Mathematical Modeling of Bacterial Regulatory Networks

Hidde de Jong

INRIA Grenoble - Rhône-Alpes
Hidde.de-Jong@inria.fr http://ibis.inrialpes.fr

UNIVERSITE JOSEPH FOURIER SCIENCES TECHNOLOGIE MEDECINE

INSTITUT NATIONAL DE RECHERCHE EN INFORMATIQUE ET EN AUTOMATIQUE

INRIA

centre de recherche GRENOBLE - RHÔNE-ALPES

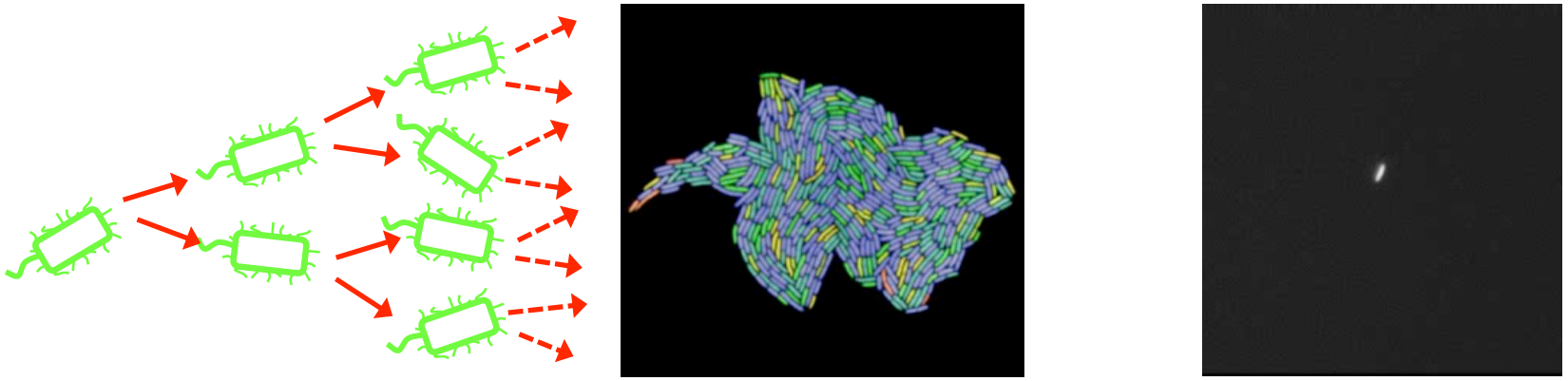
Overview

1. Gene regulatory networks in bacteria
2. Mathematical modeling of gene regulatory networks
3. Relation between network structure and dynamics
4. Stochasticity and network dynamics
5. Conclusion and challenges for modelers

Bacterial growth and adaptation

- Bacteria are geared towards growth and division

  E. coli cells have doubling times up to 20 min



Stewart *et al*. (2005), *PLoS Biol.,* 3(2): e45

- External perturbations may cause adaptation of growth rate, and more generally, may change physiology of bacterial cell

  Nutrient starvation, heat shock, osmotic stress, high population density, …

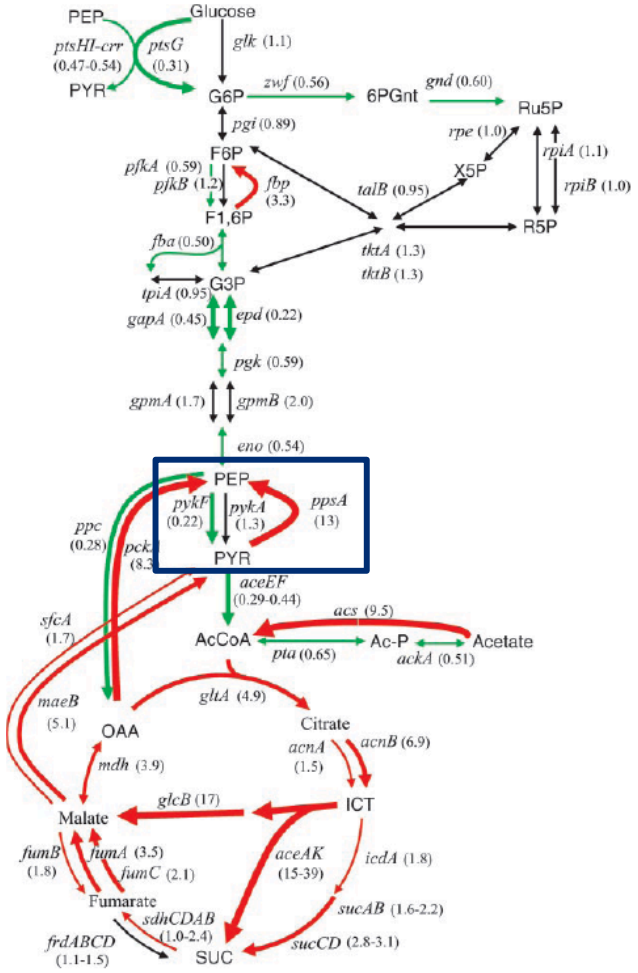
Gene regulatory networks

- The adaptation of bacteria to changes in their environment involves adjustment of gene expression levels

  Differences in expression of enzymes in central metabolism of *E. coli* during growth on glucose or acetate

  Oh *et al.* (2002), *J. Biol. Chem.*, 277(15):13175–83

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



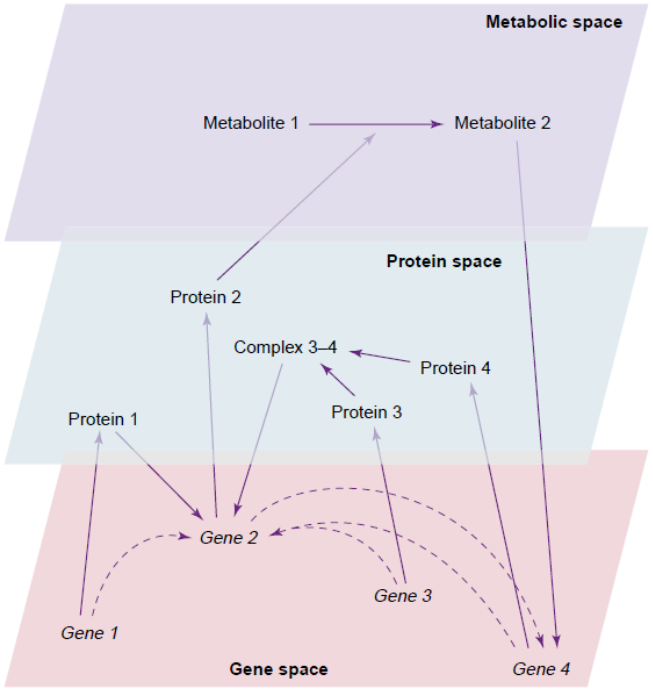
Gene regulatory networks

- **Gene regulatory networks** consist of genes, gene products (RNAs, proteins), and the regulatory effect of the latter on the expression of other genes

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

- Gene regulatory networks cannot be reduced to **direct** interactions (transcription regulation), but also include **indirect** interactions (mediated by metabolism)

  Brazhnik *et al.* (2002), *Trends Biotechnol.*, 20(11):467-72

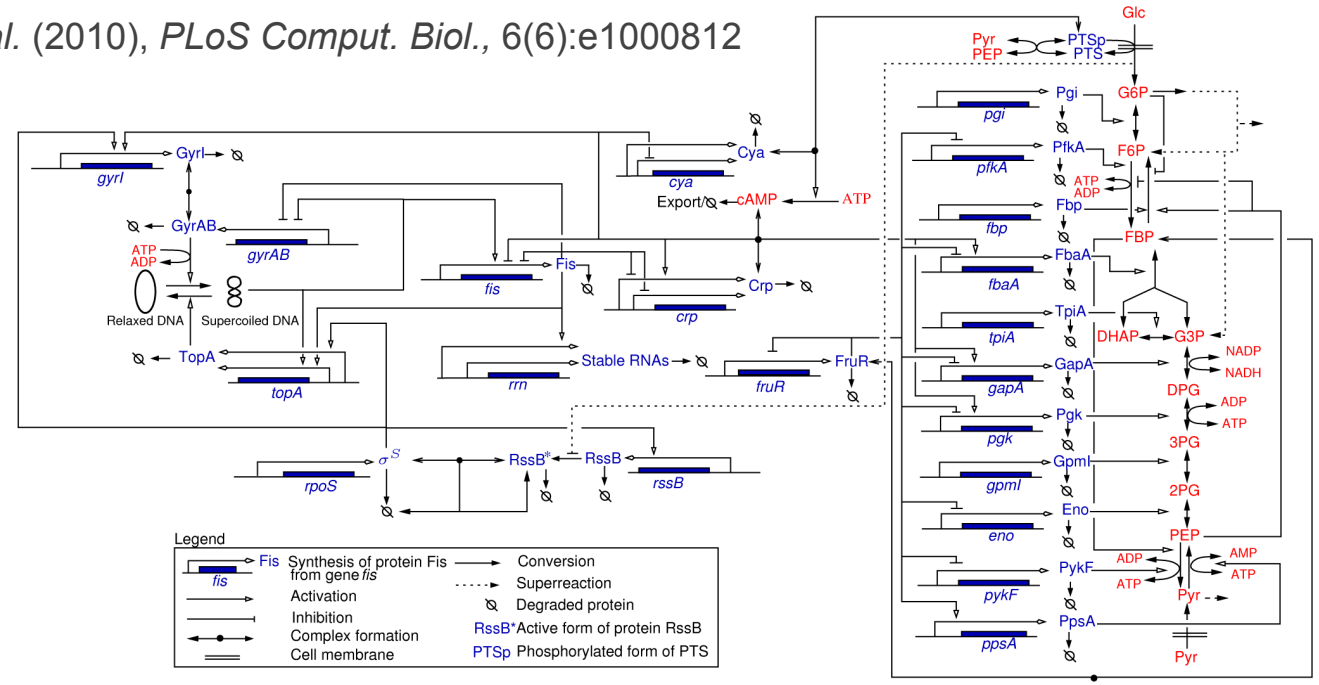


Gene regulatory networks

- **Indirect interactions** can be derived from underlying system of biochemical reactions

  Time-scale hierarchies between metabolism and gene expression allows model reduction using quasi-steady-state approximation

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



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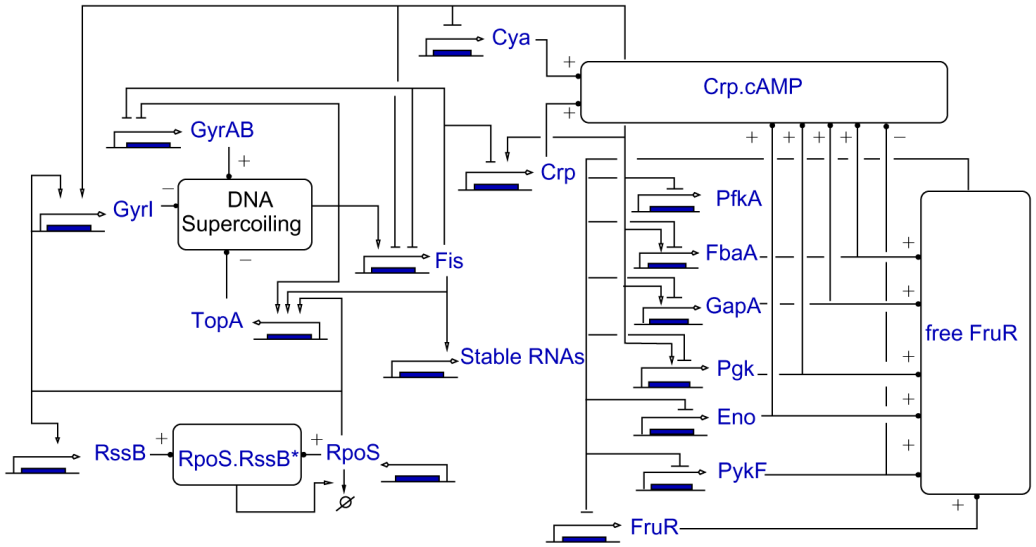


Modeling of gene regulatory networks

- Most gene regulatory networks of biological interest are large and complex

  E. coli has 4200 genes coding for several,hundreds of transcription factors

- **No global view of functioning** of network available, despite abundant knowledge on network components

  Understanding of dynamics requires **mathematical modeling** and **computer analysis and simulation**

  Discipline now often referred to as **systems biology**

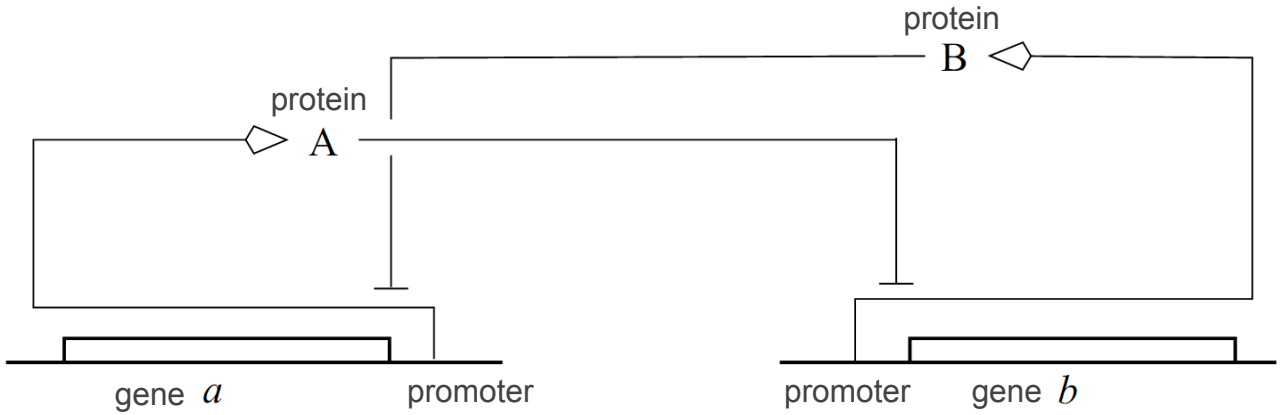
- Well-established framework for modeling of gene regulatory networks using ordinary differential equation (ODE) models

  Ultimately (often implicitly) based on kinetic theory of biochemical reactions

  Polynikis *et al.* (2009), *J. Theor. Biol.*, 261(4):511-30

Cross-inhibition network

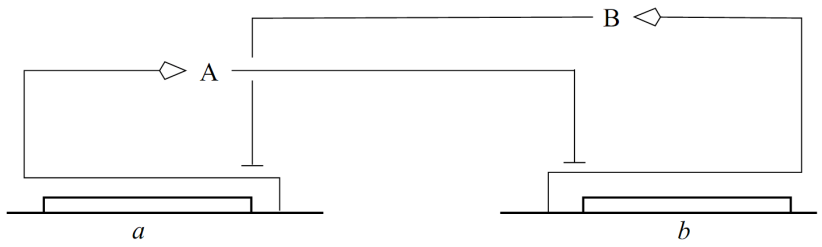
- **Cross-inhibition** network consists of two genes, each coding for transcription regulator inhibiting expression of other gene



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

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

ODE model of cross-inhibition network



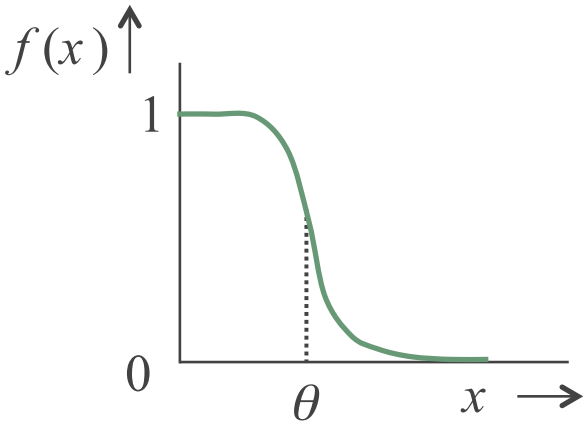
$$\dot{x}_a = \kappa_a f(x_b) - \gamma_a x_a$$
$$\dot{x}_b = \kappa_b f(x_a) - \gamma_b x_b$$

x_a = concentration protein A

x_b = concentration protein B

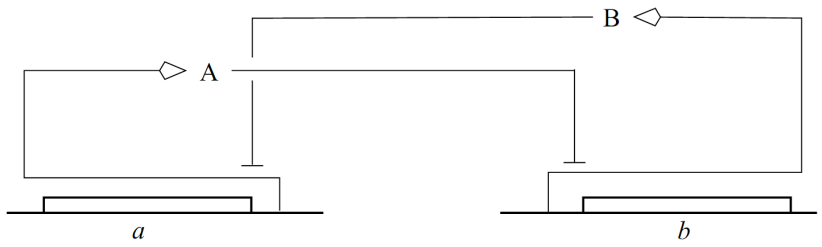
$\kappa_a, \kappa_b > 0$, production rate constants

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



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

ODE model of cross-inhibition network



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

x_b = concentration protein B

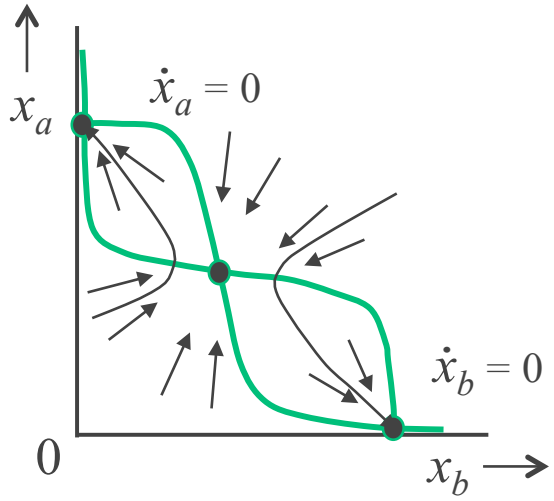
$\kappa_a, \kappa_b > 0$, production rate constants

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

- Implicit modeling assumptions:
  - Ignore intermediate gene products (mRNA)
  - Ignore gene expression machinery (RNA polymerase, ribosome)
  - Simplification of complex interactions of regulators with DNA to single response function

Bistability of cross-inhibition network

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



$$\dot{x}_a = 0 : \; x_a = \frac{\kappa_a}{\gamma_a} f(x_b)$$

$$\dot{x}_b = 0 : \; x_b = \frac{\kappa_b}{\gamma_b} f(x_a)$$

- 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

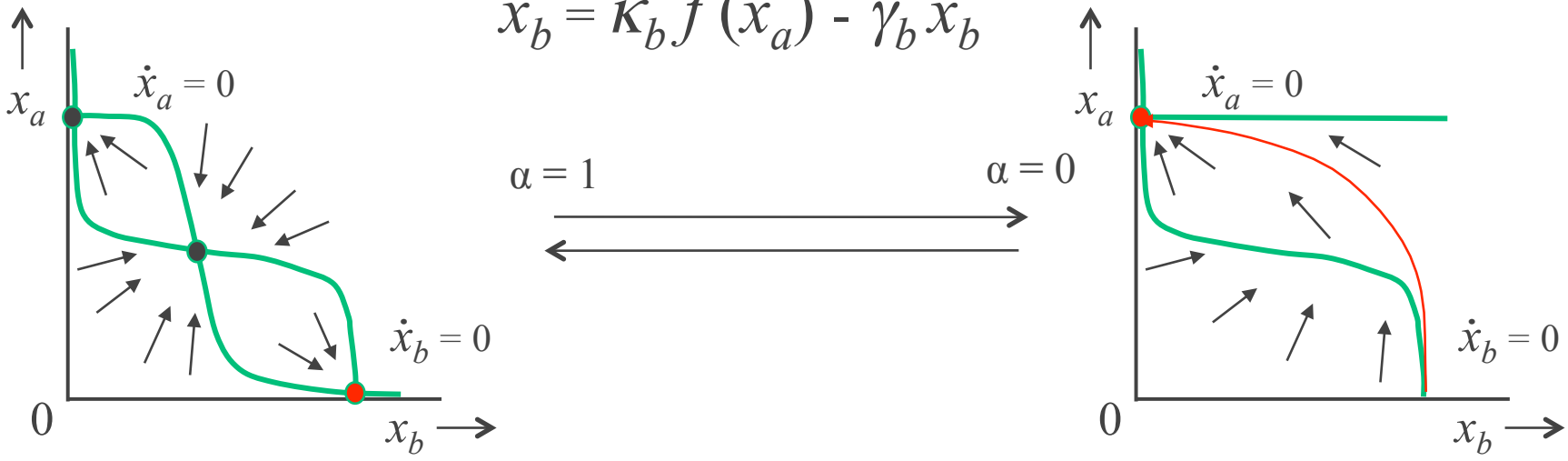
Hysteresis in cross-inhibition network

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

  Modulation of regulatory effect of one of inhibitors (α)

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

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

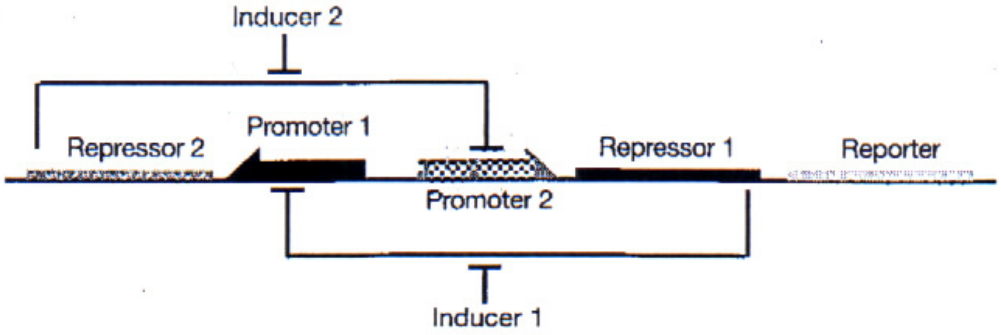


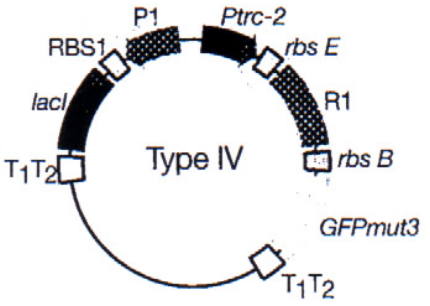
- Change in parameter causes saddle-note bifurcation

Construction of cross inhibition network

- Construction of cross inhibition network *in vivo*

Gardner *et al.* (2000), *Nature*, 403(6786): 339-342





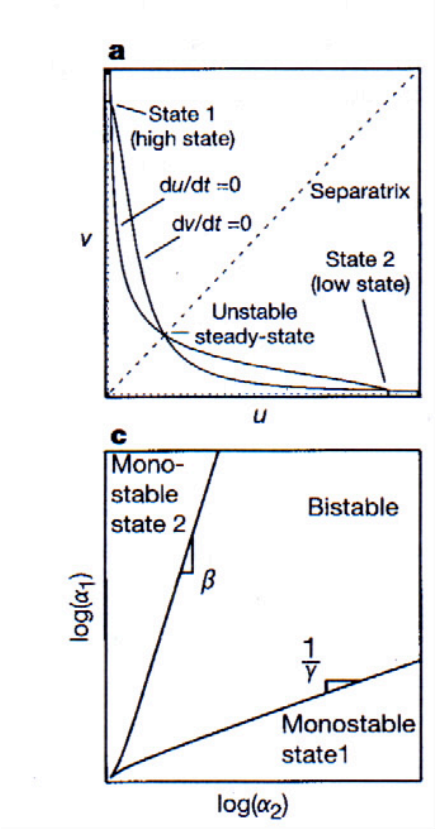
- Differential equation model of network

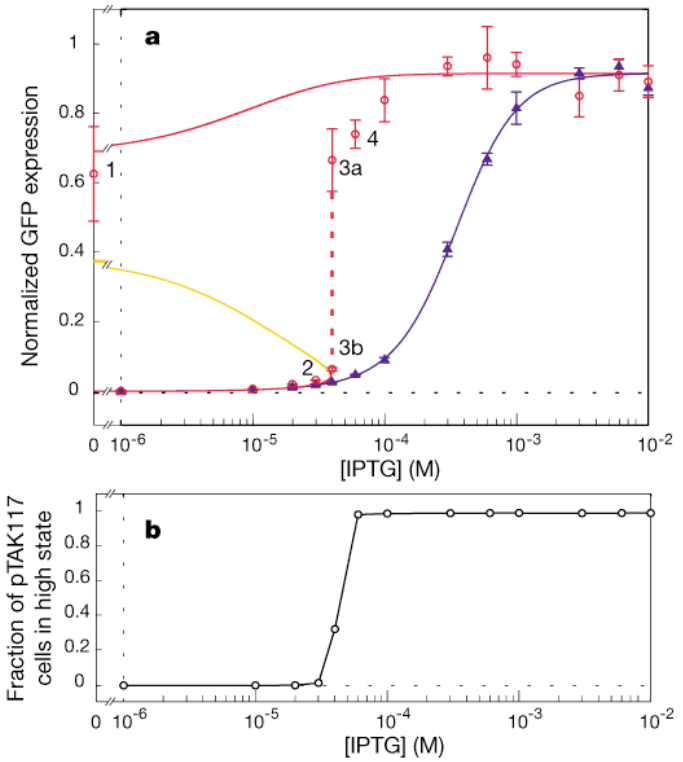
$$\dot{u} = \frac{\alpha_1}{1 + v^{\beta}} - u \qquad \dot{v} = \frac{\alpha_2}{1 + u^{\gamma}} - v$$

Experimental test of model

- Experimental test of mathematical model (bistability and hysteresis)

Gardner *et al.* (2000), *Nature*, 403(6786): 339-342

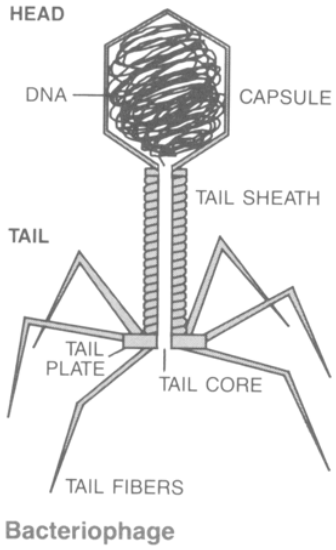


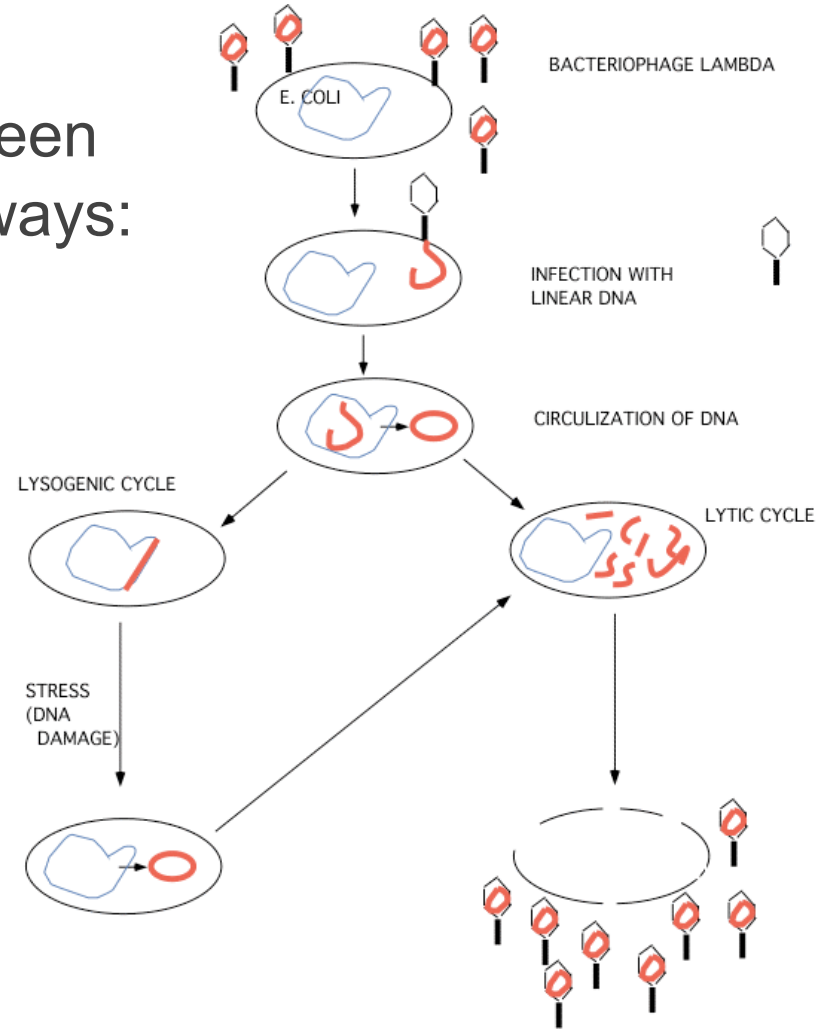


Bacteriophage λ infection of *E. coli*

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

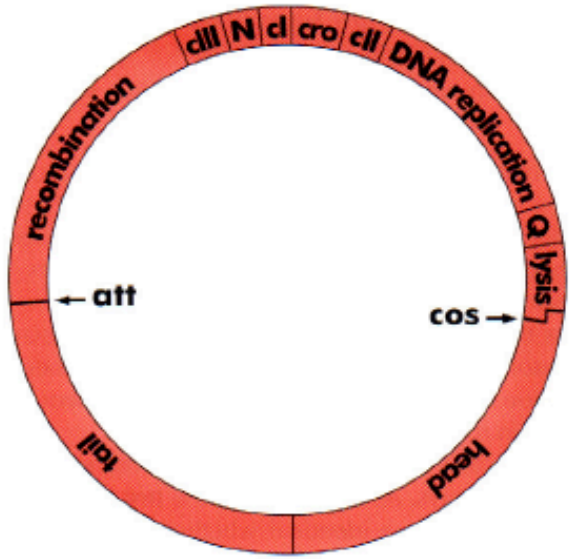
Ptashne, *A Genetic Switch*, Cell Press,1992

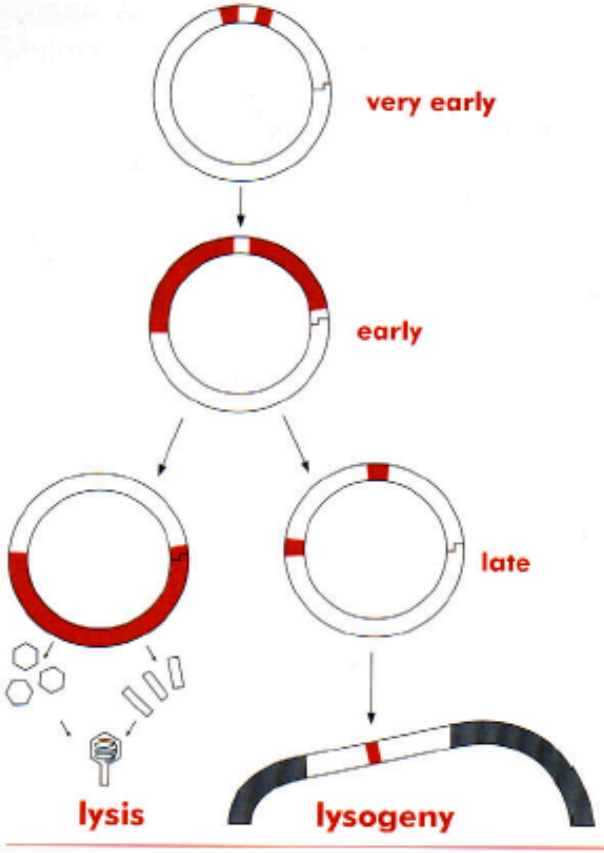




Bistability in phage λ

- Lytic and lysogenic pathways involve different patterns of gene expression

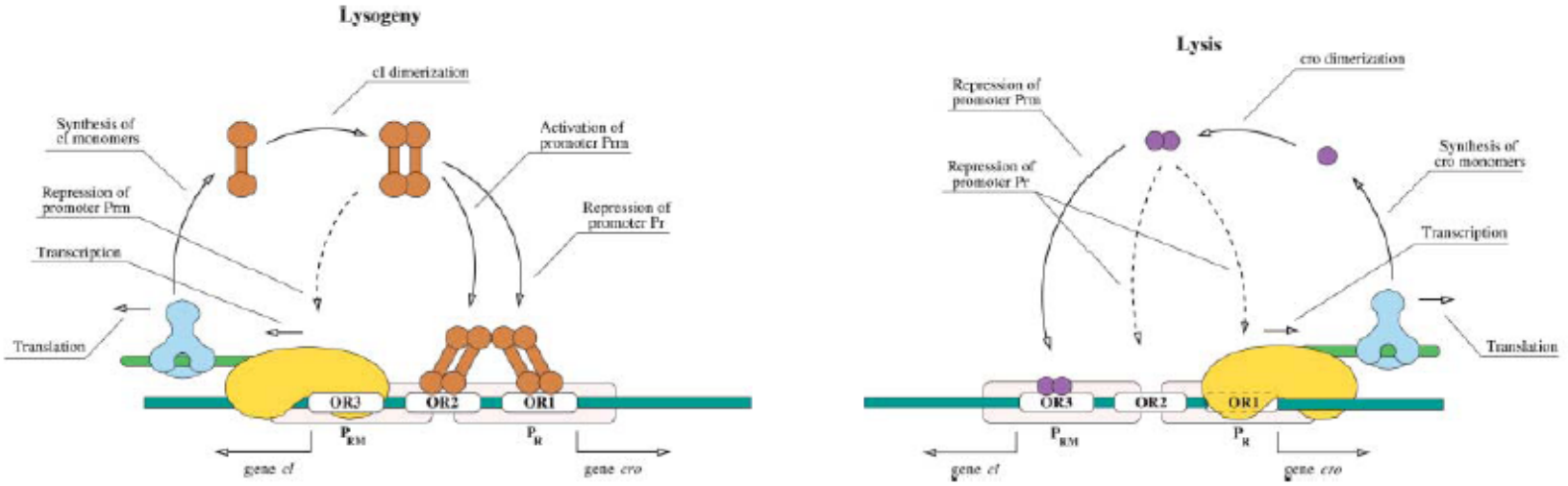




Ptashne, *A Genetic Switch*, Cell Press,1992

Control of phage λ fate decision

- Cross-inhibition motif 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

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

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

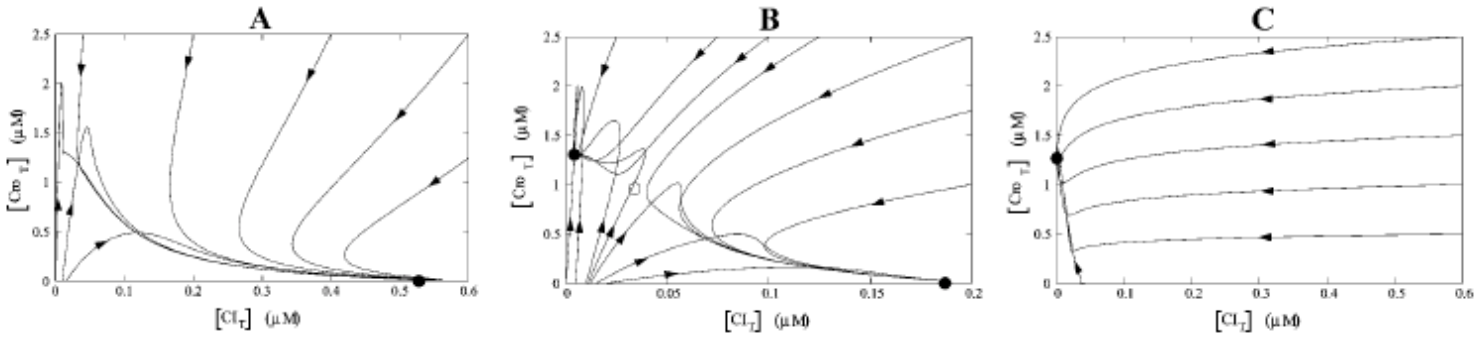
$$\frac{d[CI_T]}{dt} = \upsilon_{cI}[M_{cI}]_{\tau_{cI}} - (\gamma_{cI} + \mu)[CI_T],$$

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

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 between two monostable regimes, due to change in degradation constant



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

Extended model of phage λ infection

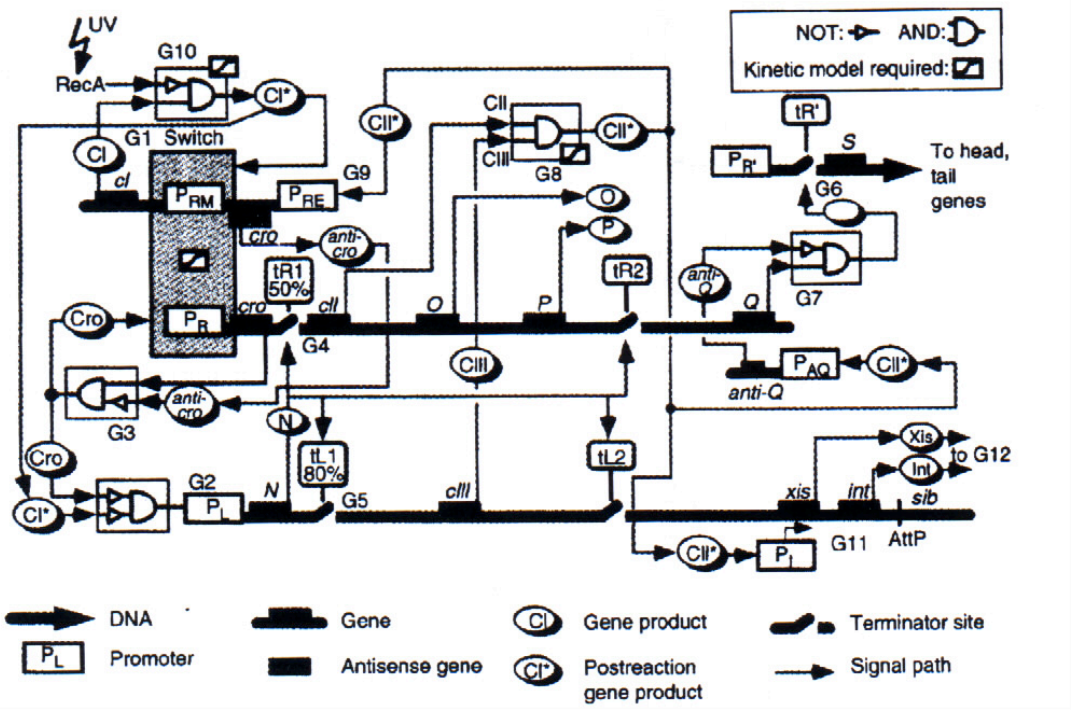
- ODE model of the **extended network** underlying decision between lysis and lysogeny

  Role of other regulatory proteins (CII, N, Q, …)

  McAdams and Shapiro (1995), *Science*, 269(5524): 650-656

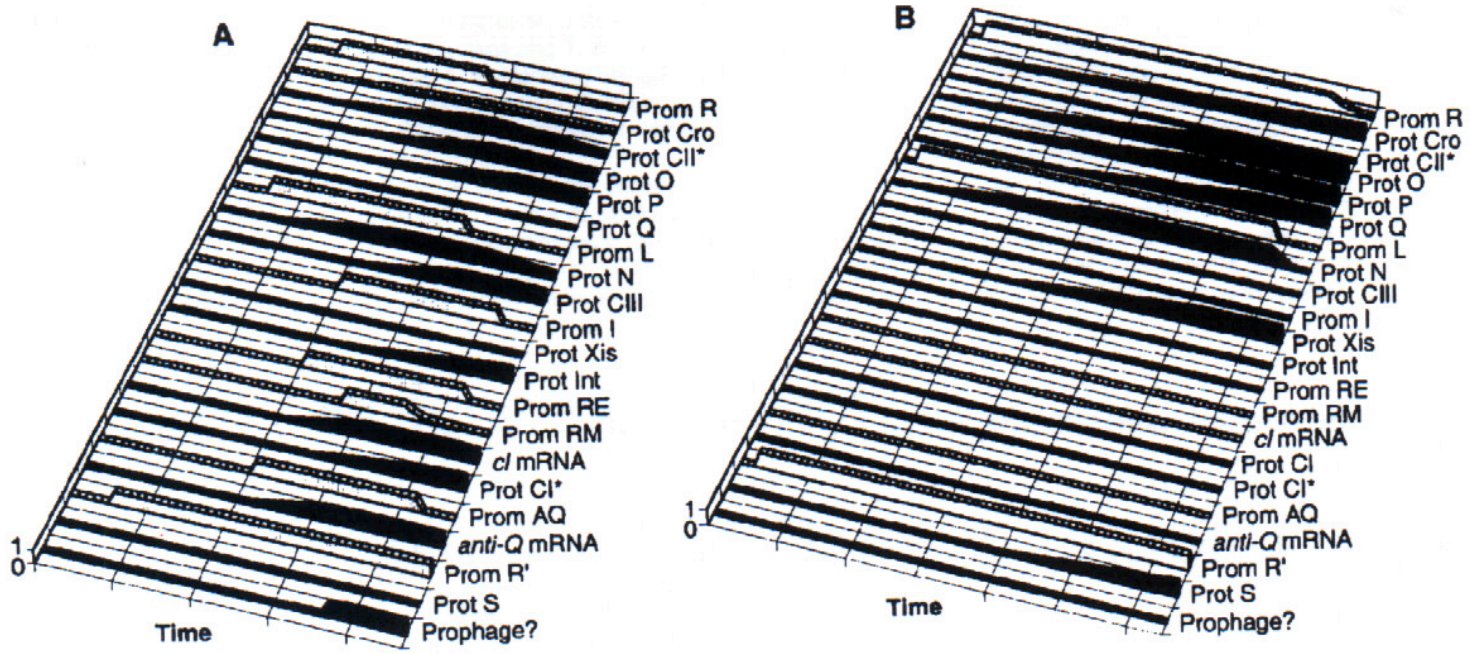
- Recent experimental work downplays importance of mutual inhibition of CI and Cro in lysis-lysogeny decision

  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

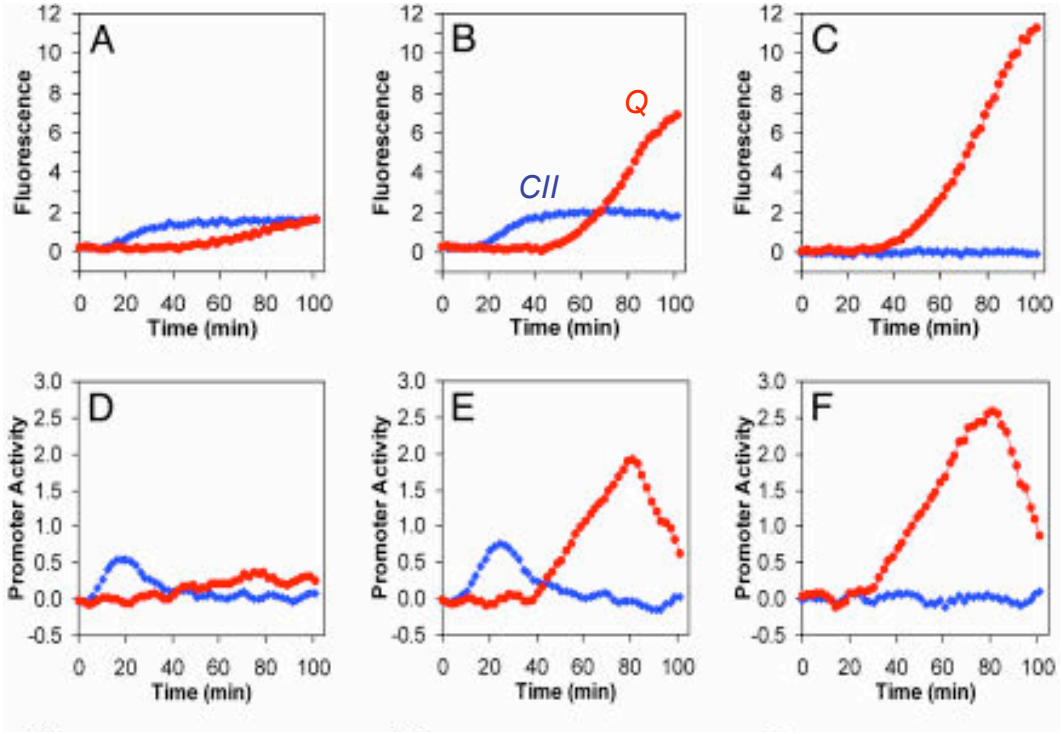


- Cell follows one of two pathways after infection

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

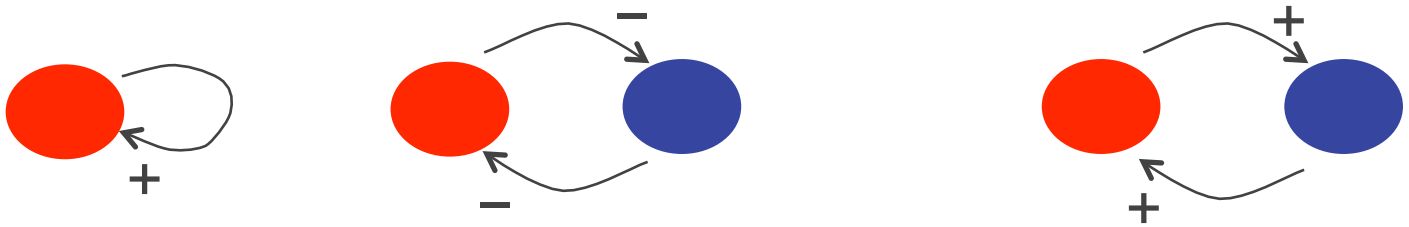
- Can we find general **design principles**, relating network structure to bistability and other properties of network dynamics?

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,* 1:123-133

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

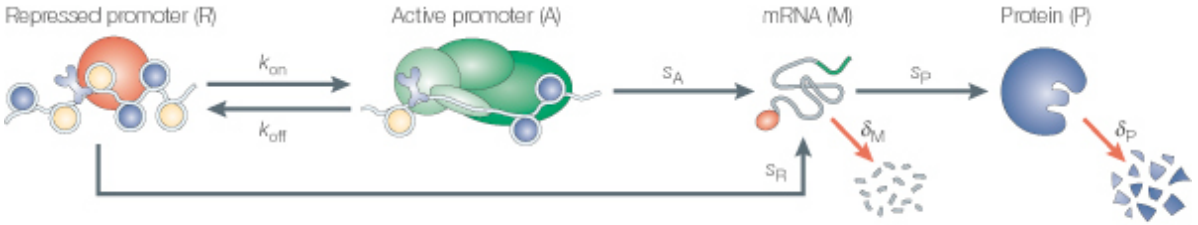
Stochasticity in gene expression

- ODE models make abstraction of underlying biochemical reaction processes involved in gene expression that may not be warranted

  Kaern *et al.* (2005), *Nat. Rev. Genet.*, 6(6):451-464

- Gene expression is **stochastic** instead of **deterministic** process

  Stochasticity gives rise to fluctuations in gene products (**noise**)



- **Discrete** number of molecules of reaction species, instead of **continuous** concentrations

  Noise amplified by low number of molecules of each species

Stochasticity in gene expression

- Major question is how cells both tolerate and exploit noise.

  Rao *et al.* (2002), *Nature*, 420(6912):231-237

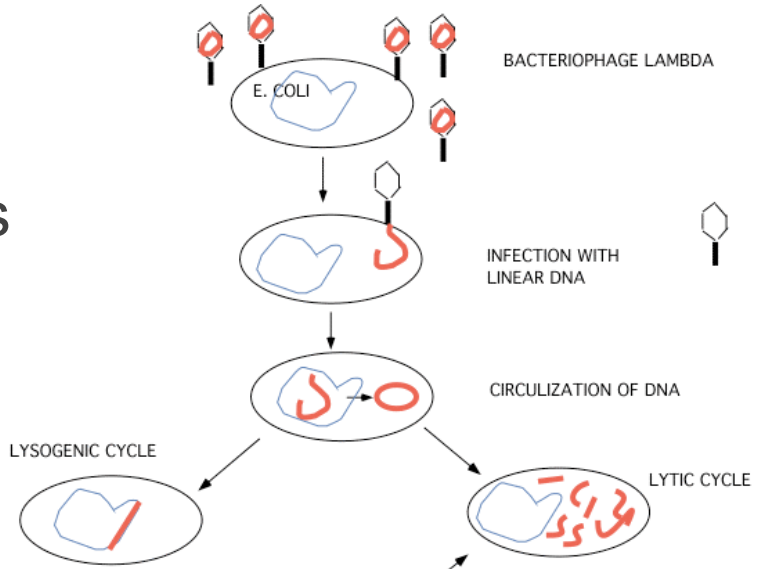
  Raj and van Oudenaarden (2008), *Cell*, 135(2):216-26

- Most cellular processes are **robust** to noise, despite stochasticity of underlying system of biochemical reactions

- Sometimes, intracellular noise drives **population heterogeneity** that may be beneficial for a species

  After infection, only fraction of cells lyse

- ODE models are not suitable for studying origin and effects of noise



Stochastic models of gene expression

- **Stochastic master equation** describes dynamics of biochemical reaction system

$$\mathrm{d}p[\boldsymbol{X}(t)=\boldsymbol{V}] / \mathrm{d}t = \sum_{j=1}^{m} p[\boldsymbol{X}(t)=\boldsymbol{V}-\boldsymbol{\nu}_j]\,\beta_j - p[\boldsymbol{X}(t)=\boldsymbol{V}]\,\alpha_j$$

  - Number of molecules of each species i at time-point t described by discrete variable $X_i(t) \in \mathrm{N}$
  - $p[X_i(t)=V_i]$ describes probability that at time t there are V_i molecules of species i
  - m is the number of different reactions
  - α_j and β_j are constants defined in terms of reaction constants and number of reactant molecules

Van Kampen (1997), *Stochastic Processes in Physics and Chemistry,* Elsevier

Stochastic simulation

- Analytical solution of master equations is not possible in most situations of practical interest
- **Stochastic simulation** predicts sequences of reactions that change state of system, starting from initial state $X(0) = V_0$

  Two different runs from identicial initial state may lead to different final states

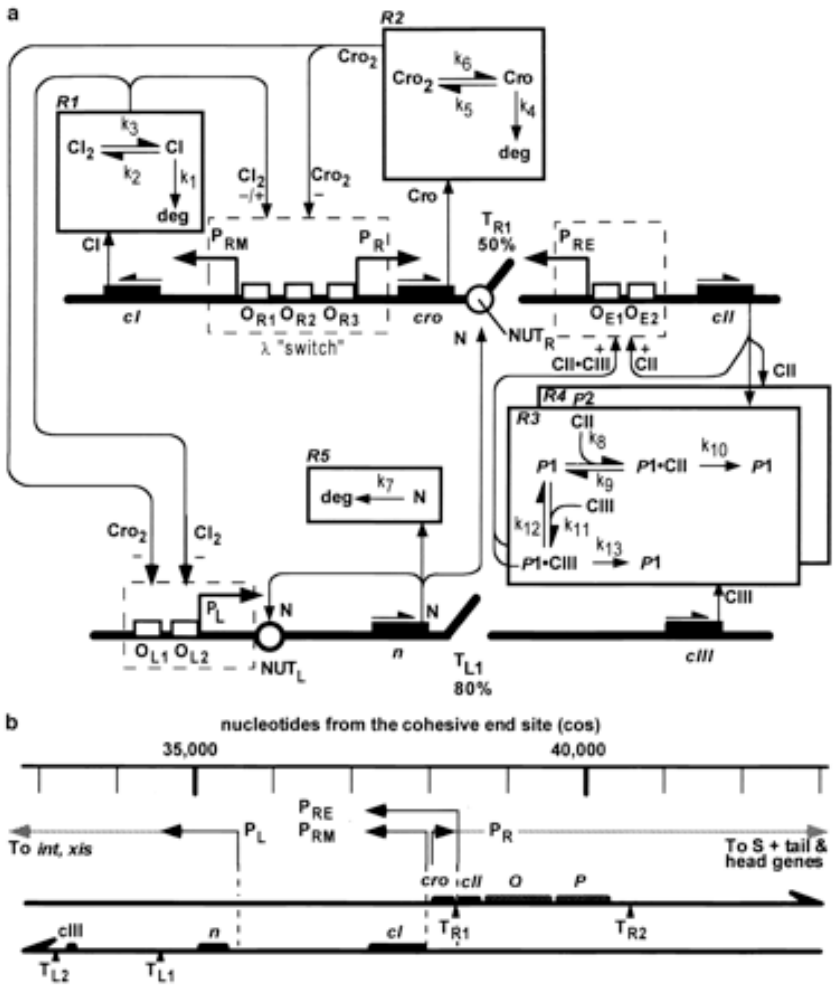
  Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

- Repeating stochastic simulation many times yields approximation of probability distribution $p(X(t)=V)$, and thus solution of stochastic master equation

  Gillespie (2002), *J. Phys. Chem.*, 81(25): 2340-61

Stochastic modeling of phage λ infection

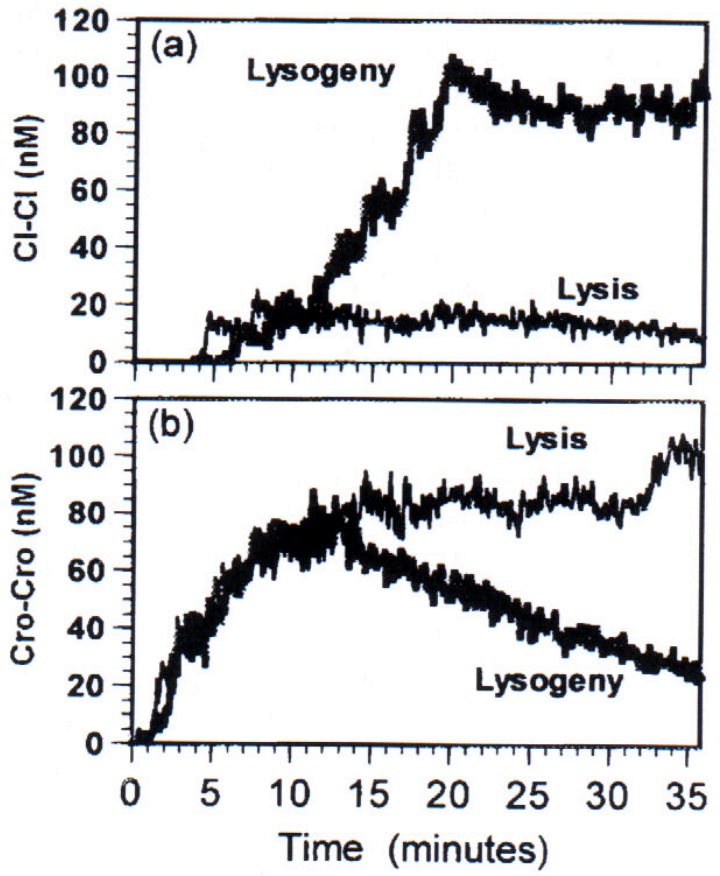
- **Stochastic model** of λ lysis-lysogeny decision network



Arkin *et al.* (1998), *Genetics*, 149(4): 1633-48

Stochastic modeling of phage λ infection

- Time evolution of Cro and CI dimer concentrations
- Due to stochastic fluctuations, from identical initial conditions cells follow one or other pathway
- Averaging over many simulations gives probability of lytic and lysogenic phenotype, corresponding to observed ratio

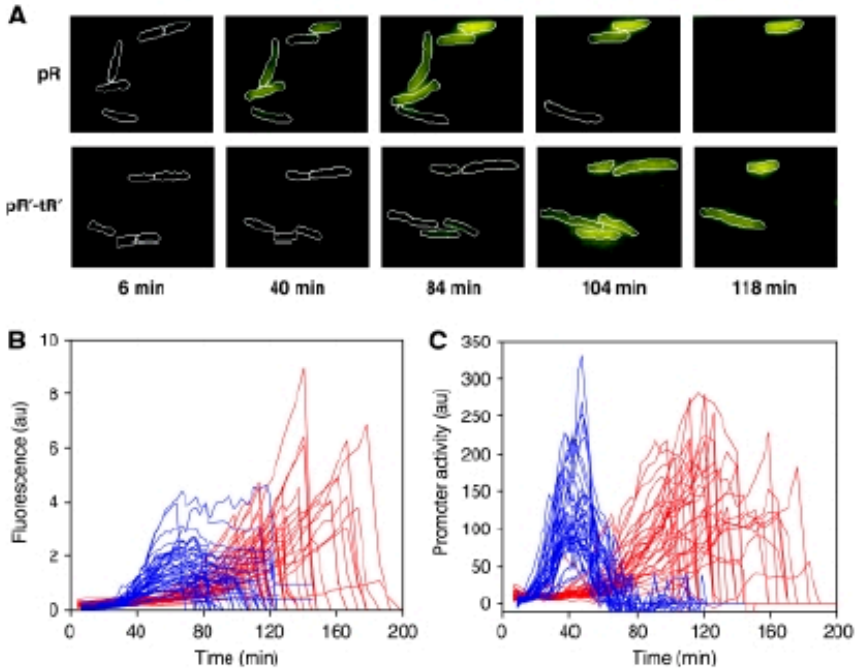


Arkin *et al.* (1998), *Genetics*, 149(4): 1633-48

Measurements of phage λ infection

- New measurement techniques allow real-time and *in-vivo* monitoring of the execution of lytic and lysogenic pathways in individual cells

  Use of reporter genes in combination with fluorescence microscopy

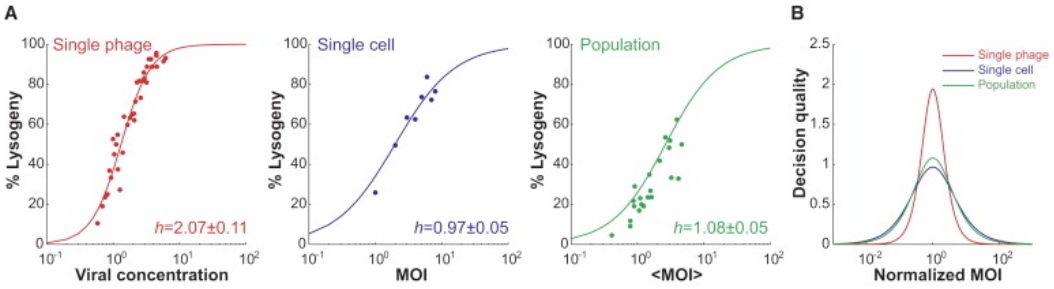


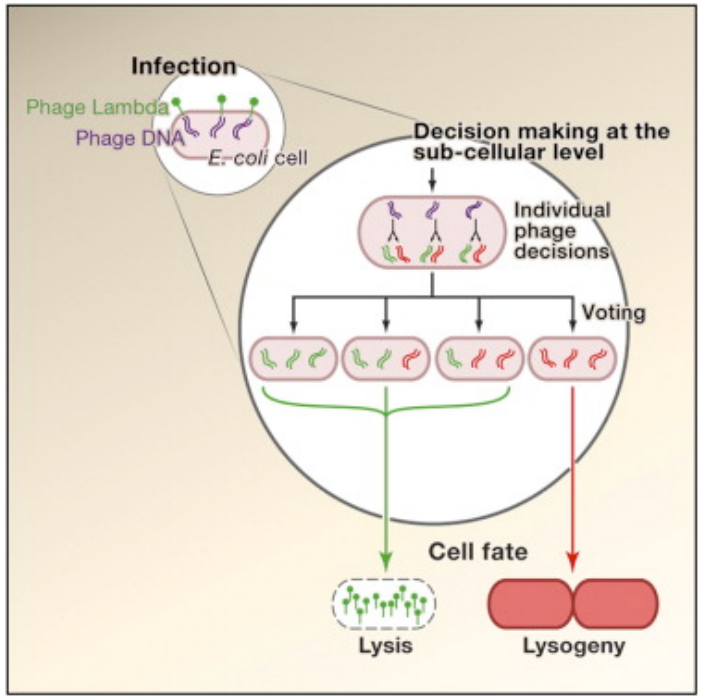
Amir *et al.* (2007), *Mol. Syst. Biol.*, 3:71

Stochasticity and hidden variables

- Is observed population heterogeneity entirely due to stochastic dynamics of biochemical reactions?
- **Hidden variables** that deterministically set outcome of what seems noisy decision process

  Deterministic voting of stochastic decision in single phages





Zeng *et al.* (2010), *Cell*, 141(4):682-91

Conclusions

- Gene regulatory networks control changes in gene expression levels in response to environmental perturbations
- Dynamic properties of bacterial regulatory networks can be studied by means of mathematical models
  - Deterministic and stochastic models capture different aspects of network functioning
- Dynamic properties can be related to structure of regulatory interactions in network
  - Positive feedback and multistability, negative feedback and oscillations
- Networks both tolerate and exploit noise due to stochasticity of underlying biochemical reaction systems
  - Relation between feedback structure and noise amplification/attenuation?

Some challenges for modelers

- Upscaling of analysis to large networks of dozens or even hundreds of genes, proteins, metabolites, …
  - Model reduction, qualitative models, and formal verification tools
- System identification and parameter estimation
  - New measurement techniques yield higher-quality data, but still noisy, sparse, heterogeneous
  - Large models on different time-scales, with many unobserved variables
- Systematic design of experimental perturbations for identification and control