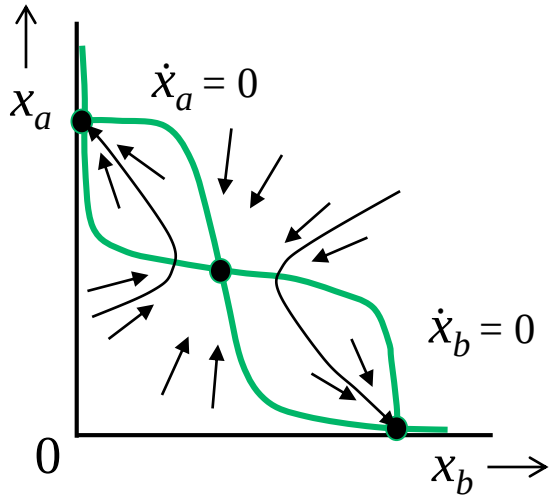
$\gamma_a , \gamma_b > 0$, degradation rate constants

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



# 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

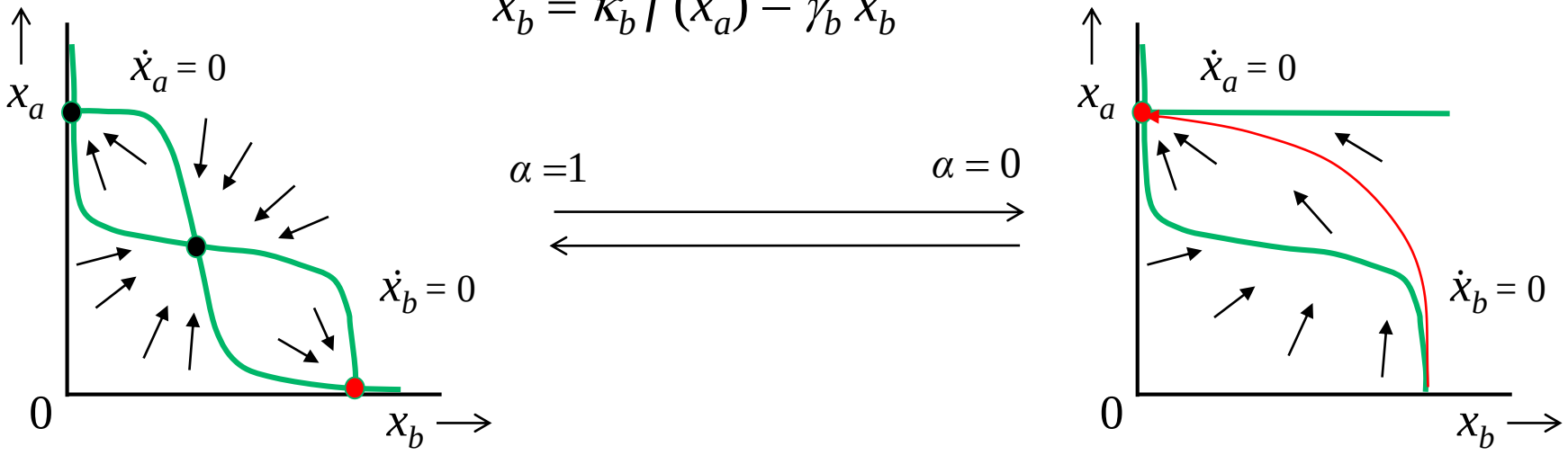
# Hysteresis in cross-inhibition network

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

  Modulation of regulatory effect of one of regulators ($\alpha$)

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

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

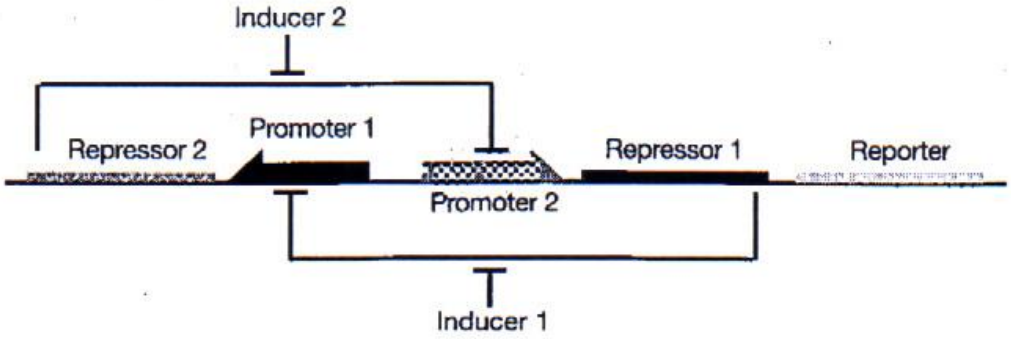


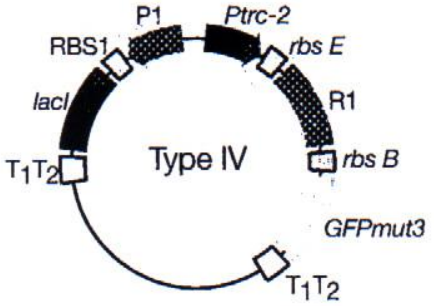

- Change in parameter causes saddle-note **bifurcation**

# Construction of cross inhibition network

- Construction of cross inhibition network *in vivo*

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



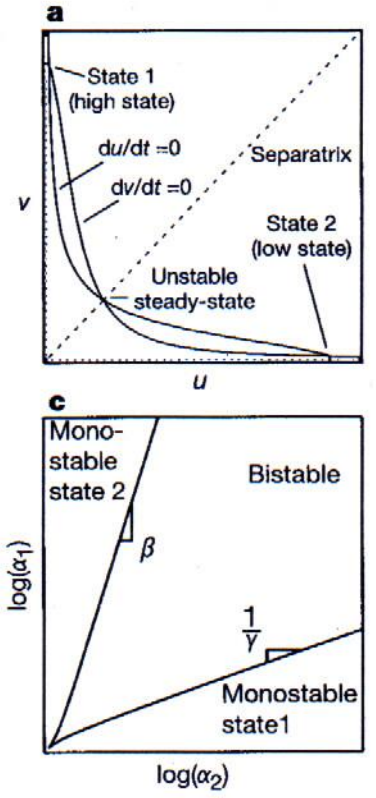

- ODE model of network

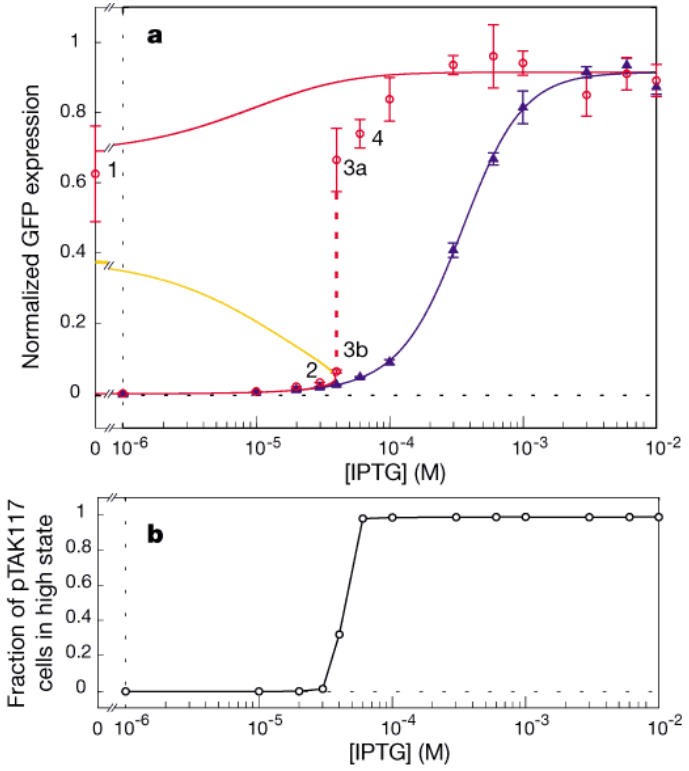
$$\dot{u} = \frac{\alpha_1}{1 + v^{\beta}} - u \qquad \dot{v} = \frac{\alpha_2}{1 + u^{\gamma}} - v$$

# Experimental test of model

- Experimental test of mathematical model (bistability and hysteresis)

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

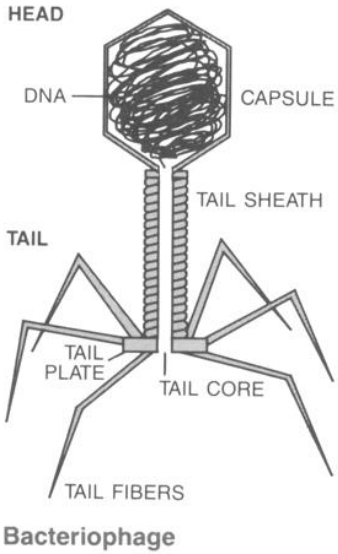


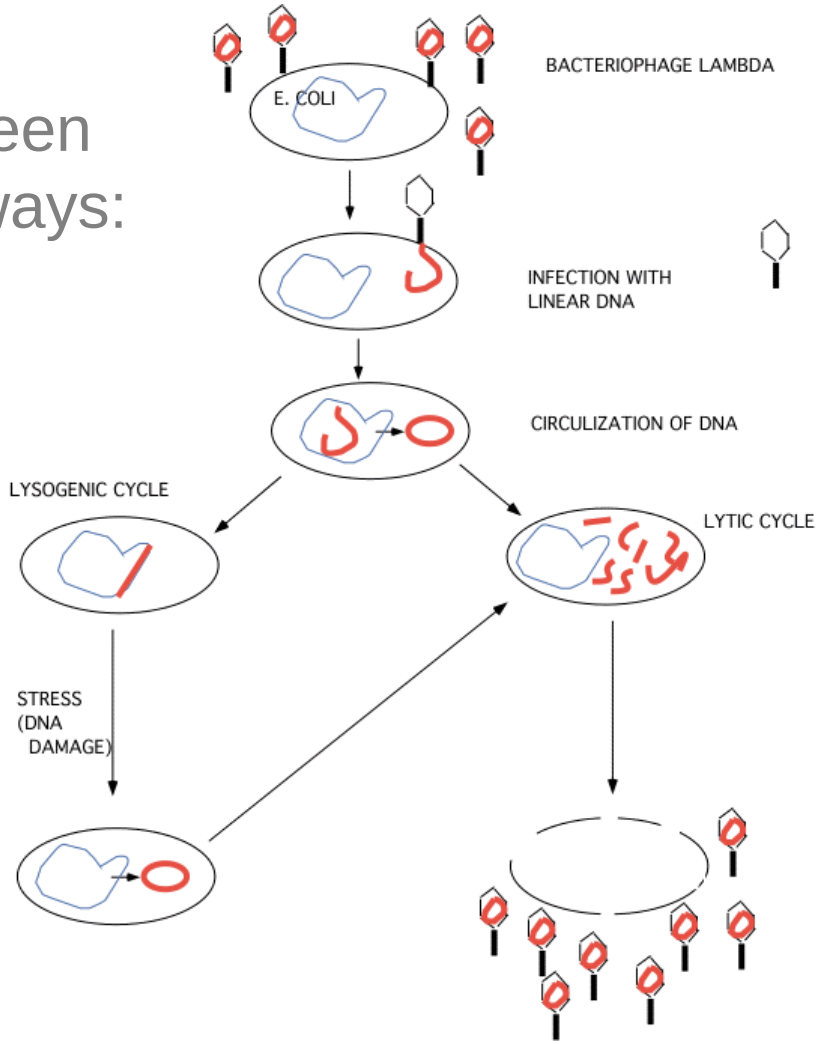



# Bacteriophage λ infection of *E. coli*

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

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



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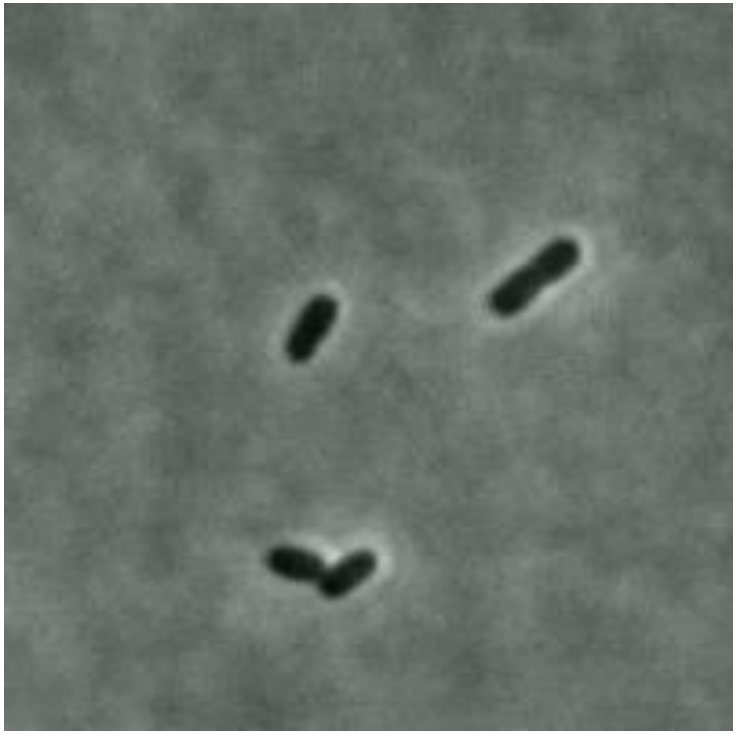
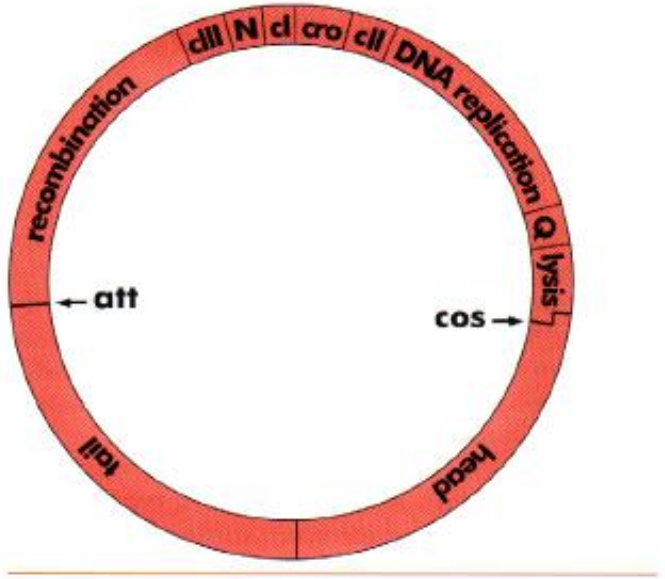
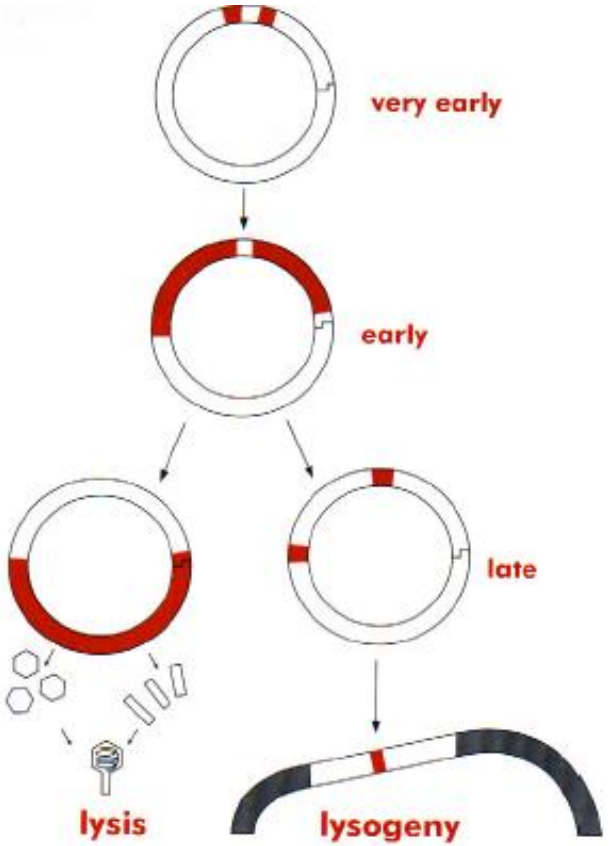


# Bistability in phage λ

- Lytic and lysogenic pathways involve different patterns of gene expression

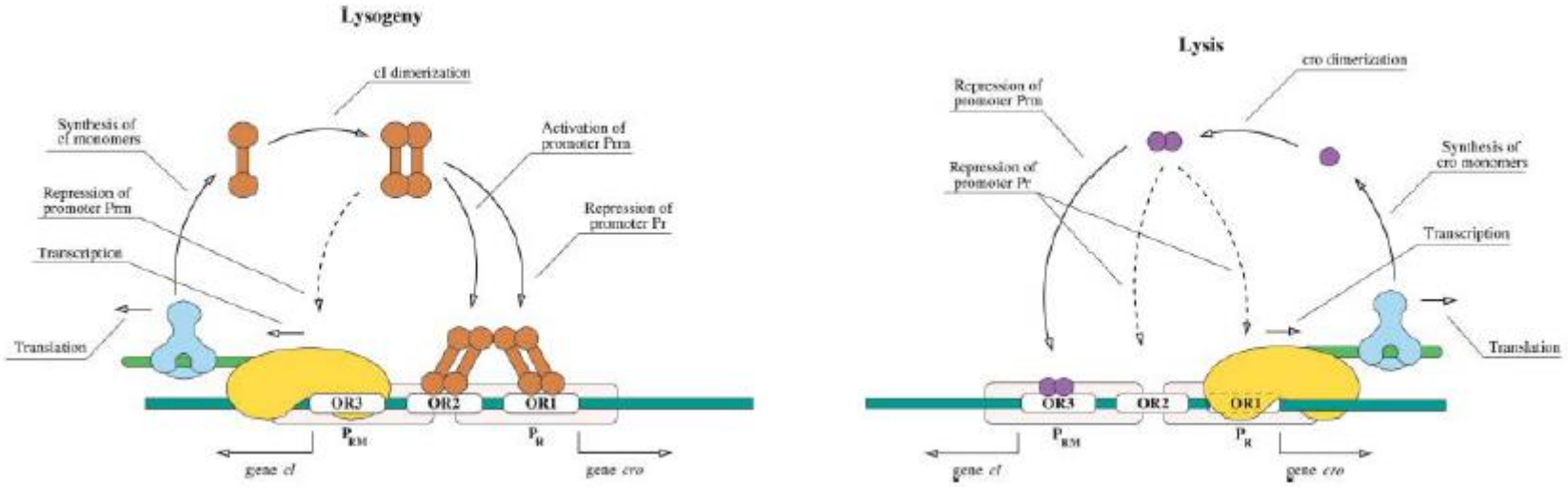



Ptashne, *A Genetic Switch*, Cell Press,1992

# 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

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

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

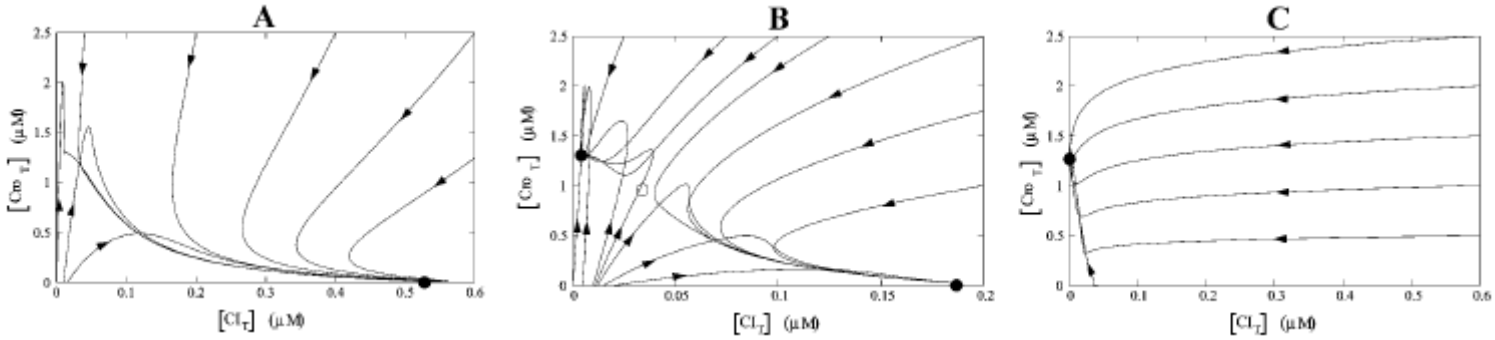
$$\frac{d[CI_T]}{dt} = \upsilon_{cI}[M_{cI}]_{\tau_{cI}} - (\gamma_{cI} + \mu)[CI_T],$$

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

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

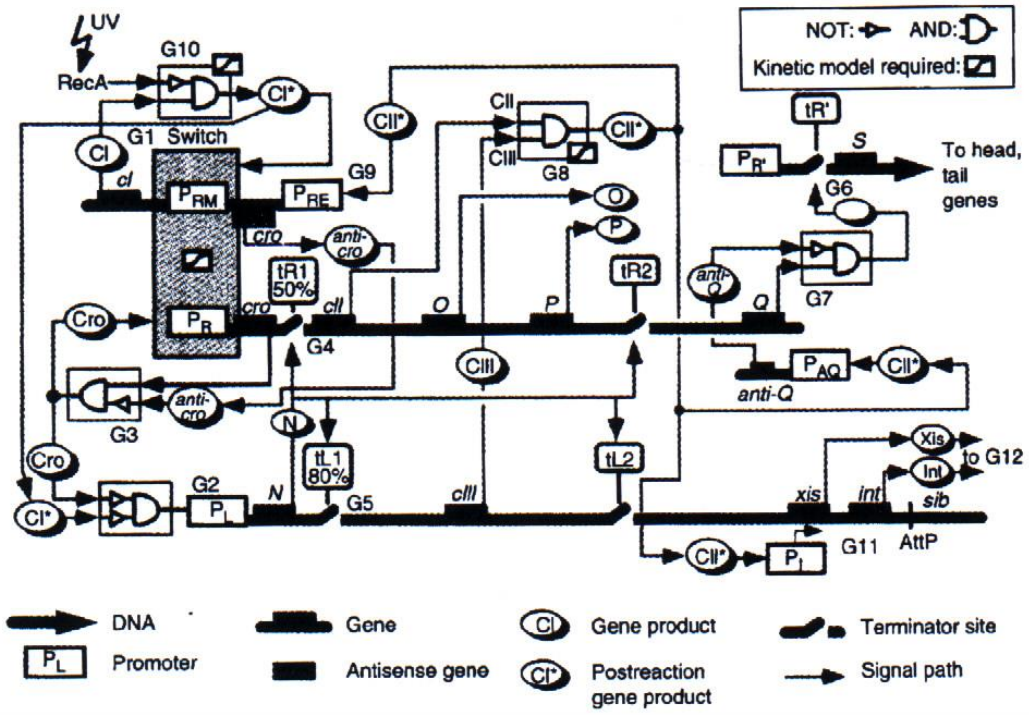
# Extended model of phage λ infection

- 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

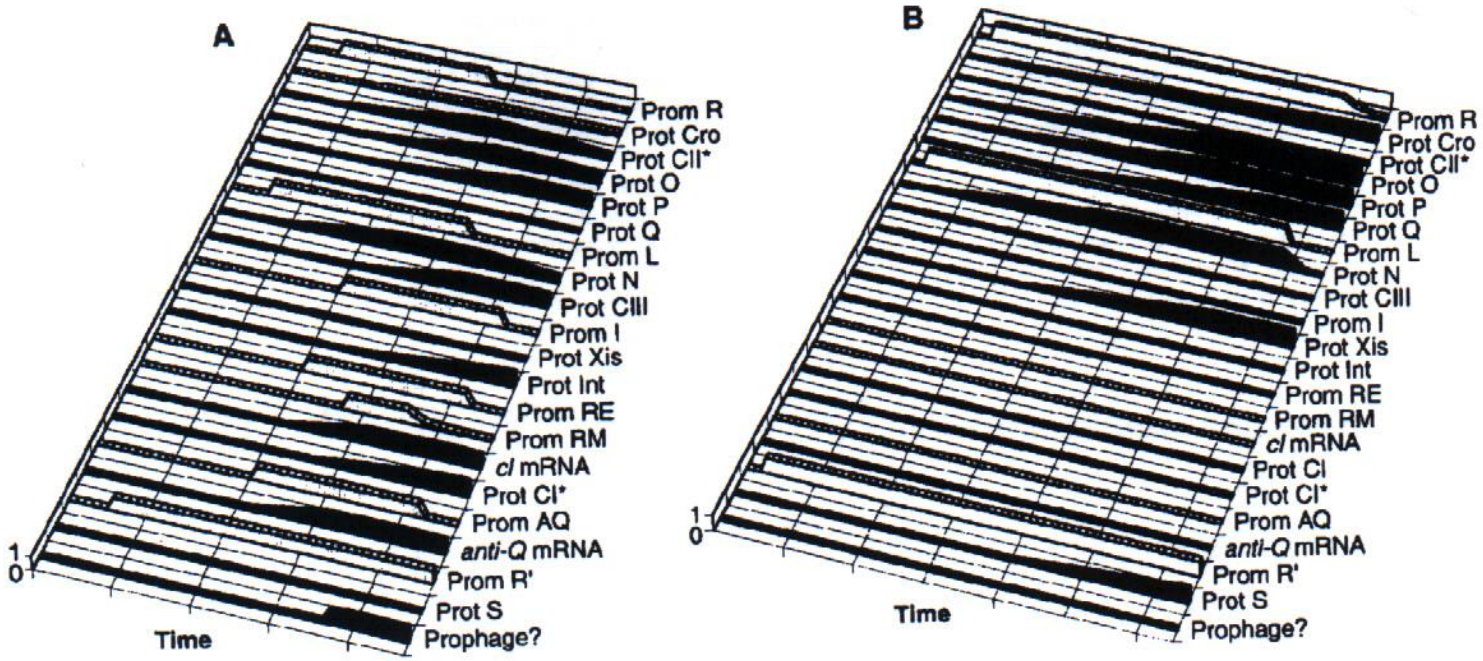
- Recent experimental work downplays importance of mutual inhibition of CI and Cro in lysis-lysogeny decision

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



# Simulation of phage λ infection

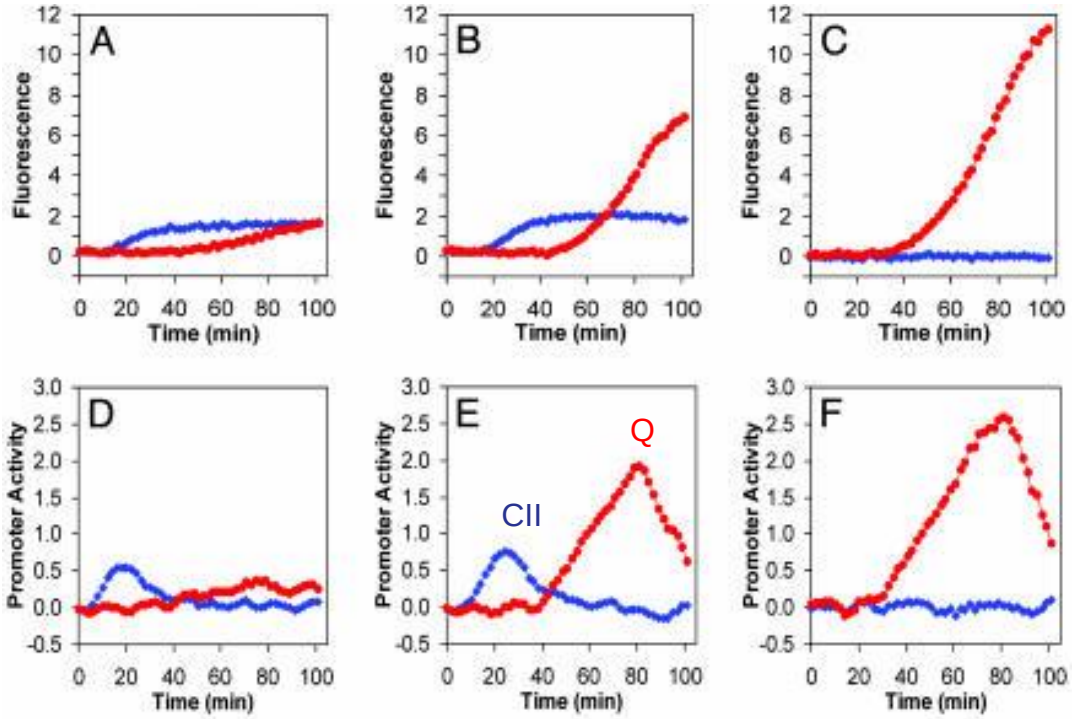
- Numerical simulation of promoter activity and protein concentrations in (a) lysogenic and (b) lytic pathways




- 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

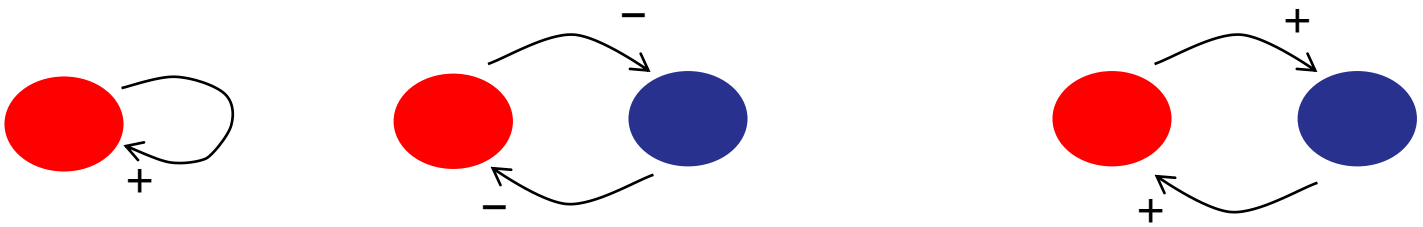
- Can we find general **design principles**, relating network structure to bistability and other properties of network dynamics?

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

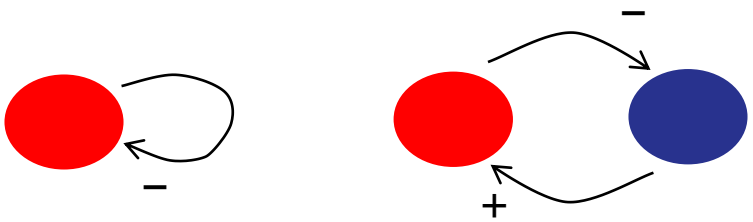
Soulé (2003), *ComPlexUs*, 1:123-33

- **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

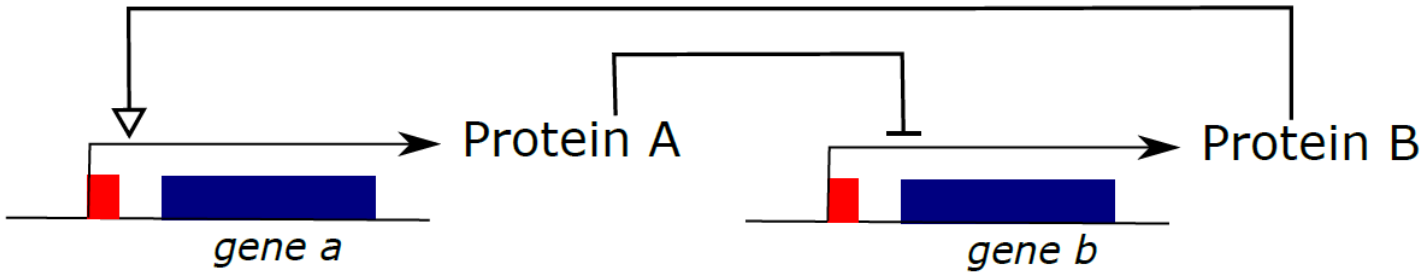



- **Condition is not sufficient**, as the actual occurrence of (stable) oscillations depends on: parameter values, nonlinearities, number of genes, …

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

# Simple oscillator network

- Question: write out the model for a simple oscillator network



# Simple oscillator network

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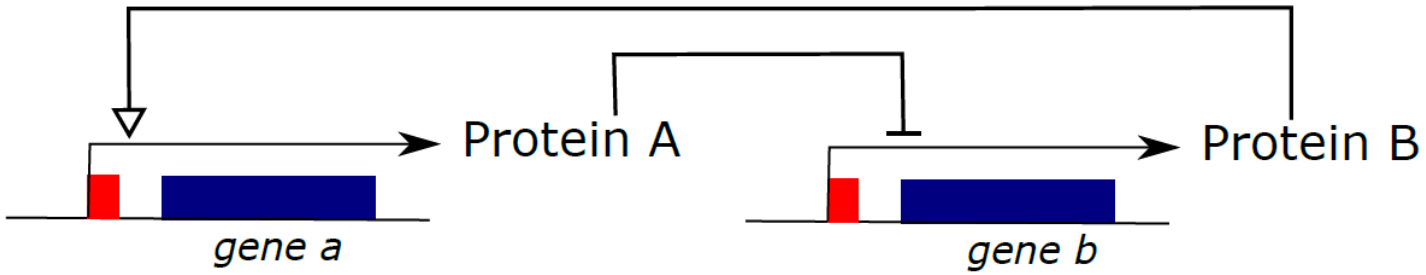
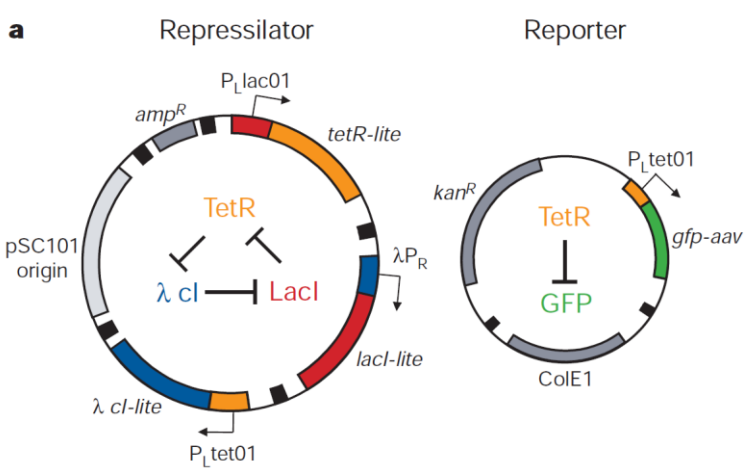


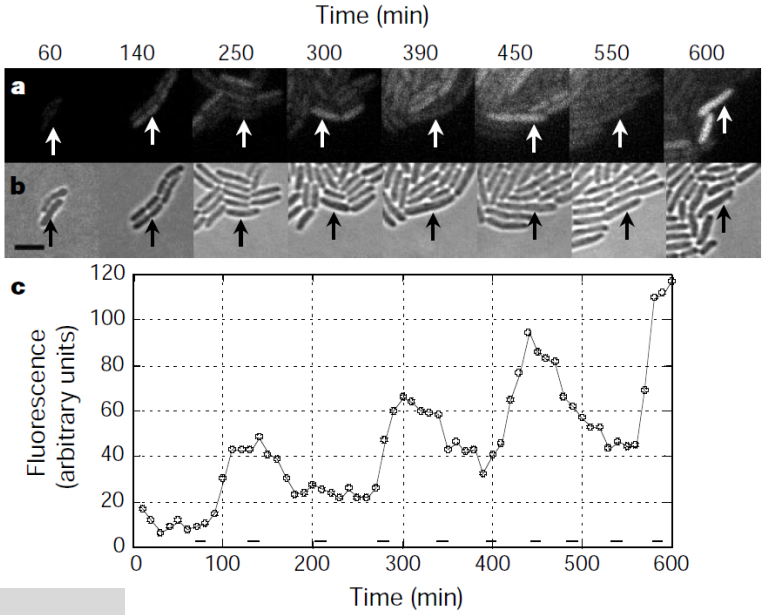

- Question: sketch nullclines in phase space and vector field

Polynikis *et al.* (2009), *J. Theor. Biol.*, 261:511-530

# Construction of oscillator network

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




- ODE model of oscillator

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

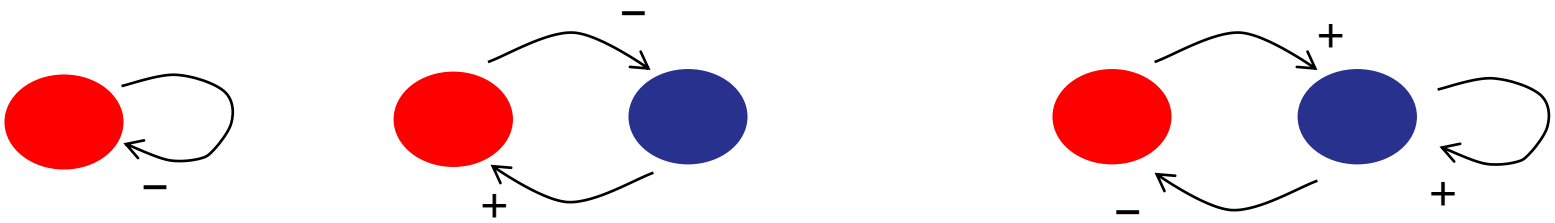
$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$

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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