Quantitative modeling of gene regulatory networks

Hidde de Jong
MICROCOSME
INRIA and UGA
Hidde.de-Jong@inria.fr

October 9, 2023

MICROCOSME: bacterial systems biology

- **MICROCOSME**: systems biology group at INRIA/Université Grenoble Alpes in Grenoble

   Microbiologists, computer scientists, mathematicians, physicists**, …**


https://team.inria.fr/microcosme

- **Objective:** analysis, engineering, and control of the growth of bacteria

  – Specific research problems shaped by **biological questions**
  – Problems often addressed by combination of **models and experiments**

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)

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
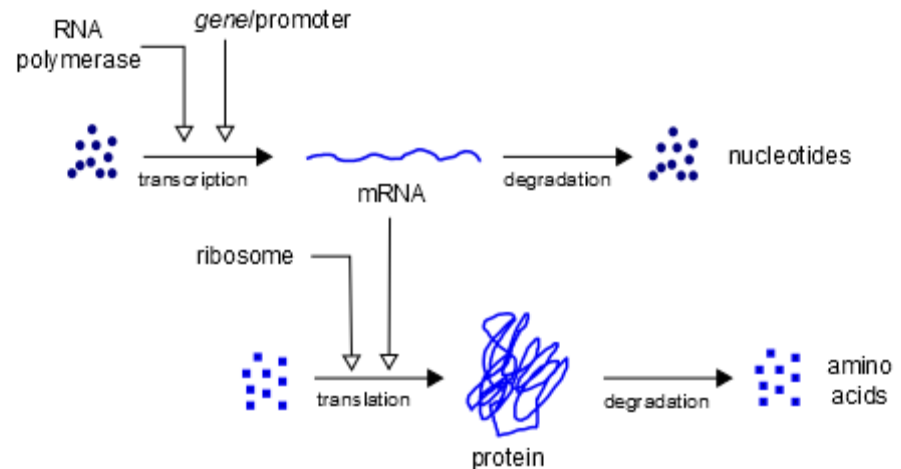 - Time-scale: min (mRNA) to h (proteins)
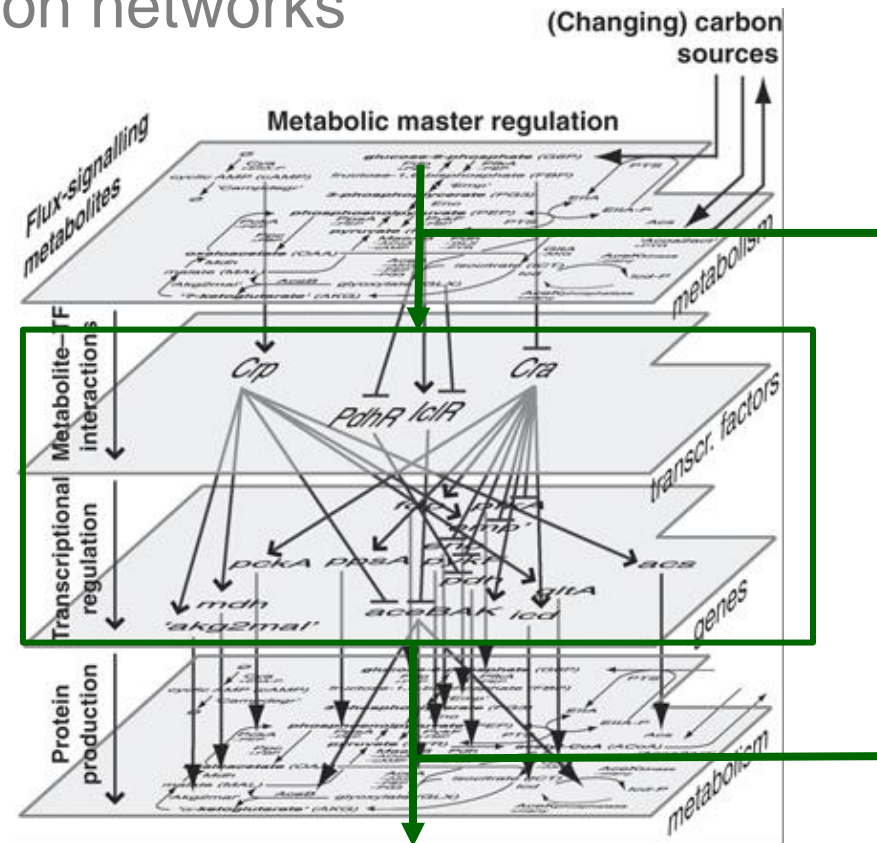
Kotte *et al.* (2010), *Mol. Syst. Biol.*, 6: 355

Gene expression

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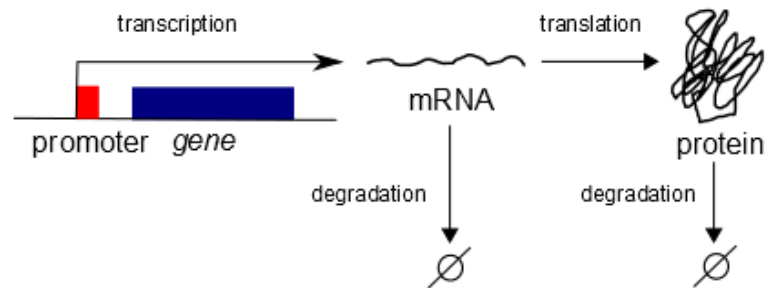 - Degradation of mRNA and protein

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
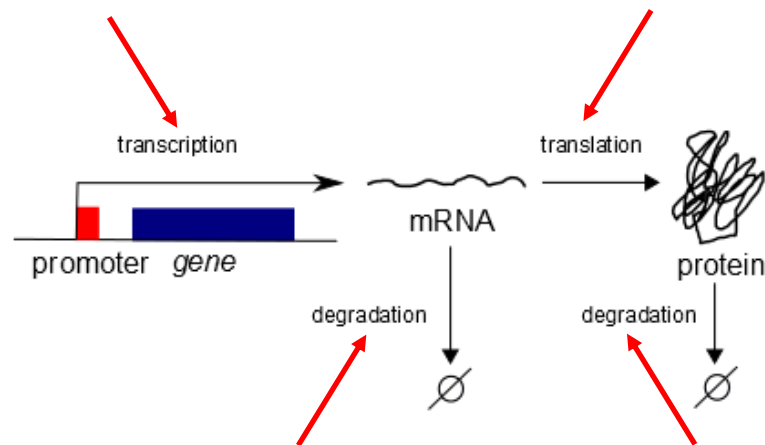
Gene regulatory networks

- **Gene regulatory networks** control changes in expression levels in response to environmental perturbations
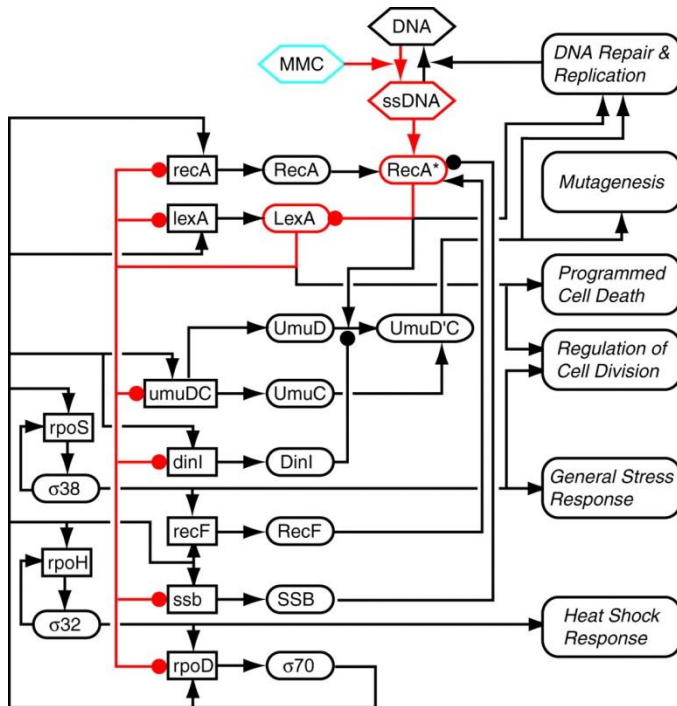
Original *lac* operon model
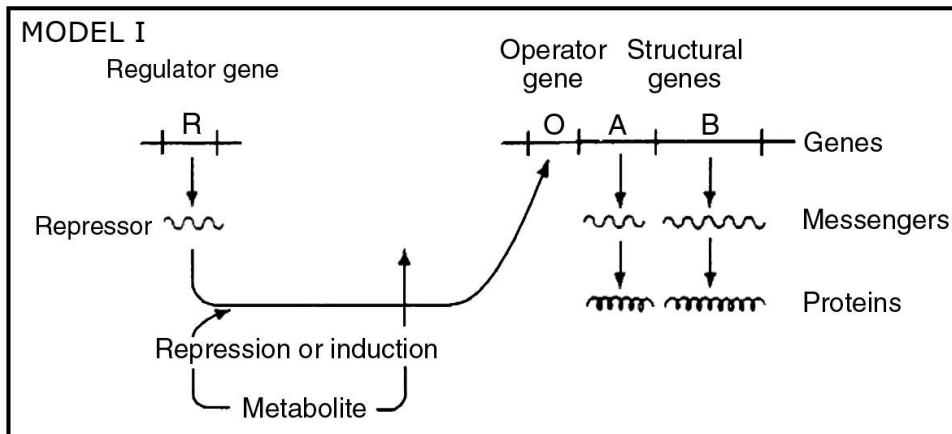
Jacob and Monod (1961), *J. Mol. Biol.*, 3(3):318-56

Gene regulatory networks

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

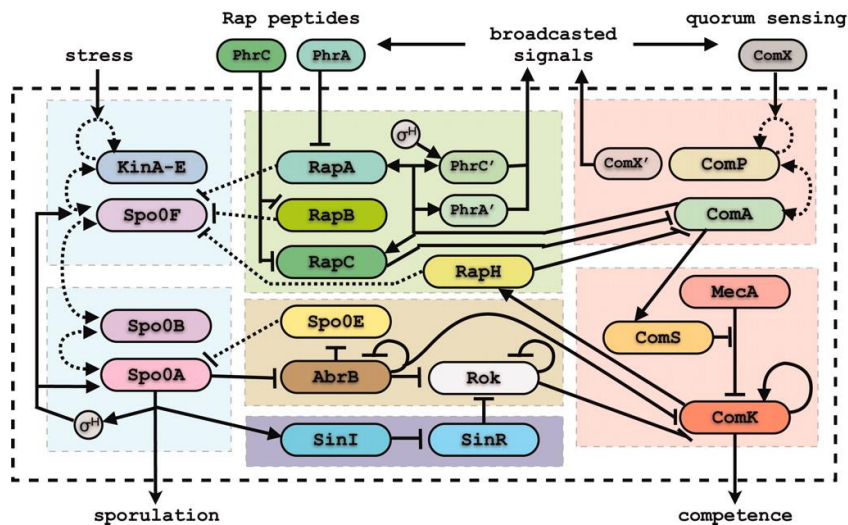SOS response network in *E. coli*

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

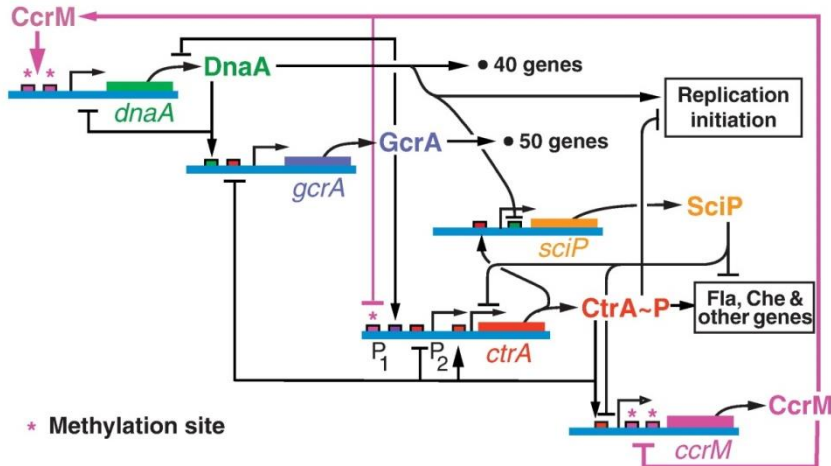Sporulation and competence network in *B. subtilis*

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

Gene regulatory networks

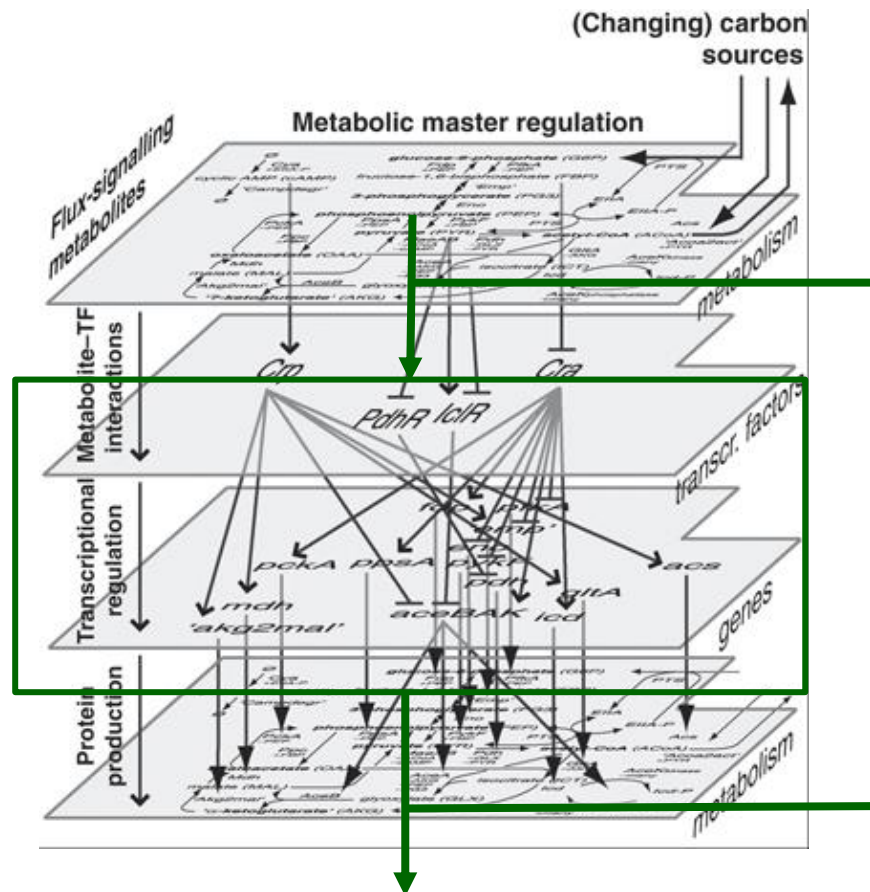- **Gene regulatory networks** control changes in expression levels in response to environmental perturbations

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
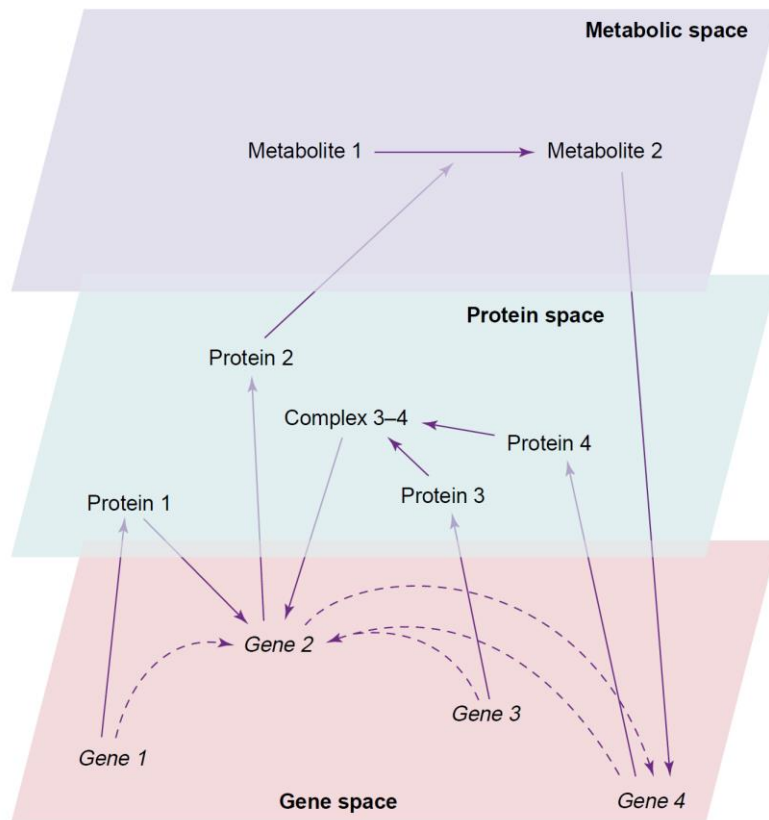

- **But**: adaptation of gene expression leads to changes in metabolism which feed back into regulatory network

- 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
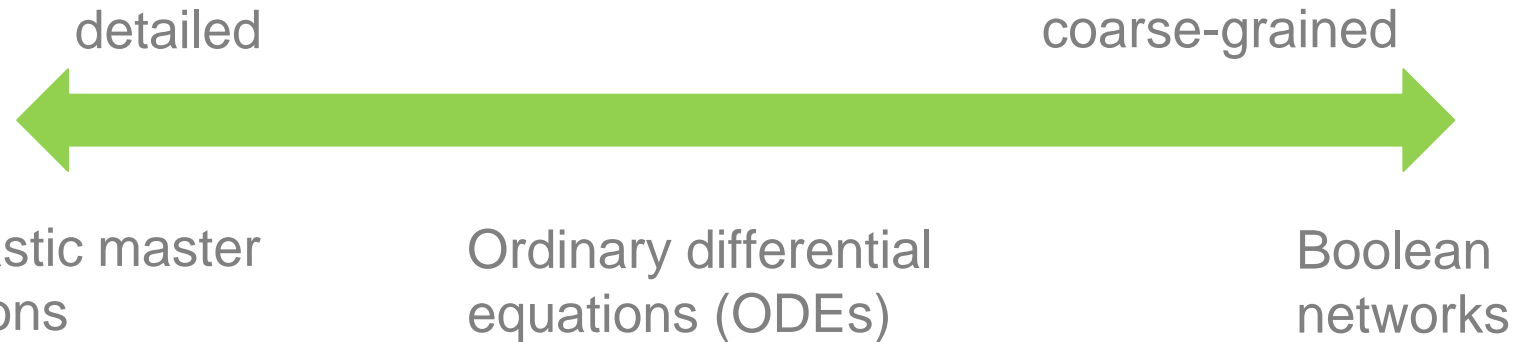

- **But**: adaptation of gene expression leads to changes in metabolism which feed back into regulatory network

- 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

detailed   coarse-grained

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 $x_i(t) \in \mathbf{R}_{\geq 0}$

  Concentration on level of (growing) cell population
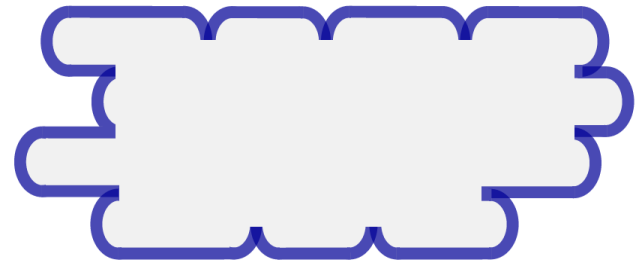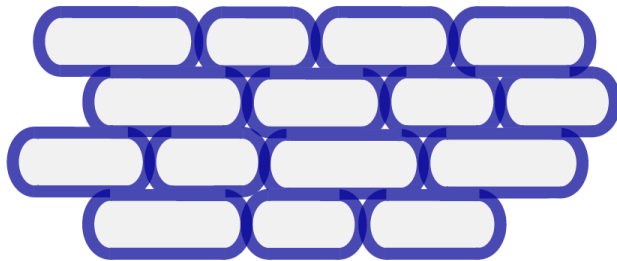
$Vol(t)$

- 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 $x_i(t) \in \mathbf{R}_{\geq 0}$

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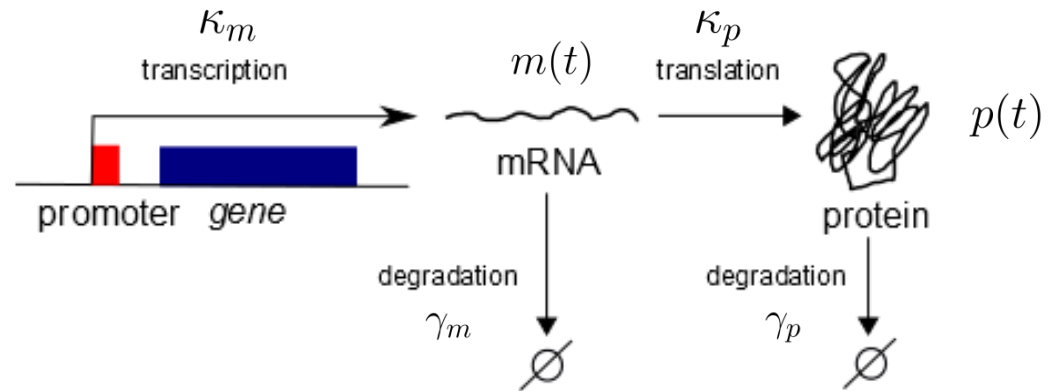
Cornish-Bowden (1995), *Fundamentals of Enzyme Kinetics*, Portland Press

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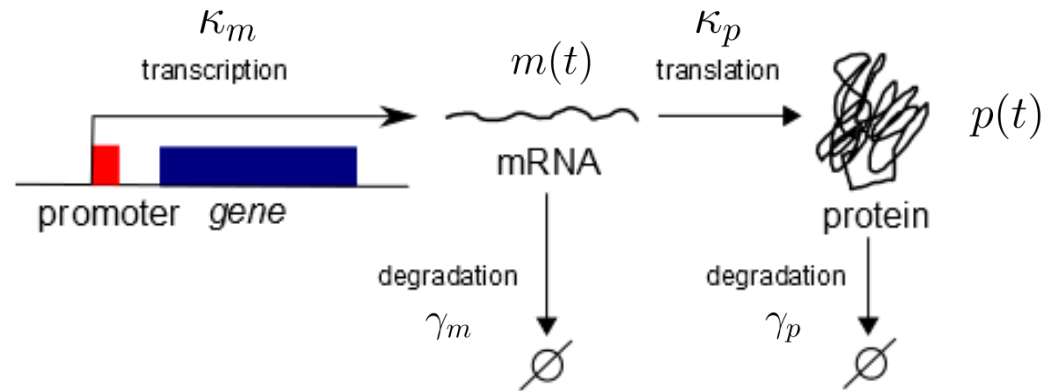
$\kappa_m, \kappa_p > 0$, synthesis rate constants

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

Modeling of gene regulatory networks

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

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

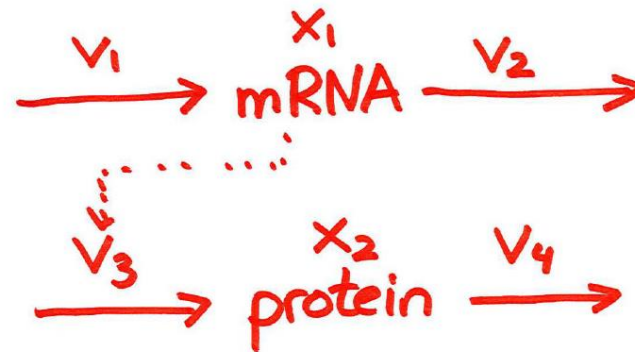$$\dot{p} = \kappa_p\, m - \gamma_p\, p$$

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

Modeling of gene regulatory networks

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

$$\dot{m} = \kappa_m - \gamma_m\, m$$
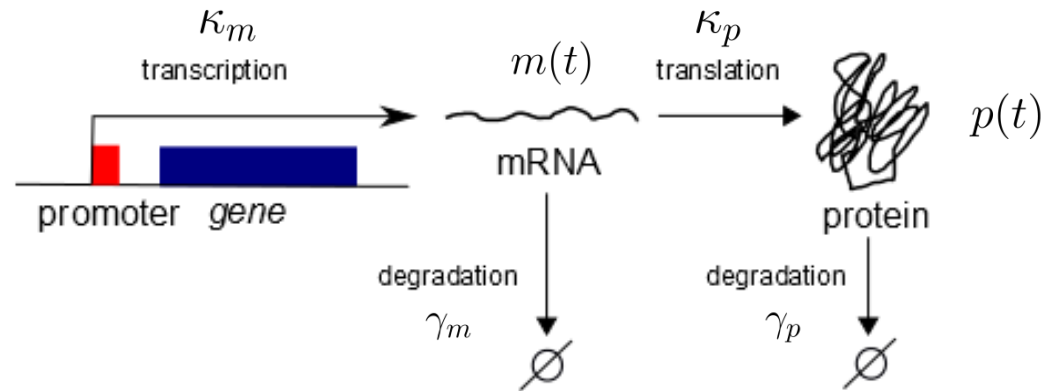$$\dot{p} = \kappa_p\, m - \gamma_p\, p$$

$$\frac{dx}{dt} = Nv = \begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{bmatrix}$$

$$v_3 = k_p \cdot x_1$$

Modeling of gene regulatory networks

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

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

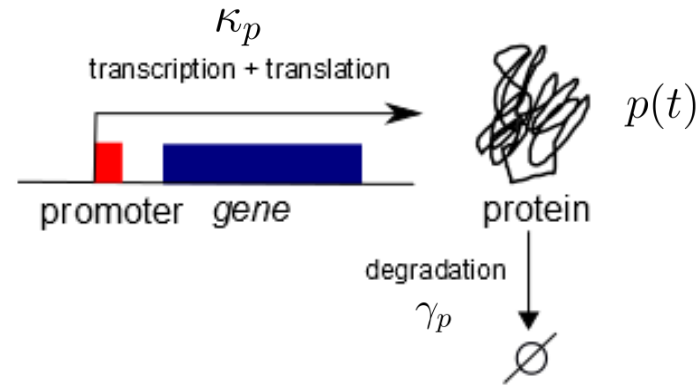$$\dot{p} = \kappa_p\, m - \gamma_p\, p$$

- **Question:** extend model with dependence on growth rate, assuming that $\mu \gg \gamma_p$ and $\mu \ll \gamma_m$

- **Question:** solve model for protein concentration at steady state. Under which conditions is growth dilution dominant?

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
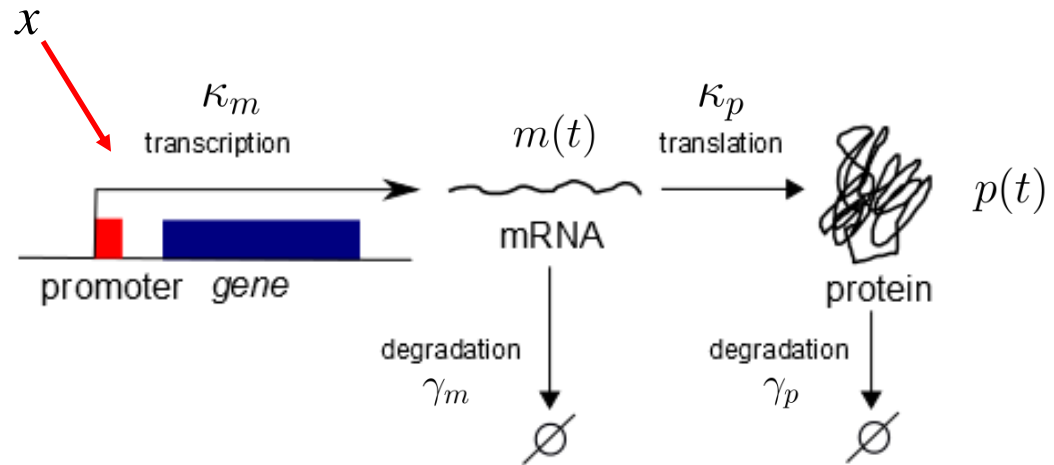
$\kappa_p > 0$, synthesis rate constant

$\gamma_p > 0$, degradation rate constant

Modeling of gene regulatory networks

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

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

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

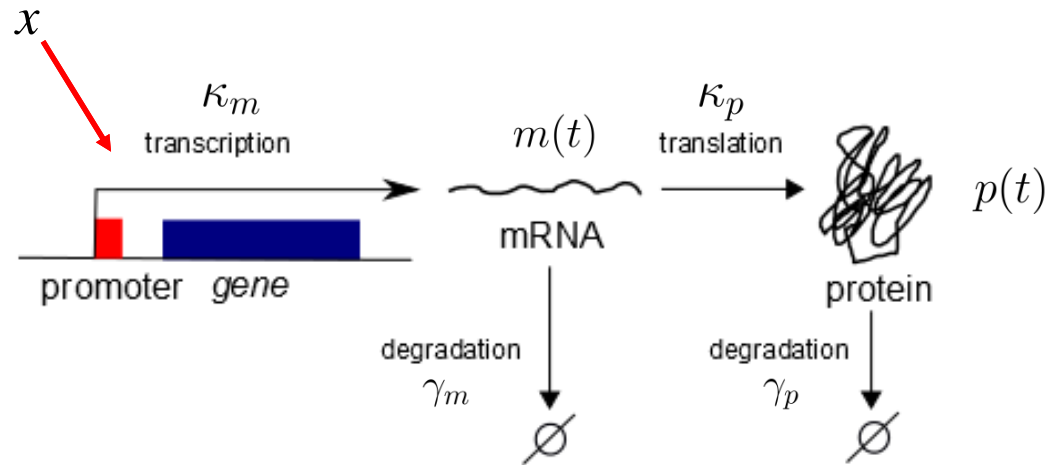- Regulation function $f(x)$ describes modulation of synthesis rate by transcription factor

    Generalization to regulation on translational and proteolytic level

Modeling of gene regulatory networks

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

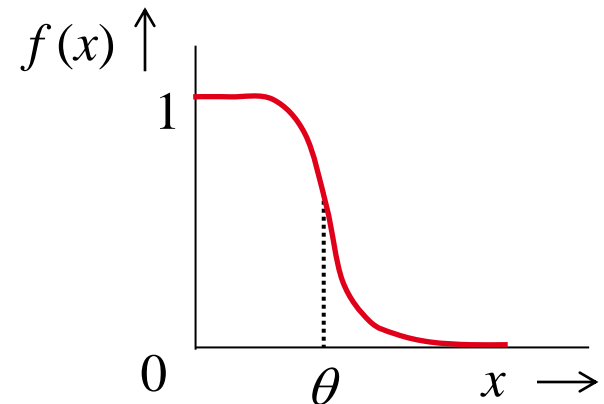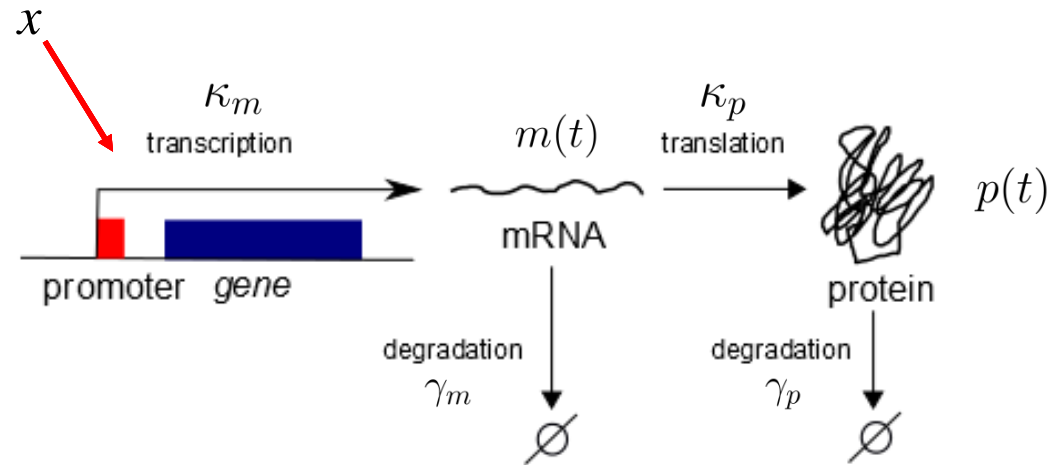$$\dot{m} = \kappa_m f(x) - \gamma_m m$$

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

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

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

$$n > 1 \text{ cooperativity}$$

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

$$\dot{m} = \kappa_m f(x) - \gamma_m m$$
$$\dot{p} = \kappa_p m - \gamma_p p$$
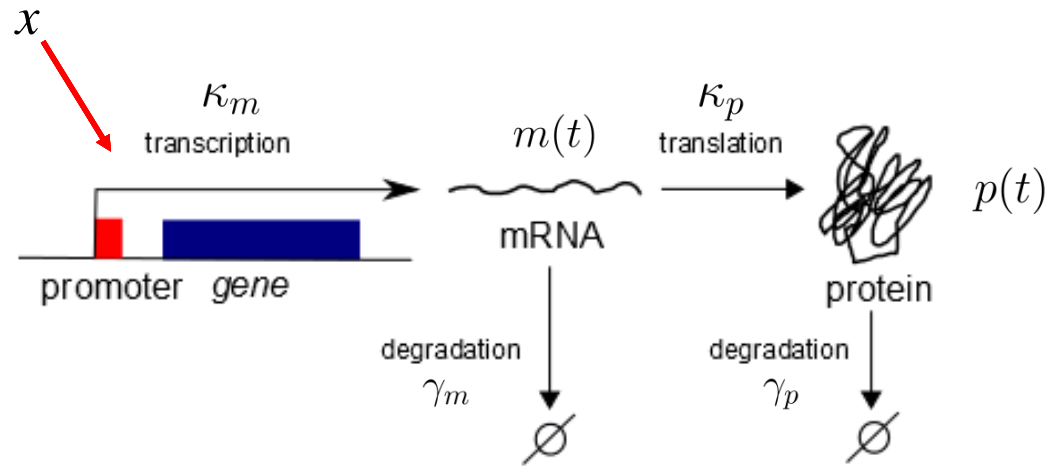
- Gene regulatory network has many genes with mutual regulatory interactions: model of coupled ODEs

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 $\boldsymbol{x}(0) = \boldsymbol{x}^0$
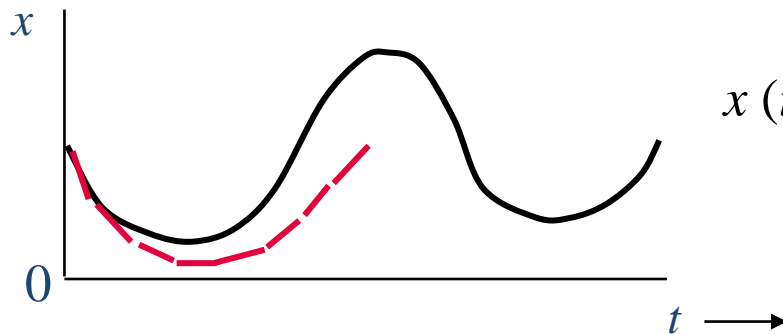
$$x(t + \Delta t) = x(t) + \int_{t}^{t + \Delta t} f(x)\, \mathrm{d}t \approx x(t) + f(x)\, \Delta t$$

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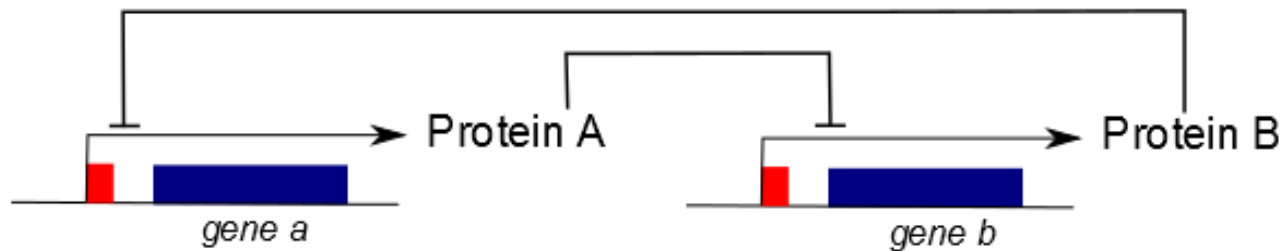
- Cross-inhibition network is example of **positive feedback**, important for phenotypic differentiation (multi-stability)

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

ODE model of cross-inhibition network
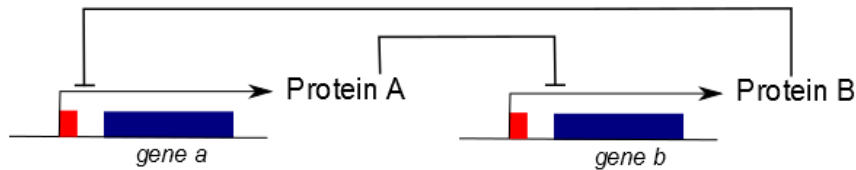
$x_a(t) \geq 0$, concentration protein A

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

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

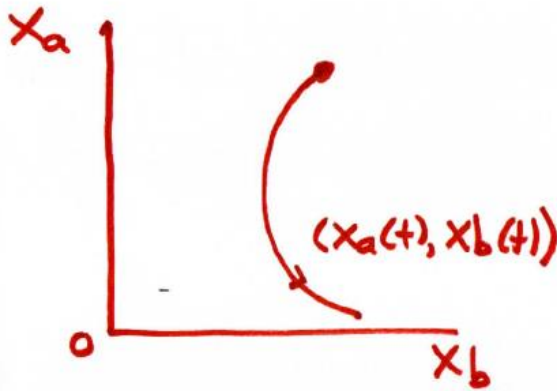γ_a, $\gamma_b > 0$, degradation rate constants

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

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

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

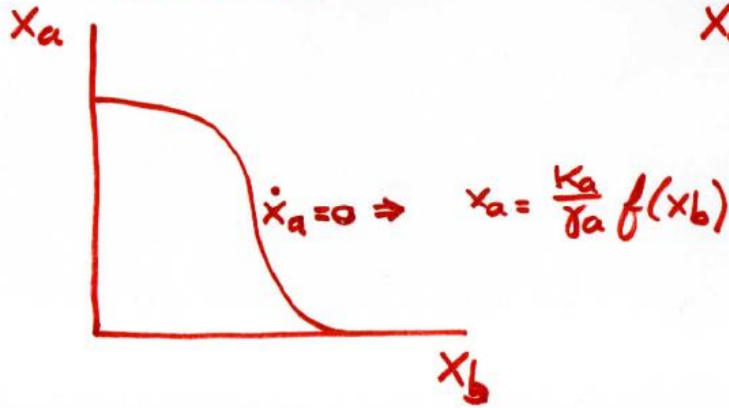$n > 1$ cooperativity
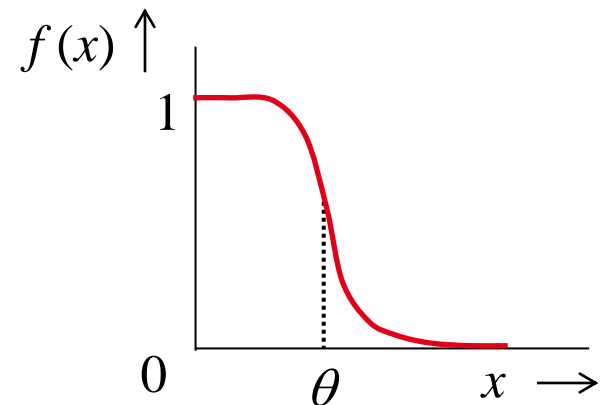
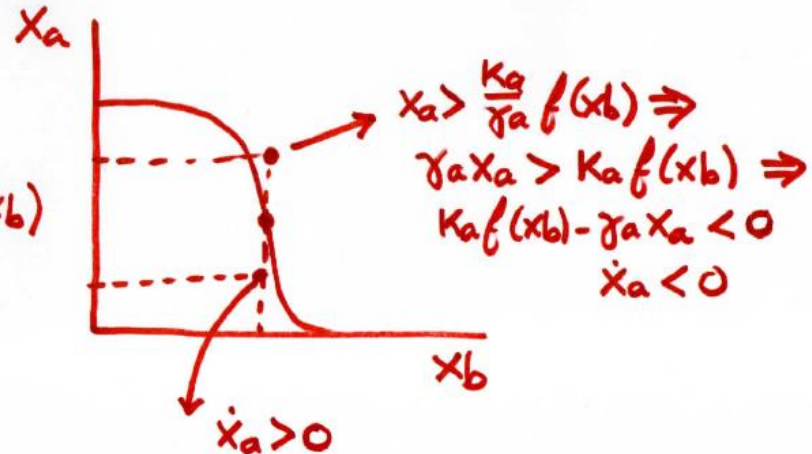# Reminders dynamical systems theory

Solutions in phase plane

x_a

$(x_a(t), x_b(t))$

0
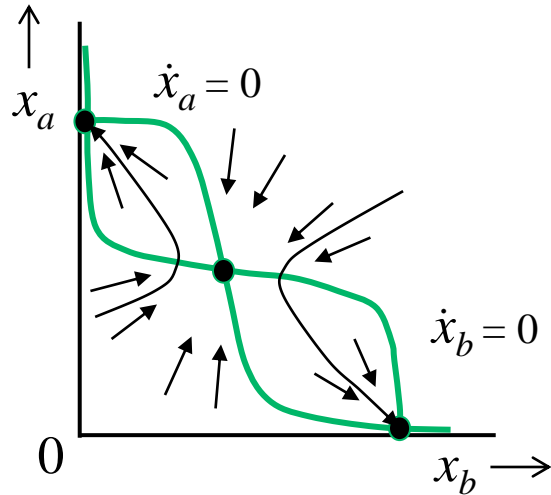
x_b

Vector field

x_a

$(\dot{x}_a, \dot{x}_b)$

x_b

Null clines

x_a

$\dot{x}_a = 0 \Rightarrow \qquad x_a = \dfrac{K_a}{\gamma_a} f(x_b)$

x_b

Direction vector field

x_a

$x_a > \dfrac{K_a}{\gamma_a} f(x_b) \Rightarrow$

$\gamma_a x_a > K_a f(x_b) \Rightarrow$

$K_a f(x_b) - \gamma_a x_a < 0$

$\dot{x}_a < 0$

$\dot{x}_a > 0$

x_b

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**)
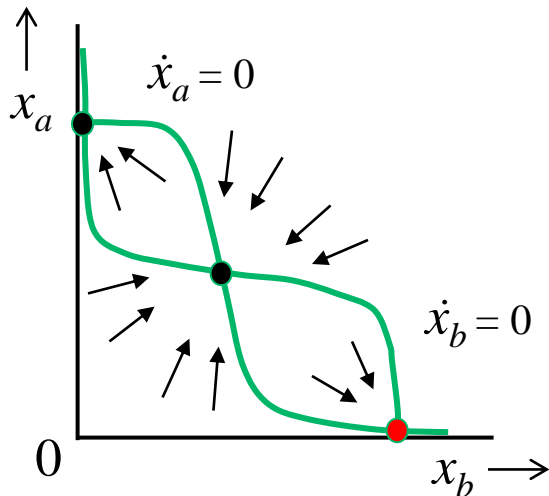- System returns to steady state after small perturbation

Hysteresis in cross-inhibition network

- Transient perturbation may cause irreversible switch from one steady state to another (**hysteresis**)
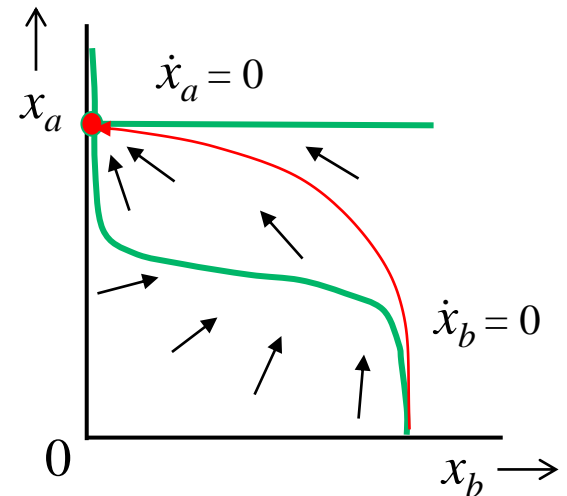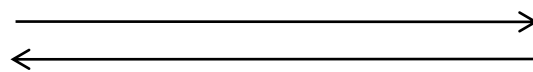
    Modulation of regulatory effect of one of regulators (α)

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

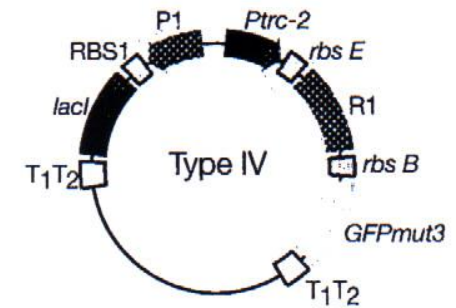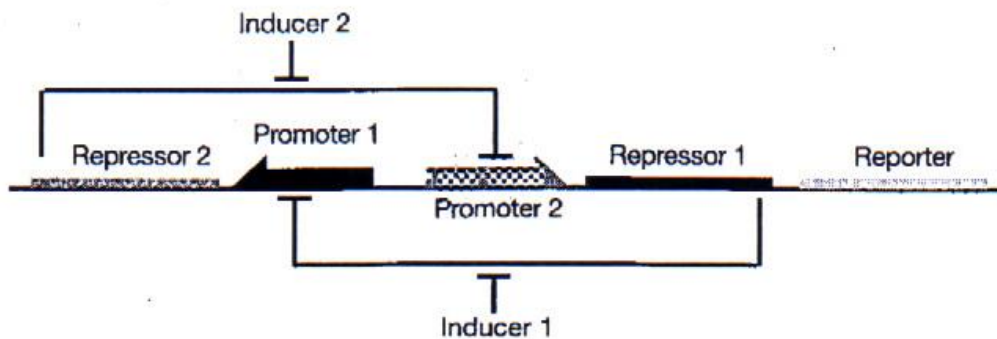$$\dot{x}_b = \kappa_b f(x_a) - \gamma_b x_b$$

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

Construction of cross inhibition network

- Construction of cross inhibition network *in vivo*

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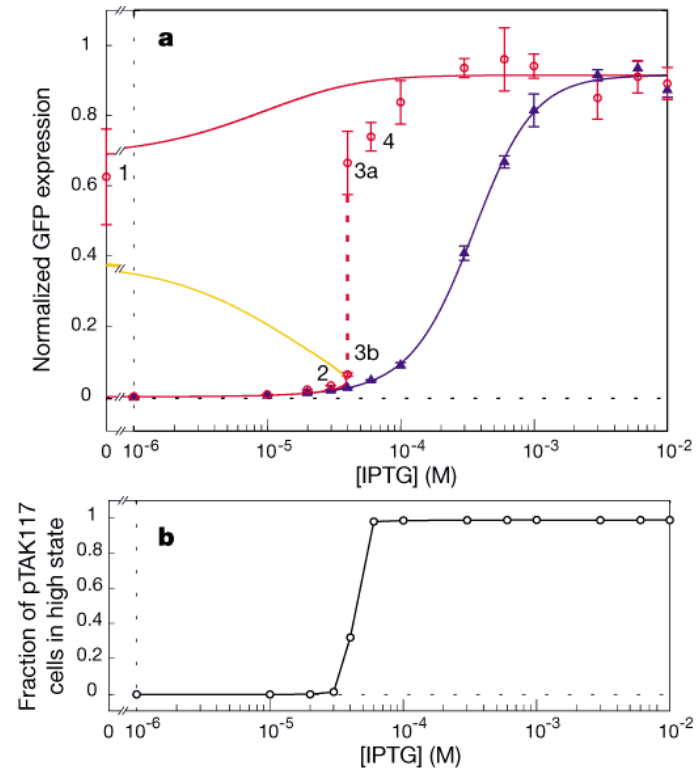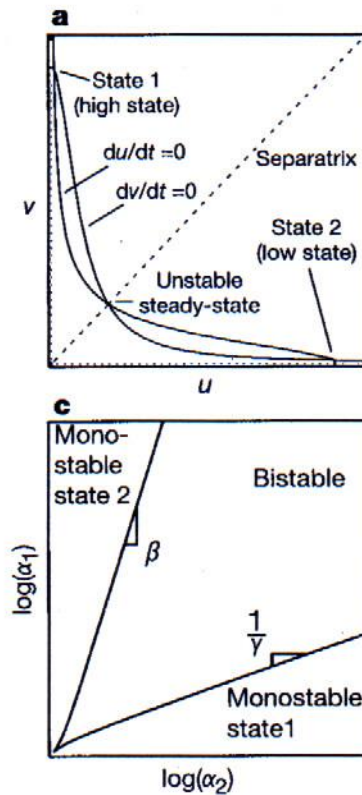

- ODE model of network

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

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

Experimental test of model

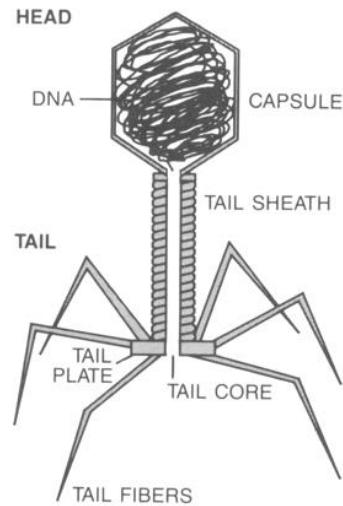- Experimental test of mathematical model (bistability and hysteresis) Gardner *et al.* (2000), *Nature*, 403(6786): 339-42

Bacteriophage λ infection of *E. coli*

- Response of *E. coli* to phage λ infection involves decision between alternative developmental pathways: **lysis** and **lysogeny**
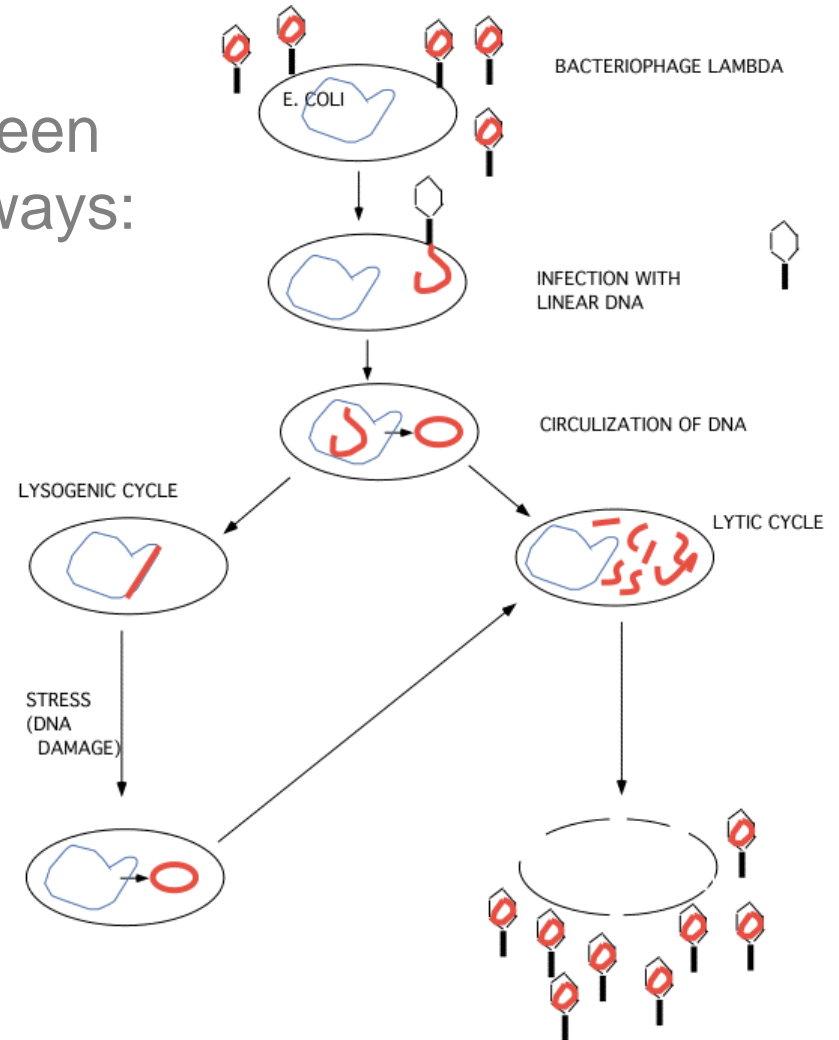
  Ptashne, *A Genetic Switch*, Cell Press,1992

Bacteriophage λ infection of *E. coli*

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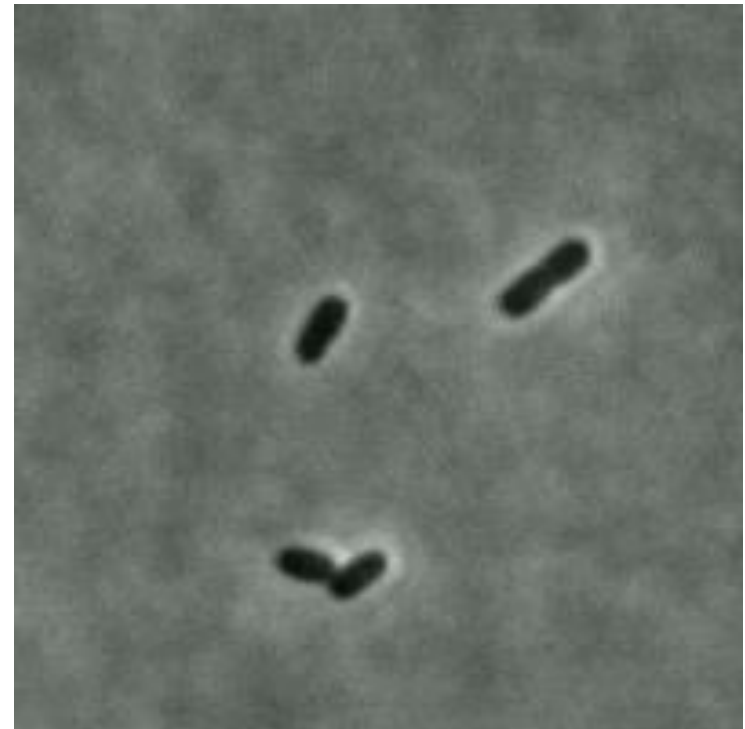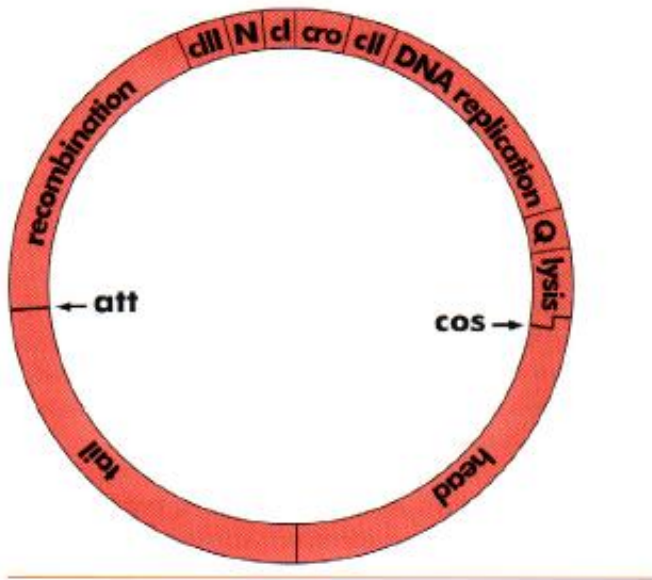 Ptashne, *A Genetic Switch*, Cell Press, 1992
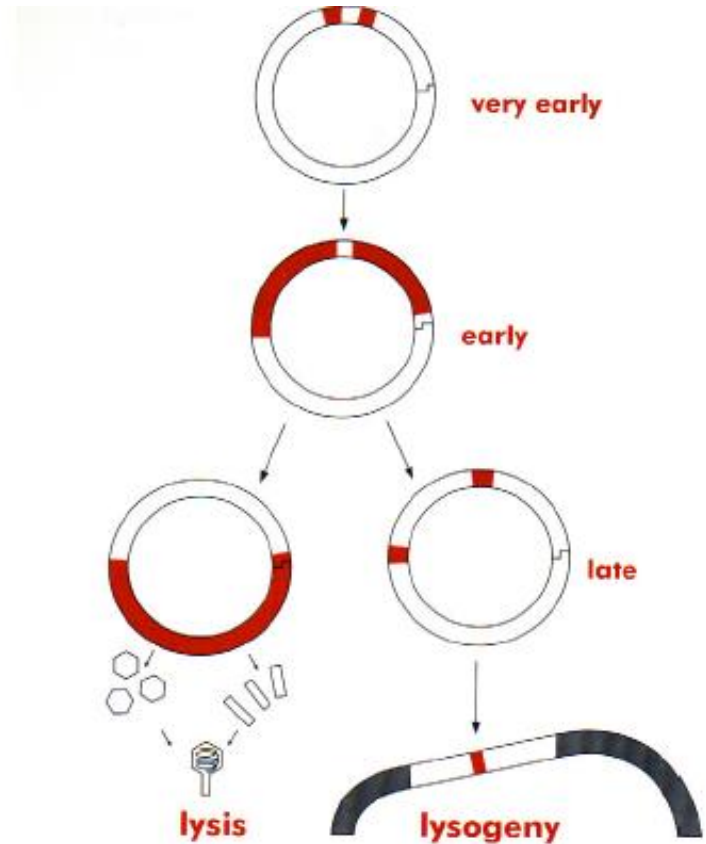
Bistability in phage λ

- Lytic and lysogenic pathways involve different patterns of gene expression
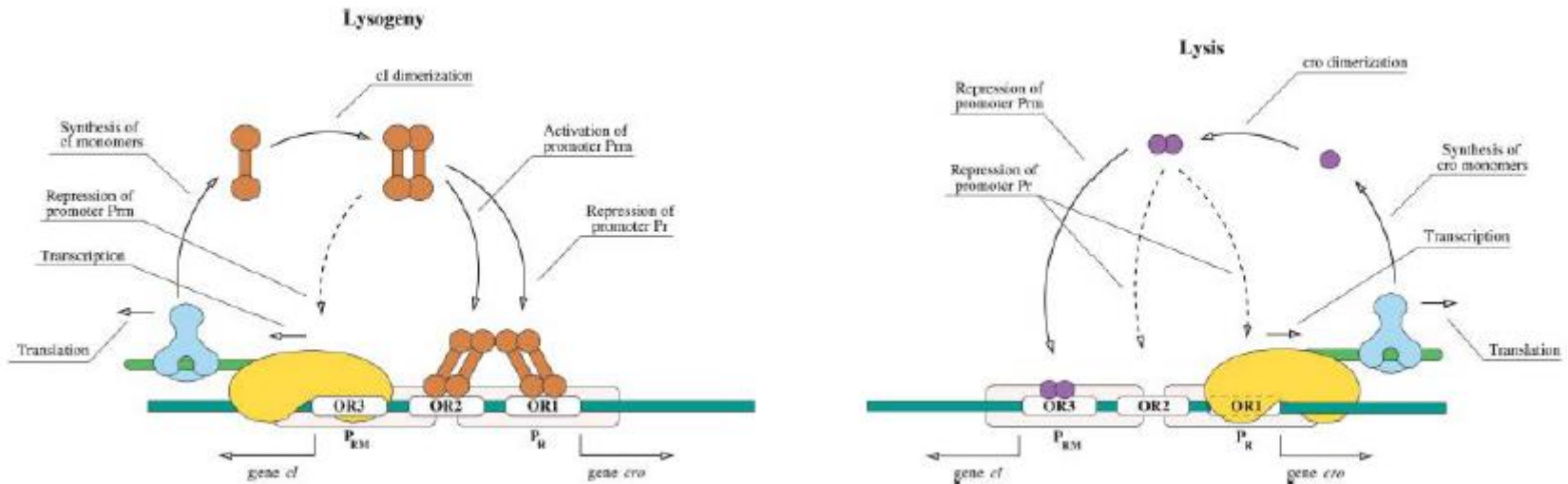

Ptashne, *A Genetic Switch*, Cell Press,1992

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

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

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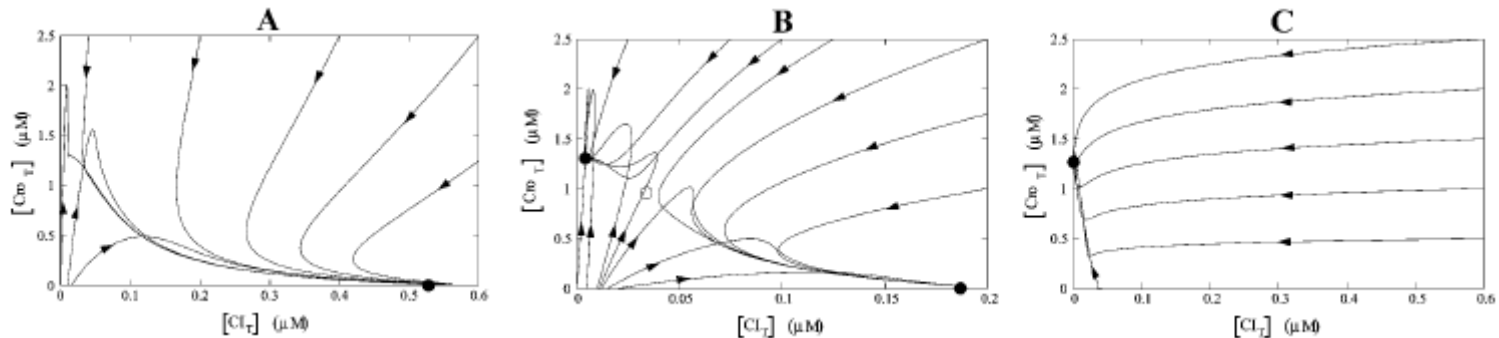
$$\frac{d[CI_T]}{dt} = v_{cI}[M_{cI}]_{\tau_{cI}} - (\gamma_{cI} + \mu)[CI_T],$$

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

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, …)

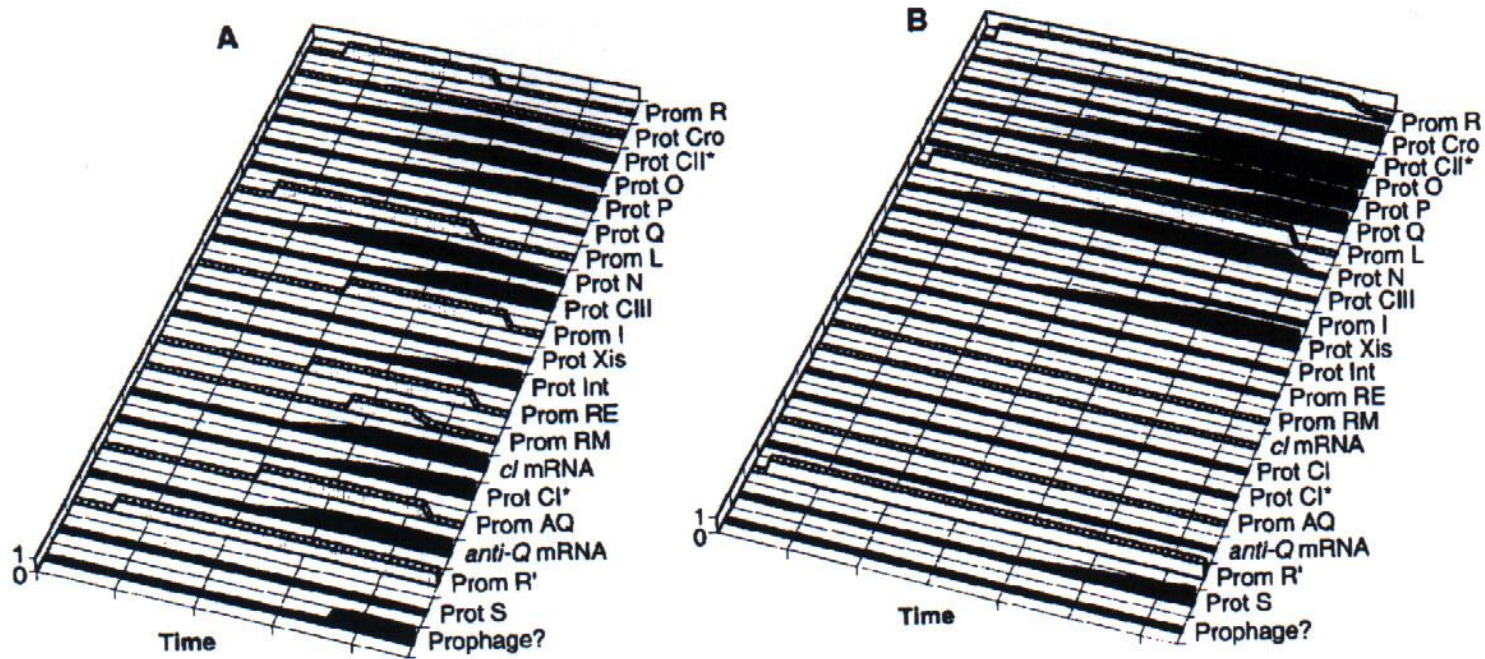McAdams and Shapiro (1995), *Science*, 269(5524):650-6

- 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
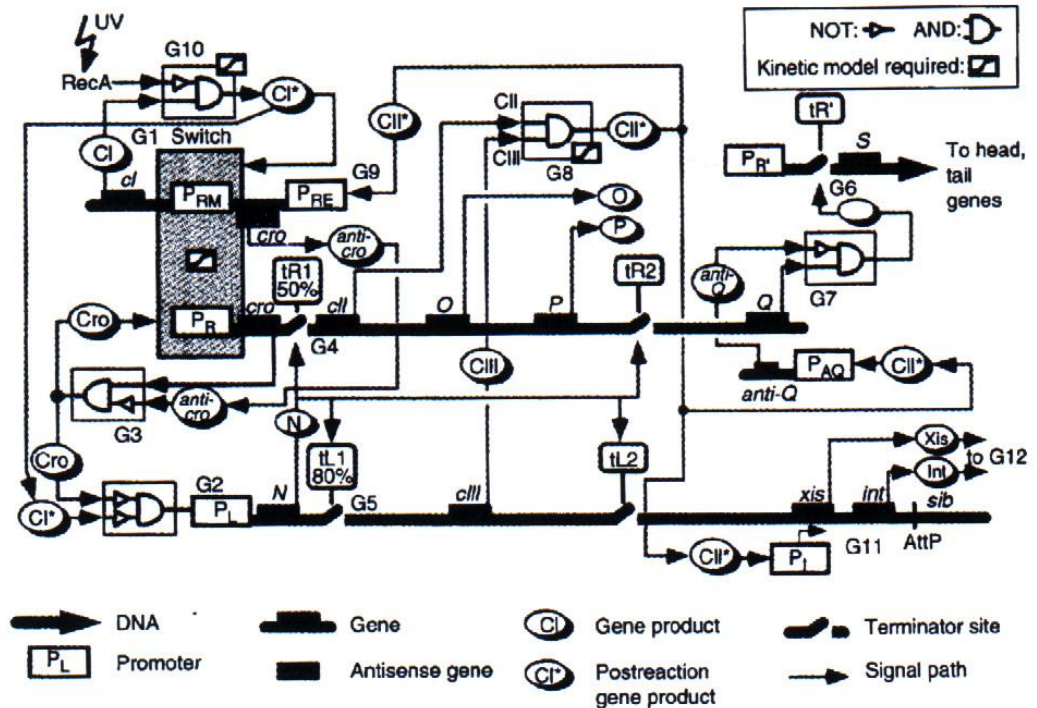
Simulation of phage λ infection

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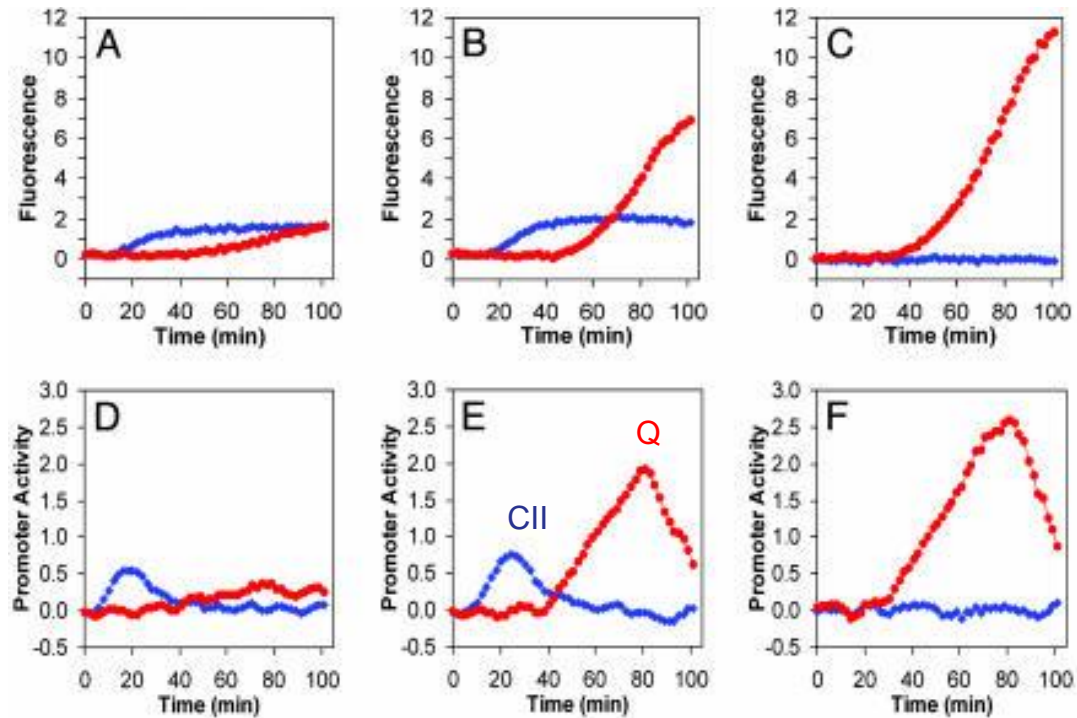
Kobiler *et al.* (2005), *Proc. Natl. Acad. Sci. USA*, 102(12): 4470-5

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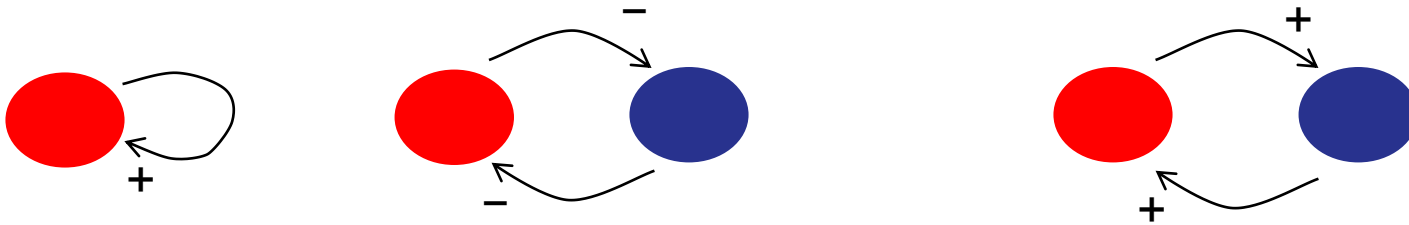
  Alon (2007), *An Introduction to Systems Biology*, Chapmann&Hall/CRC

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

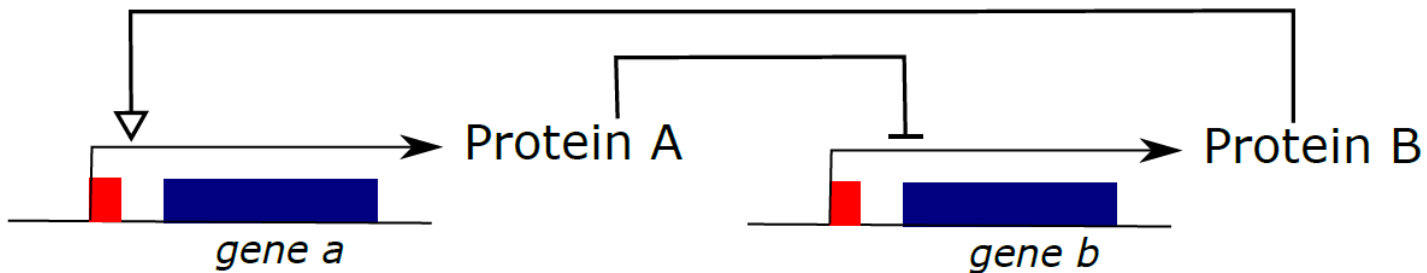Thomas and d'Ari (1990), *Biological Feedback*, CRC Press
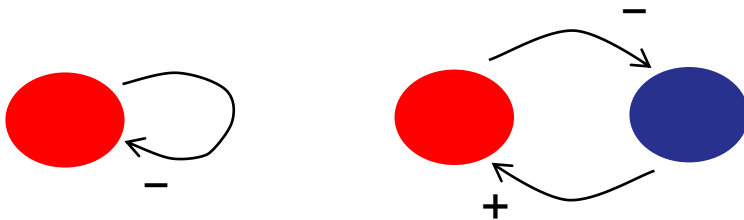
- **Condition is not sufficient**, as the actual occurrence of (stable) oscillations depends on: parameter values, nonlinearities, number of genes, …

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

Simple oscillator network

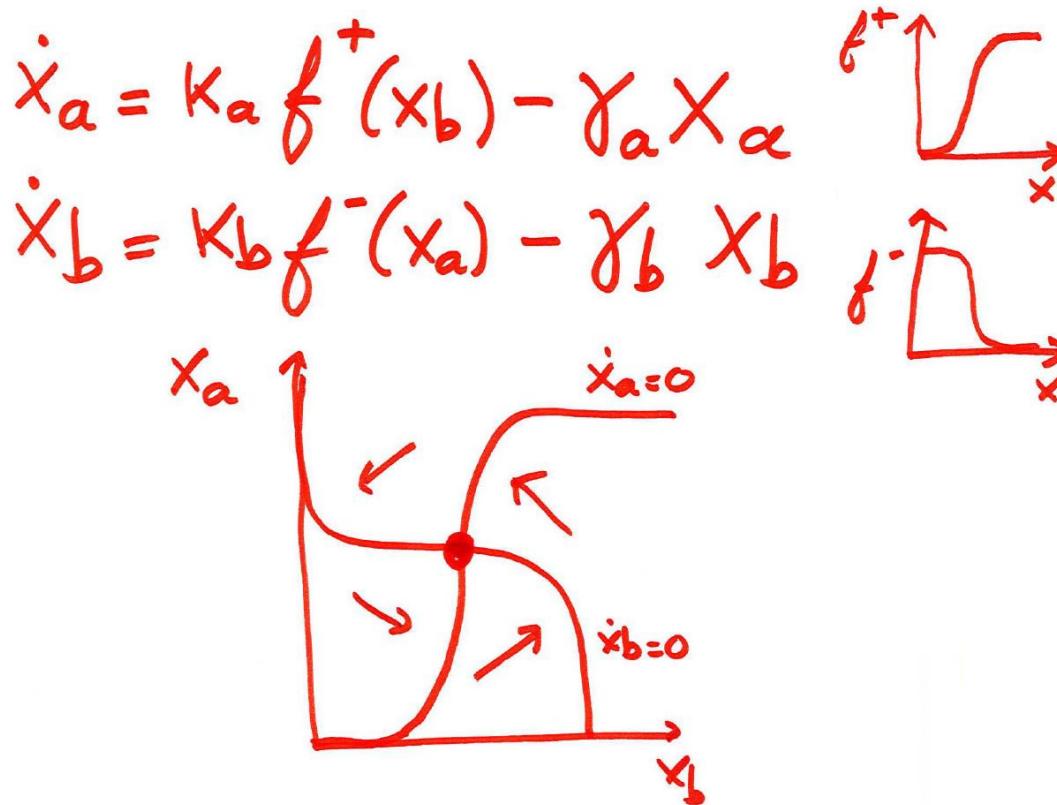- Question: write out the model for a simple oscillator network

- Question: sketch nullclines in phase space and directions of vector field

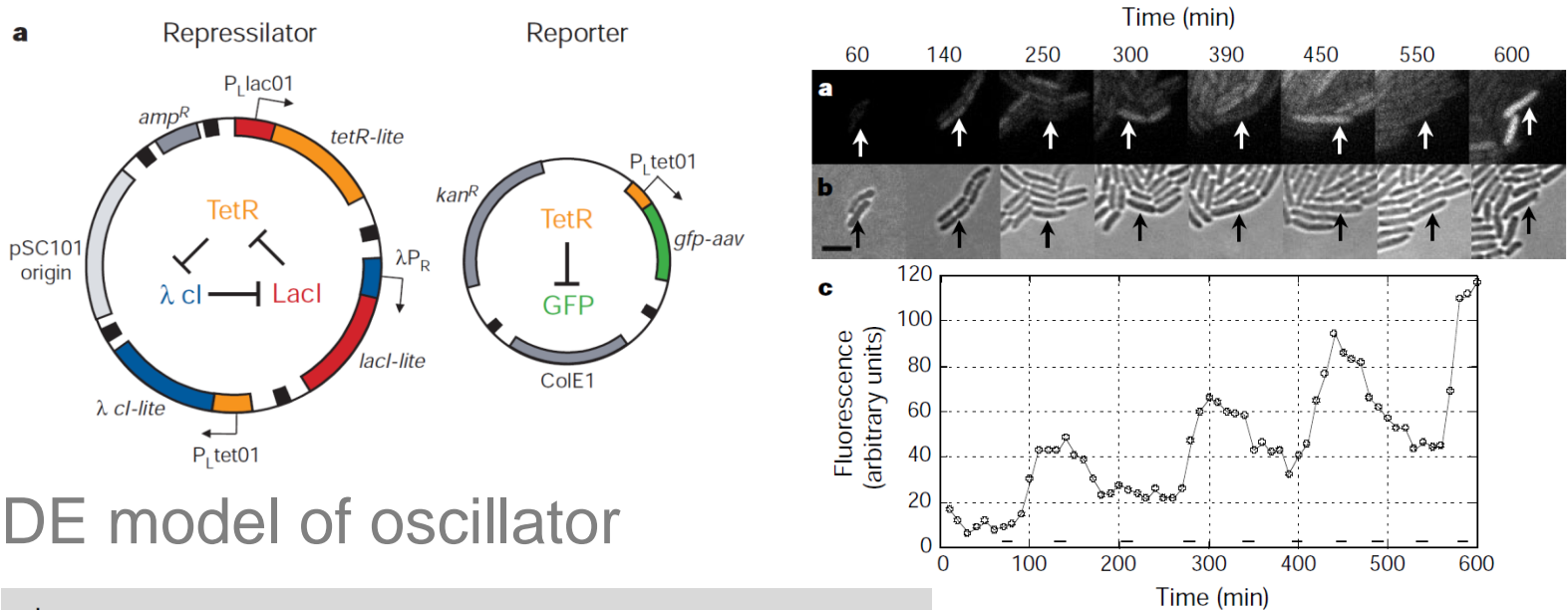Polynikis *et al.* (2009), *J. Theor. Biol.*, 261:511-530

Simple oscillator network

- Question: write out the model for a simple oscillator network
- Question: sketch nullclines in phase space and vector field

$$\dot{x}_a = K_a f^+(x_b) - \gamma_a x_a$$

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

Construction of oscillator network

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

- ODE model of oscillator

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

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

$$\begin{pmatrix} i = lacI, tetR, cI \\ j = cI, lacI, tetR \end{pmatrix}$$
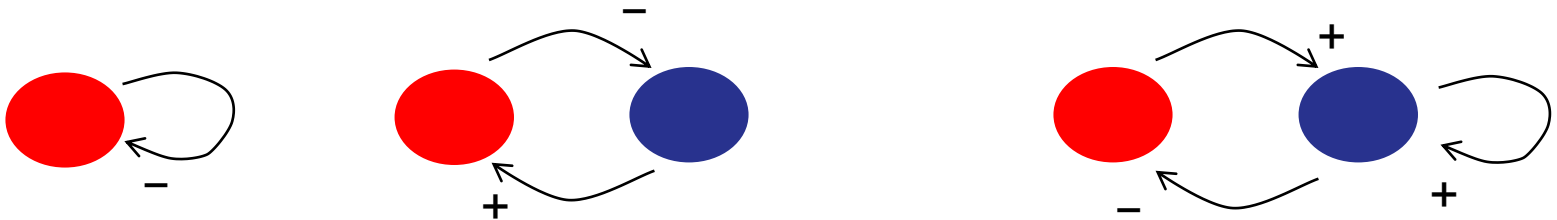
Elowitz and Leibler (2000), *Nature*, 403(6767):335-8

Necessary condition for oscillations

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

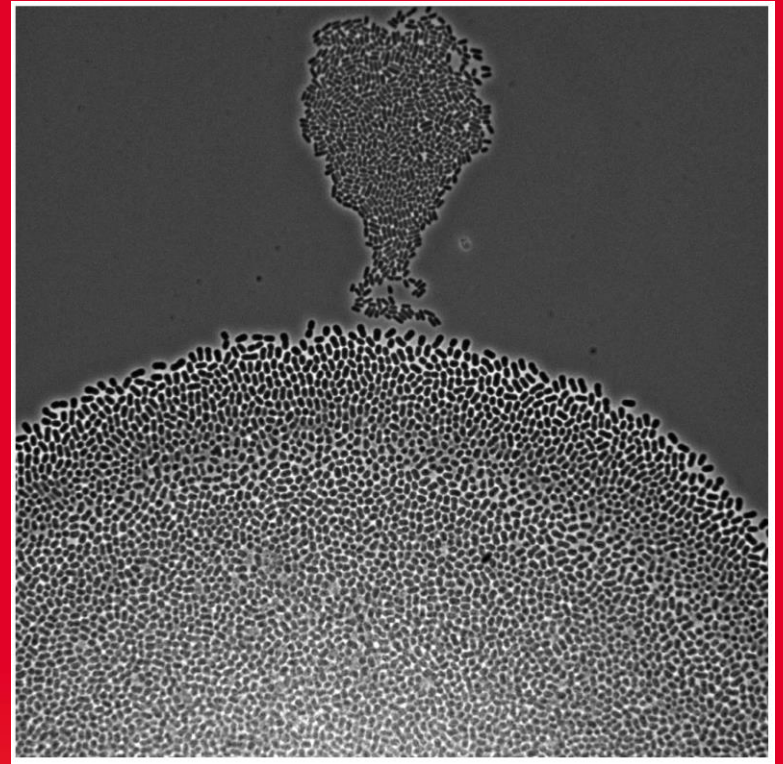 Thomas and d'Ari (1990), *Biological Feedback*, CRC Press

- **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

Thanks!

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