Qualitative Modeling and Simulation of Genetic Regulatory Networks

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Overview

1. Introduction

2. Modeling and simulation of genetic regulatory networks

3. Applications
   - Initiation of sporulation in *Bacillus subtilis*
   - Nutritional stress response in *Escherichia coli*

4. Validation of models of genetic regulatory networks

5. Conclusions
Life cycle of *Bacillus subtilis*

- *B. subtilis* can sporulate when the environmental conditions become unfavorable
Regulatory interactions

- Different types of **interactions** between genes, proteins, and small molecules are involved in the regulation of sporulation in *B. subtilis*

  - SinR represses sin operon
  - SinI inactivates SinR
  - Spo0A~P activates sin operon
  - AbrB represses sin operon
  - SinI inactivates SinR

- **Quantitative information** on kinetic parameters and molecular concentrations is usually not available
Genetic regulatory network of *B. subtilis*

- Reasonably complete **genetic regulatory network** controlling the initiation of sporulation in *B. subtilis*

- Genetic regulatory network is **large and complex**
Qualitative modeling and simulation

- Computer support indispensable for dynamical analysis of genetic regulatory networks: modeling and simulation
  - precise and unambiguous description of network
  - systematic derivation of behavior predictions

- Method for qualitative simulation of large and complex genetic regulatory networks


- Method exploits related work in a variety of domains:
  - mathematical and theoretical biology
  - qualitative reasoning about physical systems
  - control theory and hybrid systems
PL models of genetic regulatory networks

- Genetic networks modeled by class of differential equations using **step functions** to describe regulatory interactions

\[
\begin{align*}
\dot{x}_a &= \kappa_a s^-(x_a, \theta_{a2}) s^-(x_b, \theta_{b1}) - \gamma_a x_a \\
\dot{x}_b &= \kappa_b s^-(x_a, \theta_{a1}) s^-(x_b, \theta_{b2}) - \gamma_b x_b 
\end{align*}
\]

- Differential equation models of regulatory networks are **piecewise-linear (PL)**

Domains in phase space

- Phase space divided into **domains** by threshold planes

![Diagram of phase space](image)

- Different types of domains: **regulatory** and **switching** domains

  Switching domains located on threshold plane(s)
Analysis in regulatory domains

- In every regulatory domain $D$, system monotonically tends towards **target equilibrium set** $\Phi(D)$

\[
\begin{align*}
\dot{x}_a &= \kappa_a s^-(x_a, \theta_{a2}) s^-(x_b, \theta_{b1}) - \gamma_a x_a \\
\dot{x}_b &= \kappa_b s^-(x_a, \theta_{a1}) s^-(x_b, \theta_{b2}) - \gamma_b x_b \\
\Phi(D^3) &= \{(\kappa_a / \gamma_a, \theta_{b1} / \gamma_b)\}
\end{align*}
\]
Analysis in switching domains

- In every switching domain $D$, system either instantaneously traverses $D$, or tends towards **target equilibrium set** $\Phi(D)$, located in the same threshold hyperplane.

- Filippov generalization of PL differential equations

Qualitative state and state transition

- **Qualitative state** is discrete abstraction, consisting of domain $D$ and relative position of target equilibrium set $\Phi(D)$
- **Transition between qualitative states** associated with $D$ and $D'$, if trajectory starting in $D$ reaches $D'$
Closure of qualitative states and transitions between qualitative states results in **state transition graph**

Transition graph contains **qualitative equilibrium states** and/or **cycles**
Robustness of state transition graph

State transition graph, and hence qualitative dynamics, is dependent on parameter values.
Inequality constraints

- Same state transition graph obtained for two types of inequality constraints on parameters $\theta$, $\kappa$, and $\gamma$:
  - Ordering of threshold concentrations of proteins
    \[ 0 < \theta_{a1} < \theta_{a2} < \max_a \quad 0 < \theta_{b1} < \theta_{b2} < \max_b \]
  - Ordering of target equilibrium values w.r.t. threshold concentrations
    \[ \theta_{a2} < \kappa_a / \gamma_a < \max_a \quad \theta_{b2} < \kappa_b / \gamma_b < \max_b \]
Qualitative simulation

- PL model supplemented with inequality constraints results in qualitative PL model

- Given qualitative PL model, qualitative simulation determines all qualitative states that are reachable from initial state through successive transitions
Genetic Network Analyzer (GNA)

- Qualitative simulation method implemented in Java 1.4: Genetic Network Analyzer (GNA)

Graphical interface to control simulation and analyze results

de Jong et al. (2003), Bioinformatics, 19(3):336-344
Simulation of sporulation in *B. subtilis*

- Simulation method applied to analysis of regulatory network controlling the initiation of sporulation in *B. subtilis*
Model of sporulation network

- Essential part of sporulation network has been modeled by qualitative PL model:
  - 11 differential equations, with 59 inequality constraints
- Most interactions incorporated in model have been characterized on genetic and/or molecular level
- With few exceptions, inequality constraints are uniquely determined by biological data
  - If several alternative constraints are consistent with biological data, every alternative considered

Simulation of sporulation network

- Simulation of network under various physiological conditions and genetic backgrounds gives results consistent with observations.

  Sequences of states in transition graphs correspond to sporulation (spo⁺) or division (spo⁻) phenotypes.

![Transition graph with 82 states, showing initial state, division state, and 82 states between them.](image)
Simulation of sporulation network

- Behavior can be studied in detail by looking at transitions between qualitative states

Predicted qualitative temporal evolution of protein concentrations
Sporulation vs. division behaviors

$max_{ka}$

$\theta_{ka3}$  

$\theta_{kal}$

$max_{se}$

$\theta_{se3}$

$\theta_{sel}$

$max_{ab}$

$\theta_{abl}$

$max_f$

$max_{si}$

$\theta_{sil}$

KinA

Spo0E

AbrB

SigF

SinI
Analysis of simulation results

- Qualitative simulation shows that initiation of sporulation is outcome of **competing positive and negative feedback loops** regulating accumulation of Spo0A~P


- Sporulation mutants disable positive or negative feedback loops
Nutritional stress response in *E. coli*

- Response of *E. coli* to nutritional stress conditions controlled by network of **global regulators of transcription**
  - Fis, Crp, H-NS, Lrp, RpoS,…
- Network only partially known and no global view of its functioning available
- **Computational** and **experimental** study directed at understanding of:
  - How network controls gene expression to adapt cell to stress conditions
  - How network evolves over time to adapt to environment
- Projects: inter-EPST, ARC INRIA, and ACI IMPBio
  - ENS, Paris ; INRIA ; UJF, Grenoble ; UHA, Mulhouse
Data on stress response

- **Gene transcription** changes dramatically when the network is perturbed by a mutation.

- **Small signaling molecules** participate in global regulation mechanisms (cAMP, ppGpp, ...)

- The **superhelical density** of DNA modulates the activity of many bacterial promoters.
Draft of stress response network

Laget et al. (2004)
Evolution of stress response network

- Stress response network evolves rapidly towards optimal adaptation to a particular environment
- Small changes of the regulatory network have large effects on gene expression
Validation of network models

- **Bottleneck** of qualitative simulation: visual inspection of large state transition graphs

- **Goal**: develop a method that can test if state transition graph satisfies certain properties
  
  Is transition graph consistent with observed behavior?

- **Model checking** is automated technique for verifying that finite state system satisfies certain properties
  
  Clarke *et al.* (1999), *Model Checking*, MIT Press

- Computer tools are available to perform automated, efficient and reliable model checking (NuSMV)
Model checking

- Use of model-checking techniques
  - transition graph transformed into Kripke structure
  - properties expressed in temporal logic

There exists a future state where $\dot{x}_a > 0$ and $\dot{x}_b > 0$ and starting from that state, there exists a future state where $\dot{x}_a = 0$ and $\dot{x}_b < 0$.

Yes!
Summary of approach

- Test validity of *B. subtilis* sporulation models

\[ \text{EF}(\dot{x}_{hpr} > 0) \land \text{EF EG}(\dot{x}_{hpr} = 0) \]

“[The expression of the gene *hpr*] increase in proportion of the growth curve, reached a maximum level at the early stationary phase \([T1]\) and remained at the same level during the stationary phase” (Perego and Hoch, 1988)

Batt *et al.* (2004), *SPIN-04*, LNCS
Conclusions

- Implemented method for qualitative simulation of large and complex genetic regulatory networks
  
  Method based on work in mathematical biology and qualitative reasoning

- **Method validated** by analysis of regulatory network underlying initiation of sporulation in *B. subtilis*
  
  Simulation results consistent with observations

- **Method currently applied** to analysis of regulatory network controlling stress adaptation in *E. coli*
  
  Simulation yields predictions that can be tested in the laboratory
Work in progress

- **Validation of models** of regulatory networks using gene expression data
  
  Model-checking techniques

- **Search of attractors** in phase space and determination of their stability

- Further development of **computer tool** GNA
  
  Connection with biological knowledge bases, …

- **Study of bacterial regulatory networks**
  
  Sporulation in *B. subtilis*, phage Mu infection of *E. coli*, signal transduction in *Synechocystis*, stress adaptation in *E. coli*
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References


GNA web site: [http://www-helix.inrialpes.fr/article122.html](http://www-helix.inrialpes.fr/article122.html)