Validation of Qualitative Models of Genetic Regulatory Networks by Model Checking: Nutritional Stress Response in *E. coli*

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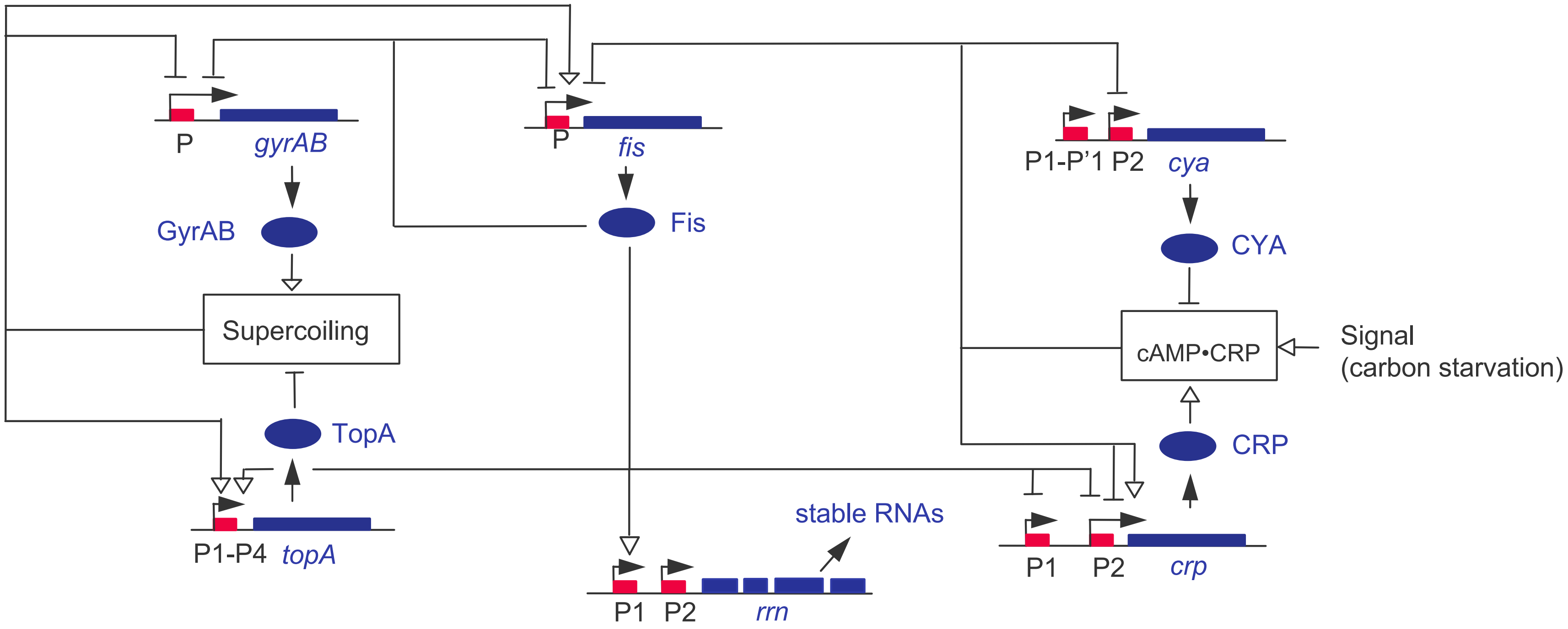
Overview

1. Introduction
2. Qualitative modeling and simulation method
3. Validation with model-checking techniques
4. Application to nutritional stress response in *E. coli*
5. Conclusions

Genetic regulatory networks

- **Genetic regulatory networks** underlie functioning and development of living organisms

  Genes, proteins, small molecules, and their regulatory interactions



- Genetic regulatory networks are **large** and **complex**

Validation of genetic network models

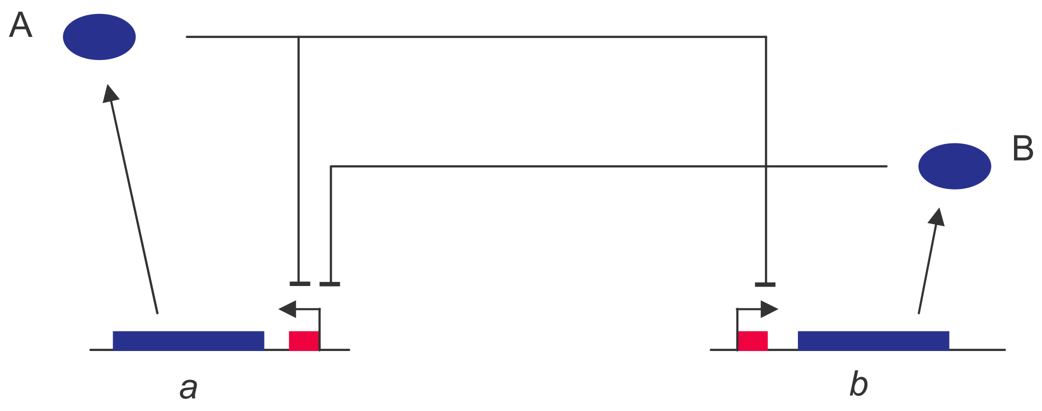
- Mathematical methods and computational tools exist for analysis of genetic networks through modeling and simulation
- Problem of **model validation**: check consistency between experimental data and predictions
- Major constraints for model validation:
  - predictions **suitable for comparison** with available experimental data
  - **automatic** and **efficient** comparison between experimental data and predictions
- Approach:
  - use of **qualitative modeling and simulation** method
  - combination with **model-checking** techniques

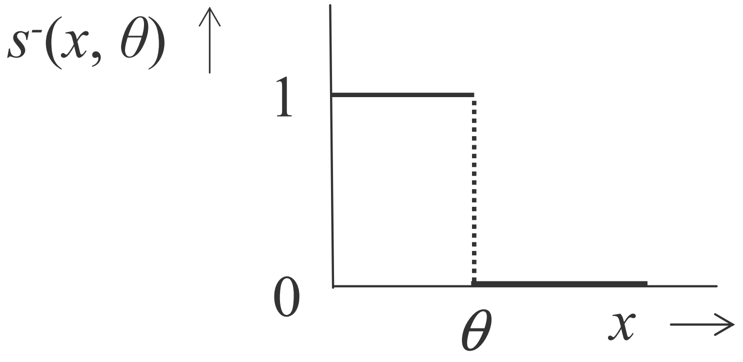
PL differential equation models

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

$$\dot{x}_a = \kappa_a\, s^-(x_a, \theta_{a2})\, s^-(x_b, \theta_b) - \gamma_a x_a$$

$$\dot{x}_b = \kappa_b\, s^-(x_a, \theta_{a1}) - \gamma_b x_b$$





x : protein concentration

θ : threshold concentration

κ, γ: rate constants

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

Glass and Kauffman, *J. Theor. Biol.*, 73

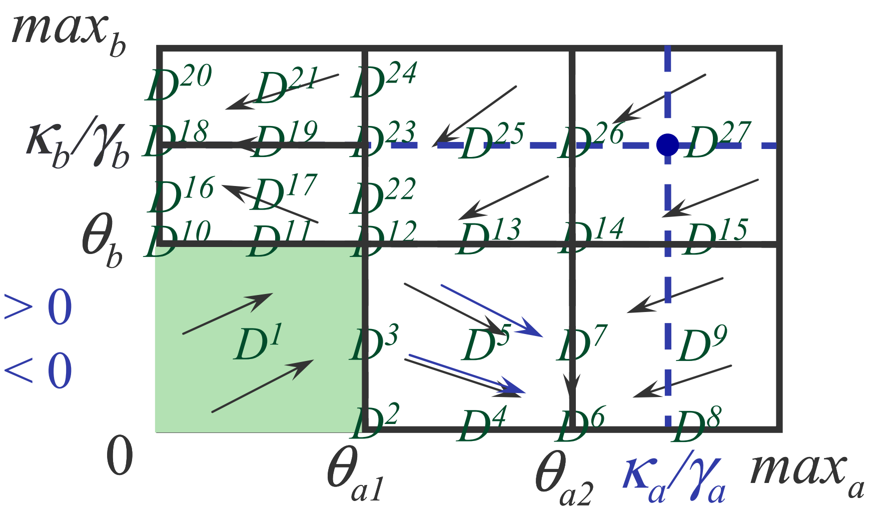
Qualitative analysis of network dynamics

- Method for **qualitative analysis** of dynamics of genetic regulatory networks:
  - discrete transition system obtained by **qualitative abstraction** of the dynamics, based on hyperrectangular partition of phase space
  - **inequality constraints** define regions in parameter space yielding the same discrete transition system
  - **symbolic computation** of transition system using inequality constraints and **tailored algorithms** (upscalability)
  - implementation in the computer tool **Genetic Network Analyzer (GNA)**
  - **application** to several bacterial systems

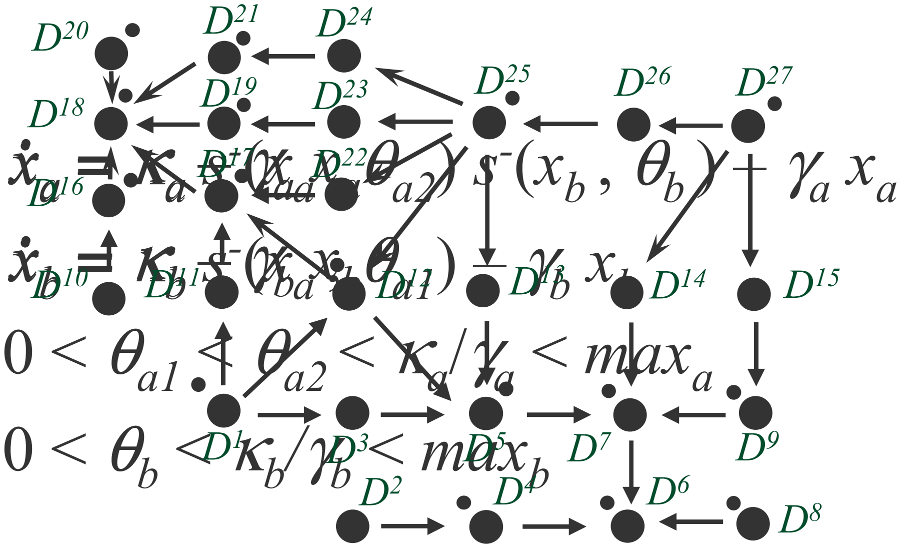
Gouzé and Sari, *Dyn. Syst.*, 03 de Jong *et al.*, *Bioinformatics*, 03 de Jong *et al.*, *Bull. Math. Biol.*, 04

Qualitative analysis of network dynamics

- Analysis of the dynamics in phase space
- Phase space partition: unique derivative sign pattern in domains
- Qualitative abstraction yields discrete transition system



D^5: $\dot{x}_a > 0$, $\dot{x}_b < 0$



$$\dot{x}_a = \kappa_a\, s^+(x_a, \theta_{a2})\, s^-(x_b, \theta_b) - \gamma_a x_a$$

$$\dot{x}_b = \kappa_b\, s^-(x_a, \theta_{a1}) - \gamma_b x_b$$

$$0 < \theta_{a1} < \theta_{a2} < \kappa_a/\gamma_a < max_a$$

$$0 < \theta_b < \kappa_b/\gamma_b < max_b$$

D^1: $\dot{x}_a > 0$, $\dot{x}_b > 0$

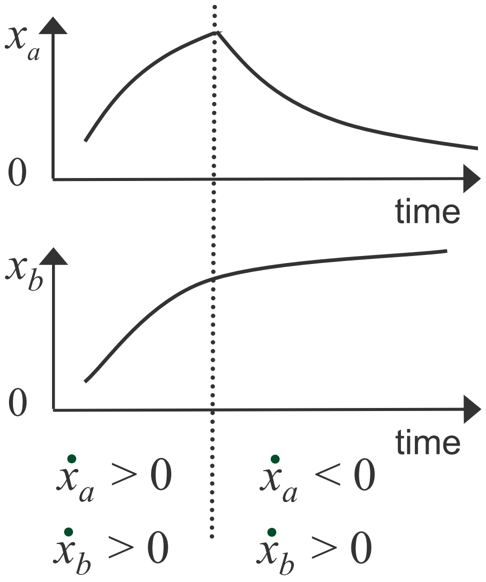
D^5: $\dot{x}_a > 0$, $\dot{x}_b < 0$

D^7: $\dot{x}_a = 0$, $\dot{x}_b < 0$

- Abstraction preserves **unicity** of **derivative sign pattern**

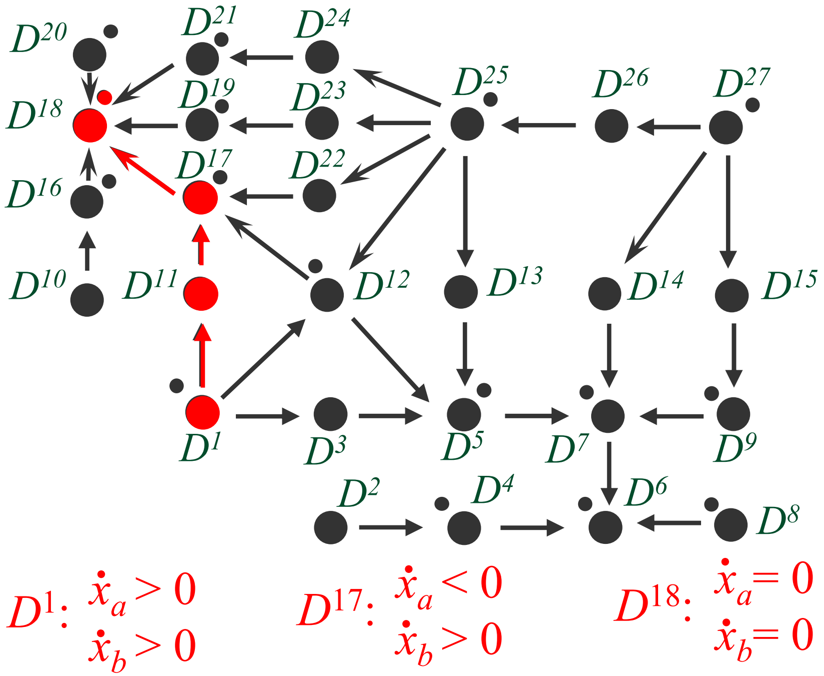
Validation of qualitative models

- Predictions well adapted to comparison with available experimental data: **changes of derivative sign patterns**



Concistency?

Yes



- Model validation: comparison of derivative sign patterns in observed and predicted behaviors
- Need for **automatic** and **efficient** comparison

Model-checking approach

- **Model checking** is automated technique for verifying that discrete transition system satisfies certain temporal properties
- CTL model-checking framework:
  - set of **atomic propositions** AP
  - discrete transition system $KS = \langle S, R, L \rangle$,

    where S set of states, R transition relation, L labeling function over AP
  - temporal properties expressed in **Computation Tree Logic (CTL)**

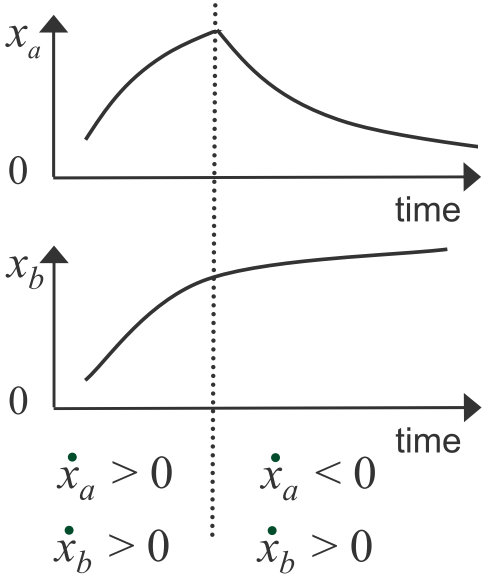
    p, $\neg f_1$, $f_1 \wedge f_2$, $f_1 \vee f_2$, $f_1 \rightarrow f_2$, $\mathbf{EX}f_1$, $\mathbf{AX}f_1$, $\mathbf{EF}f_1$, $\mathbf{AF}f_1$, $\mathbf{EG}f_1$, $\mathbf{AG}f_1$, $\mathbf{E}f_1\mathbf{U}f_2$, $\mathbf{A}f_1\mathbf{U}f_2$,
    where $p \in AP$ and f_1, f_2 CTL formulas
- Computer tools are available to perform efficient and reliable model checking (*e.g.*, NuSMV, SPIN, CADP)

Validation using model checking

- Atomic propositions

$$AP = \{x_a = 0,\ x_a < \theta_a^{1},\ \ldots,\ x_b < max_b,\ \dot{x}_a < 0,\ \dot{x}_a = 0,\ \ldots,\ \dot{x}_b > 0\}$$

- Observed property expressed in CTL

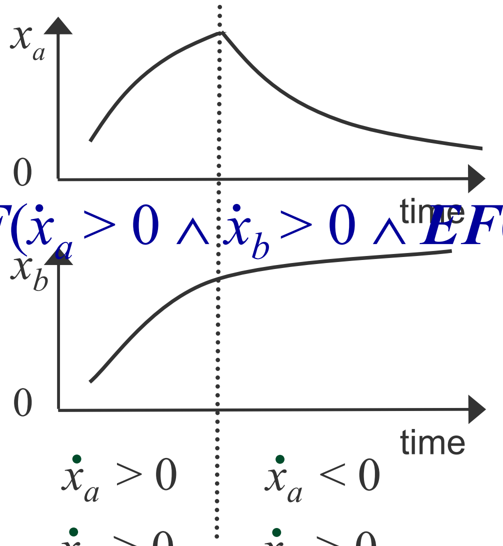


There *E*xists a *F*uture state where $\dot{x}_a > 0$ and $\dot{x}_b > 0$
and starting from that state,
there *E*xists a *F*uture state where $\dot{x}_a < 0$ and $\dot{x}_b > 0$

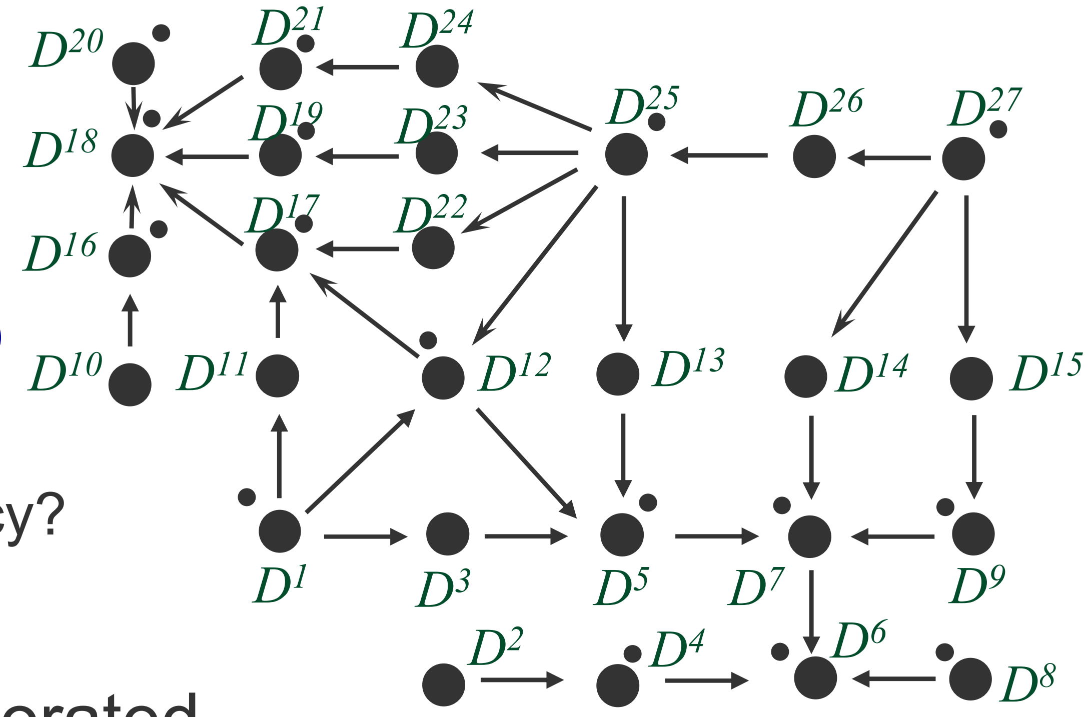
$$\boldsymbol{EF}(\dot{x}_a > 0 \wedge \dot{x}_b > 0 \wedge \boldsymbol{EF}(\dot{x}_a < 0 \wedge \dot{x}_b > 0)\ )$$

Validation using model checking

- Compute discrete transition system using qualitative simulation



$$EF(\dot{x}_a > 0 \wedge \dot{x}_b > 0 \wedge EF(\dot{x}_a < 0 \wedge \dot{x}_b > 0))$$



Concistency?

Yes

Model corroborated

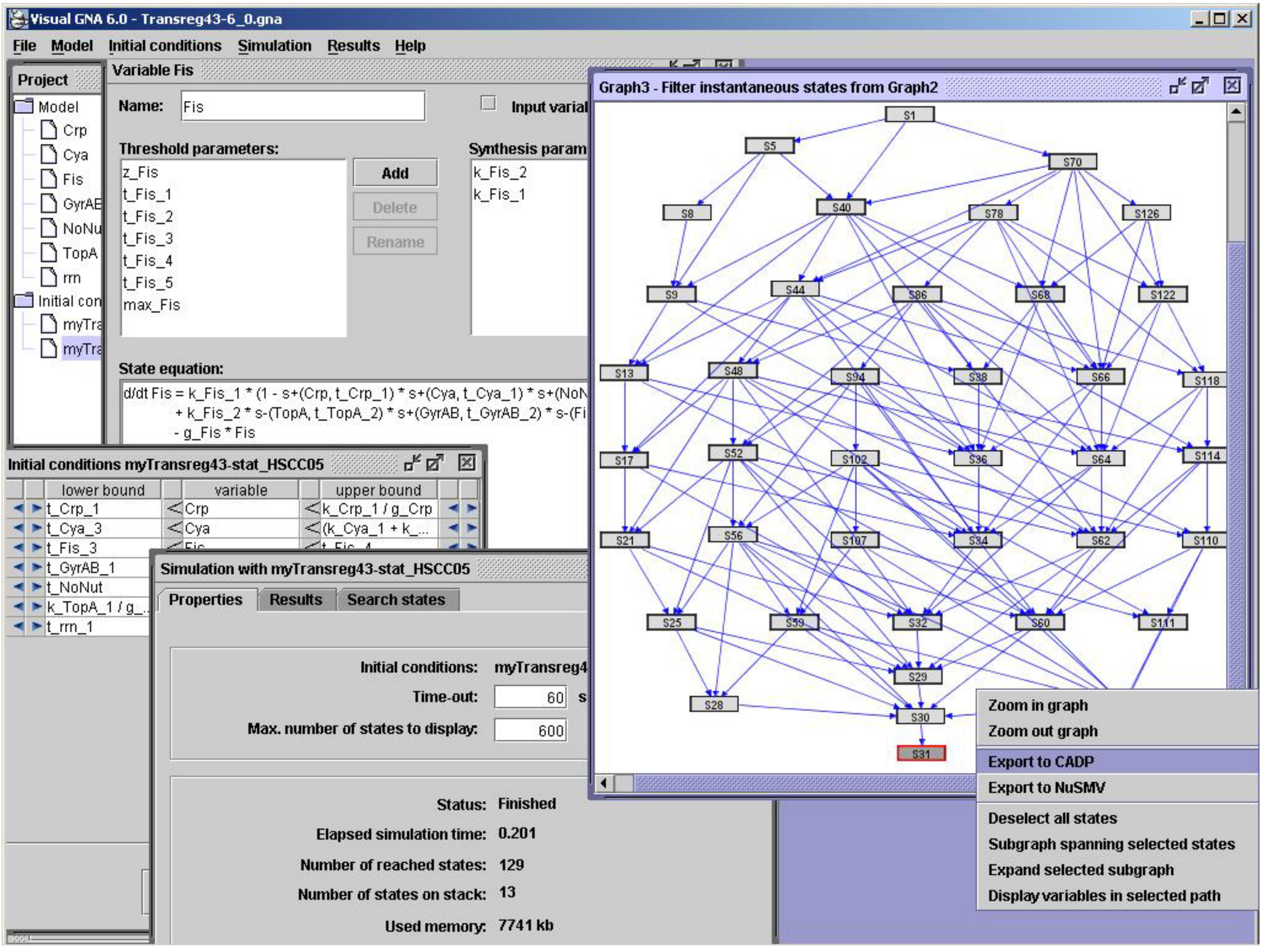
- Use of model checkers to check consistency between experimental data and predictions

Genetic Network Analyzer

❖ Model validation approach implemented in **new version** of GNA

- Tailored algorithms for symbolic computation of transition system

- Export functionalities to model checkers (NuSMV, CADP)



Name: Fis

Threshold parameters: z_Fis, t_Fis_1, t_Fis_2, t_Fis_3, t_Fis_4, t_Fis_5, max_Fis

Synthesis param: k_Fis_2, k_Fis_1

State equation:

d/dt Fis = k_Fis_1 * (1 - s+(Crp, t_Crp_1) * s+(Cya, t_Cya_1) * s+(NoN
+ k_Fis_2 * s-(TopA, t_TopA_2) * s+(GyrAB, t_GyrAB_2) * s-(Fi
- g_Fis * Fis

lower bound		variable		upper bound
t_Crp_1	<	Crp	<	k_Crp_1 / g_Crp
t_Cya_3	<	Cya	<	(k_Cya_1 + k_...
t_Fis_3	<	Fis	<	t_Fis_4
t_GyrAB_1				
t_NoNut				
k_TopA_1 / g_...				
t_rrn_1				

Initial conditions: myTransreg4

Time-out: 60 s

Max. number of states to display: 600

Status: Finished

Elapsed simulation time: 0.201

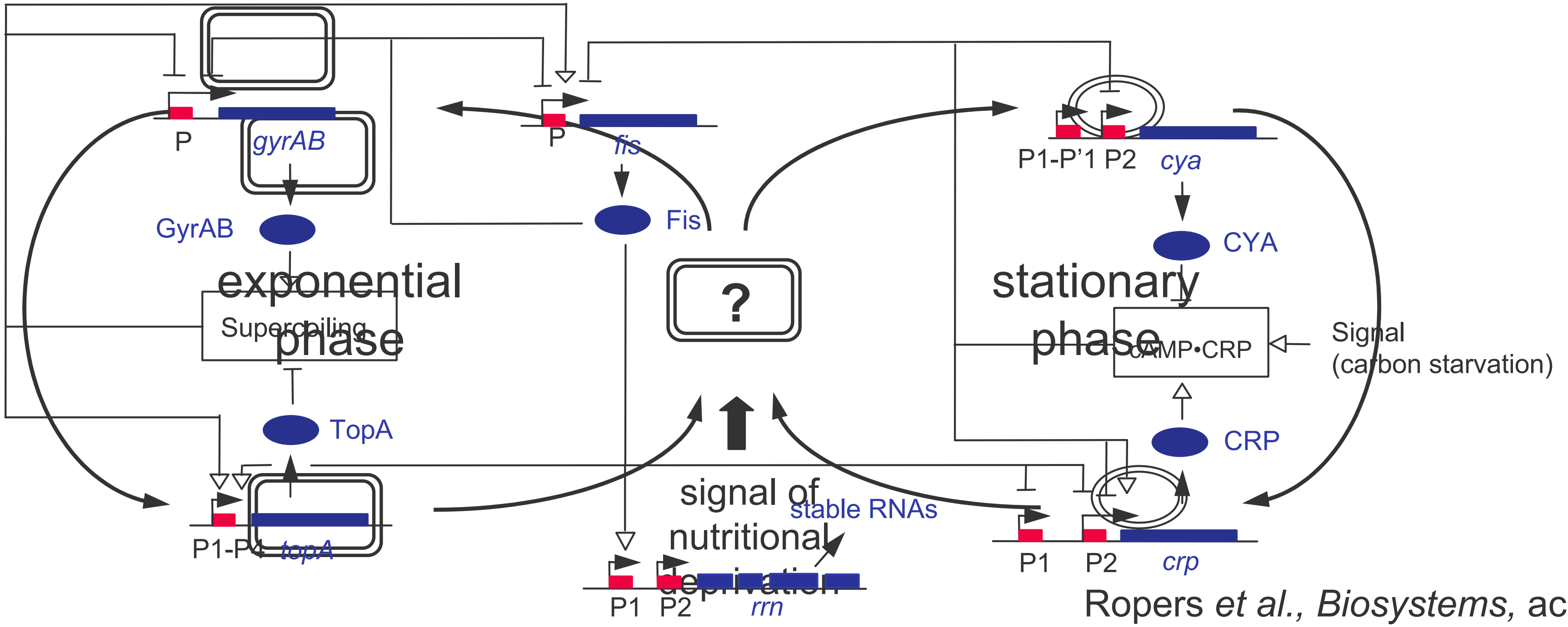
Number of reached states: 129

Number of states on stack: 13

Used memory: 7741 kb

Nutritional stress response in *E. coli*

- In case of nutritional stress, *E. coli* population abandons growth and enters stationary phase

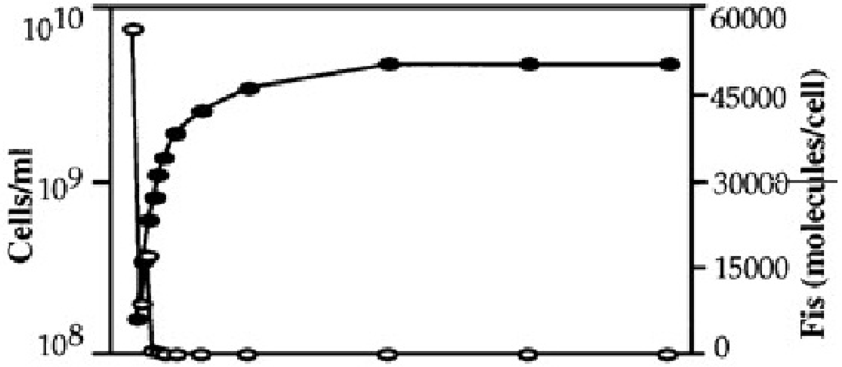


Ropers *et al.*, *Biosystems*, accepted

- Decision to abandon or continue growth is controlled by complex genetic regulatory network
- Model: 7 PLDEs, 40 parameters and 54 inequality constraints

Validation of stress response model

- Qualitative simulation of carbon starvation:
  - 66 reachable domains (< 1s.)
  - single attractor domain (asymptotically stable equilibrium point)
- Experimental data on Fis:



"Fis concentration decreases and becomes steady in stationary phase"

Ali Azam *et al.*, *J. Bacteriol.*, 99

CTL formulation:

$$EF(\dot{x}_{fis} < 0 \wedge EF(\dot{x}_{fis} = 0 \wedge x_{rrn} < \theta_{rrn}))$$

Model checking with NuSMV: property true (< 1s.)

Validation of stress response model

- Other properties:
  - "*cya* transcription is negatively regulated by the complex cAMP-CRP"

Kawamukai *et al.*, *J. Bacteriol.*, 85

$$\boldsymbol{AG}(x_{crp} > \theta^{3}_{crp} \wedge x_{cya} > \theta^{3}_{cya} \wedge x_s > \theta_s \rightarrow \boldsymbol{EF}\, \dot{x}_{cya} < 0)$$

True

  - "DNA supercoiling decreases during transition to stationary phase"

Balke and Gralla, *J. Bacteriol.*, 87

$$\boldsymbol{EF}(\ (\dot{x}_{gyrAB} < 0 \vee \dot{x}_{topA} > 0) \wedge x_{rrn} < \theta_{rrn})$$

False

- **Model-driven experiments** for more extensive tests of validity

Time-series measurements of molecular concentrations in parallel and at high sampling rate

Conclusions

- Use of qualitative modeling and simulation method yielding predictions **suitable for comparison** with experimental data
- Combination with model-checking techniques to achieve **automatic** and **efficient** comparison
- Approach **implemented** and **applied** to nutritional stress response in *E. coli*
- Model-checking used in combination with different formalisms
  - generalized logical models Bernot *et al.*, *J. Theor. Biol.*, 04
  - concurrent systems Chabrier *et al., Theor. Comput. Sci.*, 04; Eker *et al., PSB,* 02
  - hybrid automata Ghosh *et al., HSCC,* 03
- Further work: integration of tailored model checker into GNA

- Thanks for your attention!