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, …

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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*

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**

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**References**

Szallasi et al. (2006), *System Modeling in Cellular Biology*, MIT Press
Bolouri (2008), *Computational Modeling of Gene Regulatory Networks*, Imperial College Press
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
  $$\frac{dx}{dt} = \dot{x} = f(x),$$
  where $x = [x_1, \ldots, x_n]$ and $f(x)$ is rate law

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

Modeling of gene regulatory networks

• ODE model of gene expression, distinguishing transcription and translation

\[
\begin{align*}
\dot{m} &= \kappa_m - (\gamma_m + \mu) \cdot m \\
\dot{p} &= \kappa_p \cdot m - (\gamma_p + \mu) \cdot p
\end{align*}
\]

\[m(t) \geq 0, \text{ concentration mRNA}\]

\[p(t) \geq 0, \text{ concentration protein}\]

\[\kappa_m, \kappa_p > 0, \text{ synthesis rate constants}\]

\[\gamma_m, \gamma_p > 0, \text{ degradation rate constants}\]

\[\mu \geq 0, \text{ growth rate}\]
Modeling of gene regulatory networks

• ODE model of gene expression, collapsing transcription and translation

\[
\dot{p} = \kappa_p - (\gamma_p + \mu) p
\]

\[p(t) \geq 0, \text{ concentration protein}\]
\[\kappa_p > 0, \text{ synthesis rate constant}\]
\[\gamma_p > 0, \text{ degradation rate constant}\]
\[\mu \geq 0, \text{ growth rate}\]
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

\[
\begin{align*}
\dot{m} &= \kappa_m f(x) - (\gamma_m + \mu) m \\
\dot{p} &= \kappa_p m - (\gamma_p + \mu) p
\end{align*}
\]

- 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

\[
\begin{align*}
\dot{m} &= \kappa_m f(x) - (\gamma_m + \mu) m \\
\dot{p} &= \kappa_p m - (\gamma_p + \mu) p
\end{align*}
\]

- Regulation function \( f(x) \) typically has \textbf{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

\[ \frac{dm}{dt} = \kappa_m f(x) - (\gamma_m + \mu) m \]
\[ \frac{dp}{dt} = \kappa_p m - (\gamma_p + \mu) p \]

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


\[
x(t + \Delta t) = x(t) + \int_{t}^{t+\Delta t} f(x) \, dt \approx x(t) + f(x) \Delta t
\]
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

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

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

\[ x_a(t) \geq 0, \text{ concentration protein A} \]
\[ x_b(t) \geq 0, \text{ concentration protein B} \]

\[ \kappa_a, \kappa_b > 0, \text{ synthesis rate constants} \]
\[ \gamma_a, \gamma_b > 0, \text{ degradation rate constants} \]
Bistability of cross-inhibition network

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

- 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

\[
\dot{x}_a = 0 \Rightarrow x_a = \left( \frac{\kappa_a}{\gamma_a} \right) f(x_b) \\
\dot{x}_b = 0 \Rightarrow x_b = \left( \frac{\kappa_b}{\gamma_b} \right) f(x_a)
\]
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 ($\alpha$)

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

  $\alpha = 1$  $\alpha = 0$

- Change in parameter causes saddle-note bifurcation
Construction of cross inhibition network

- Construction of cross inhibition network *in vivo*
  

- 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)

Bacteriophage $\lambda$ infection of *E. coli*

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

Bistability in phage $\lambda$

- Lytic and lysogenic pathways involve different patterns of gene expression

Control of phage $\lambda$ 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 $\lambda$ fate decision

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

\[
\frac{d[M_{ci}]}{dt} = k_{ci}^{i}[O_{R}]f_{RM}^{i}([CI_{2}]_{rM}, [CI_{2}]_{rM}) \\
+ k_{ci}^{s}[O_{R}]f_{RM}^{s}([CI_{2}]_{rM}, [Cro_{2}]_{rM}) - (\gamma_{M} + \mu)[M_{ci}],
\]

\[
\frac{d[M_{cro}]}{dt} = k_{cro}[O_{R}]f_{R}([CI_{2}]_{rM}) - (\gamma_{M} + \mu)[M_{cro}],
\]

\[
\frac{d[CI_{r}]}{dt} = v_{ci}[M_{ci}]_{\tau_{ci}} - (\gamma_{ci} + \mu)[CI_{r}],
\]

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

Analysis of phage $\lambda$ 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

Extended model of phage $\lambda$ infection

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


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

Simulation of phage $\lambda$ 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 $\lambda$ 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

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*
  – …


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

Necessary condition for bistability

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

  ![Diagram of positive feedback loops](image)

  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, …

Construction of oscillator network

- Construction of oscillator *in vivo*: repressilator
  
  Elowitz and Leibler (2000), *Nature*, 403(6767):335-8

- ODE model of oscillator

\[
\frac{dm_i}{dt} = -m_i + \frac{\alpha}{(1 + p_j^n)} + \alpha_0 \\
\frac{dp_i}{dt} = -\beta(p_i - m_i)
\]

\[
(i = lacI, tetR, cl) \\
(j = cl, lacI, tetR)
\]
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.

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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