Modeling and simulation of gene regulatory networks 3

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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, …

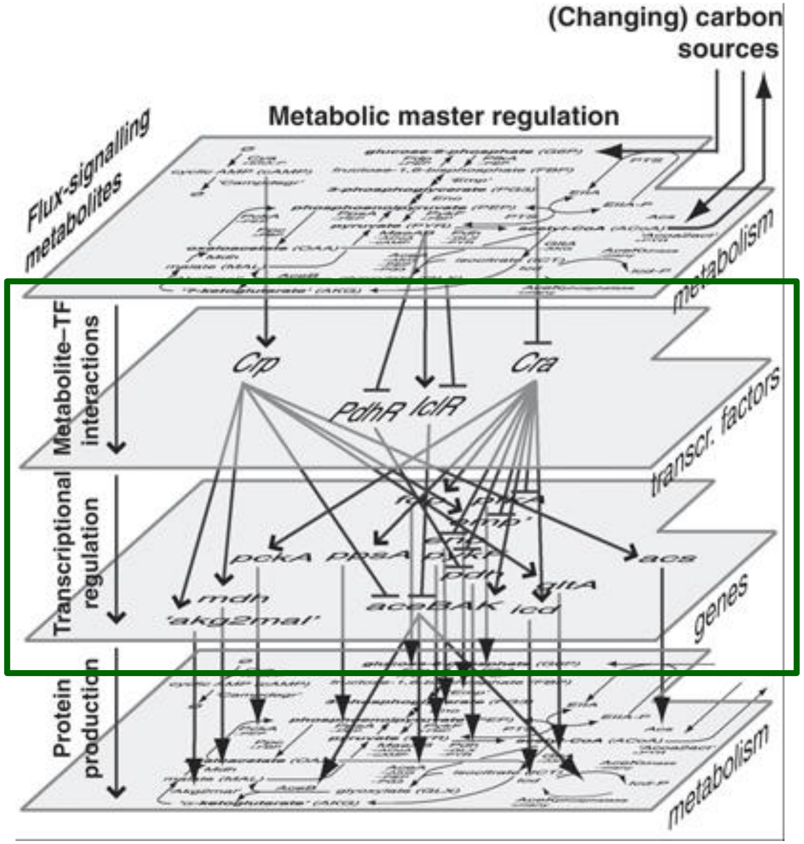
http://ibis.inrialpes.fr

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*

Kotte *et al.* (2010), *Mol. Syst. Biol.*, 6:355

Modeling of gene regulatory networks

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

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

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

- Practical problems encountered by modelers:
  - Knowledge on molecular mechanisms rare
  - Quantitative information on kinetic parameters and molecular concentrations absent
  - Large models

Qualitative modeling and simulation

- Intuition: essential properties of network dynamics **robust** against reasonable model simplifications
- **Qualitative** modeling and simulation of large and complex gene regulatory networks using **simplified** models

    de Jong, Gouzé *et al.* (2004), *Bull. Math. Biol.*, 66(2):301-40

- Relation with discrete, logical models of gene regulation

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

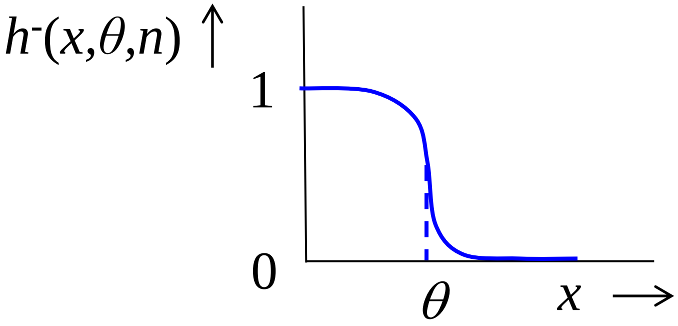
    Kauffman (1993), *The Origins of Order*, Oxford University Press

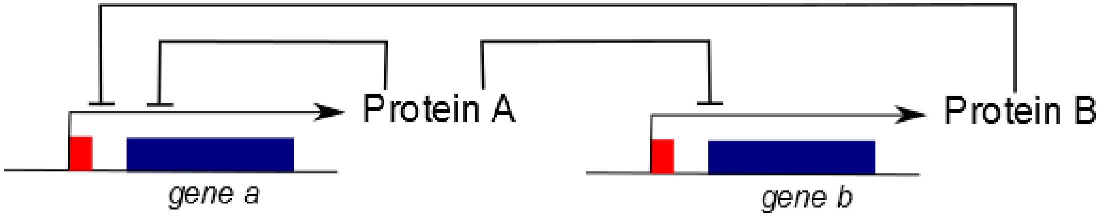
Ordinary differential equation models

- Gene regulatory networks modeled by ODE models using **sigmoid functions** to describe regulatory interactions

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

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





x : protein concentration
θ : threshold concentration
κ, γ : rate constants
n : steepness parameter

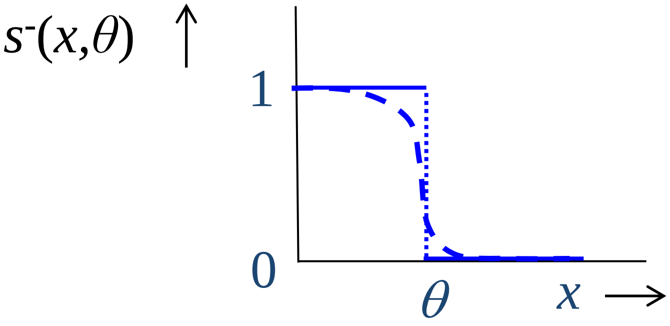
- Expressions of sigmoid functions account for **combinatorial control** of gene expression (AND, OR, NOR, …)

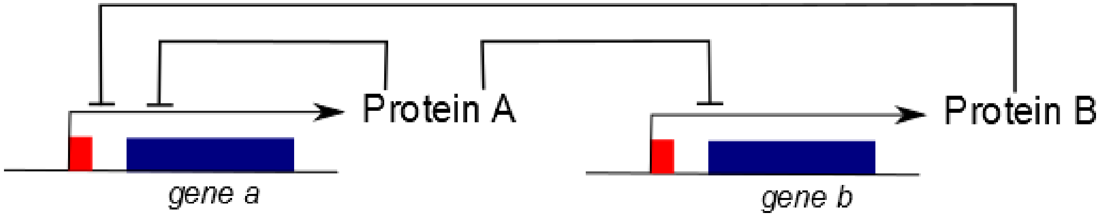
PL differential equation models

- ODE models approximated by means of **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

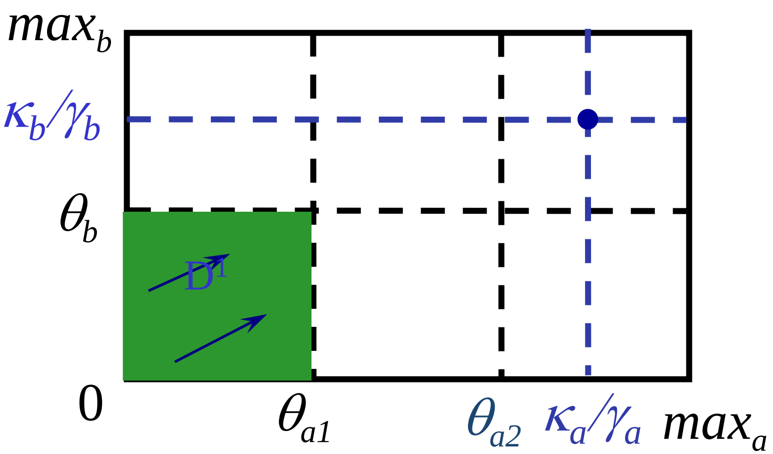
- Piecewise-linear (PL)DE models of gene regulatory networks

Glass and Kauffman (1973), *J. Theor. Biol.*, 39(1):103-29

Mathematical analysis of PL models

- Analysis of local dynamics of PL models

  Monotone convergence towards **focal point** in regions separated by thresholds



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

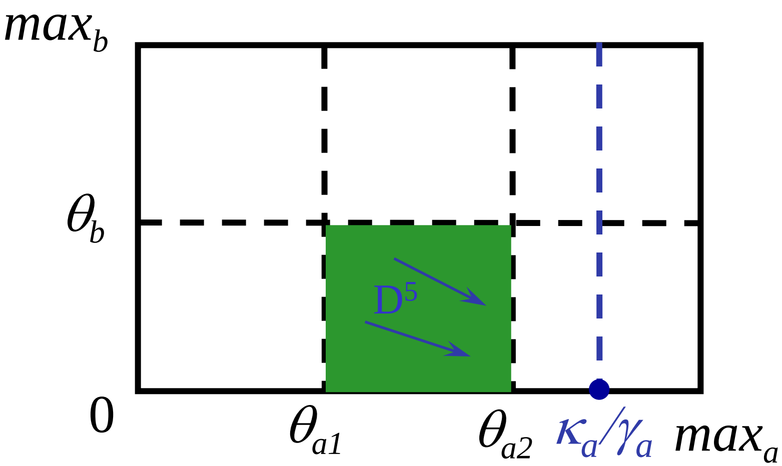
$$\dot{x}_a = \kappa_a s^-(x_a, \theta_{a2})\, s^-(x_b, \theta_b) - \gamma_a x_a$$
$$\dot{x}_b = \kappa_b s^-(x_a, \theta_{a1}) - \gamma_b x_b$$

Glass and Kauffman (1973), *J. Theor. Biol.*, 39(1):103-29

Mathematical analysis of PL models

- Analysis of local dynamics of PL models

Monotone convergence towards **focal point** in regions separated by thresholds



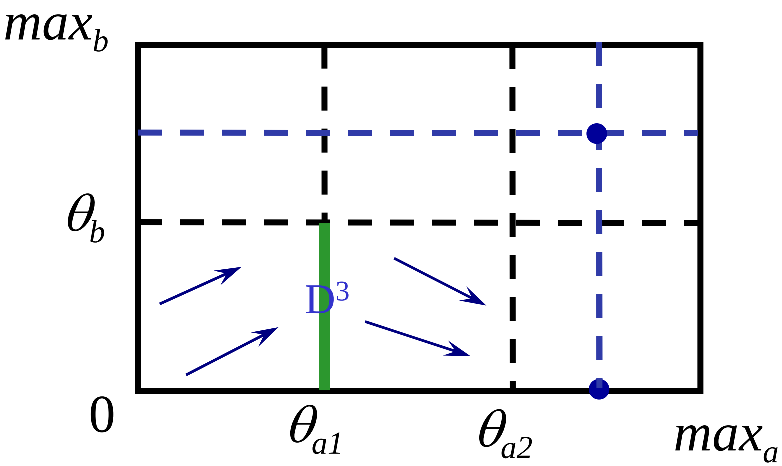
$$\dot{x}_a = \kappa_a - \gamma_a\, x_a$$
$$\dot{x}_b = -\,\gamma_b\, x_b$$

$$\dot{x}_a = \kappa_a\, s^-(x_a, \theta_{a2})\, s^-(x_b, \theta_b) - \gamma_a\, x_a$$
$$\dot{x}_b = \kappa_b\, s^-(x_a, \theta_{a1}) - \gamma_b\, x_b$$

Glass and Kauffman (1973), *J. Theor. Biol.*, 39(1):103-29

Mathematical analysis of PL models

- Analysis of local dynamics of PL models
  - Instantaneous crossing of regions located on thresholds, or …



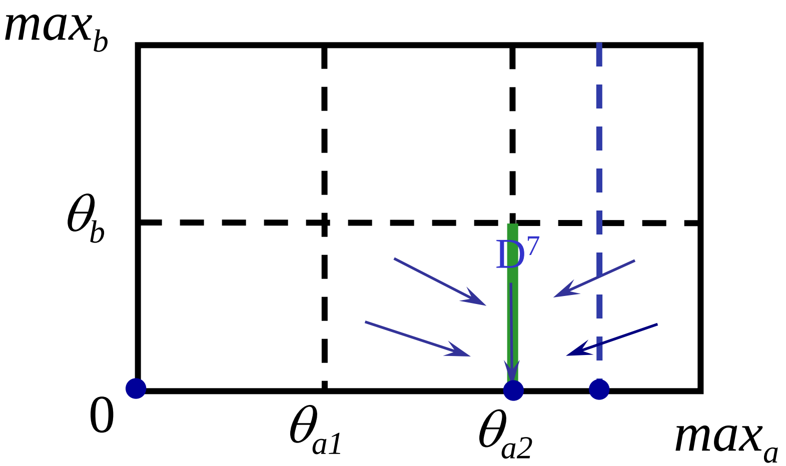
$$\dot{x}_a = \kappa_a\, s^-(x_a, \theta_{a2})\, s^-(x_b, \theta_b) - \gamma_a\, x_a$$

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

Mathematical analysis of PL models

- Analysis of local dynamics of PL models

  … quasi-monotone convergence towards **focal sets** located on threshold hyperplanes



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

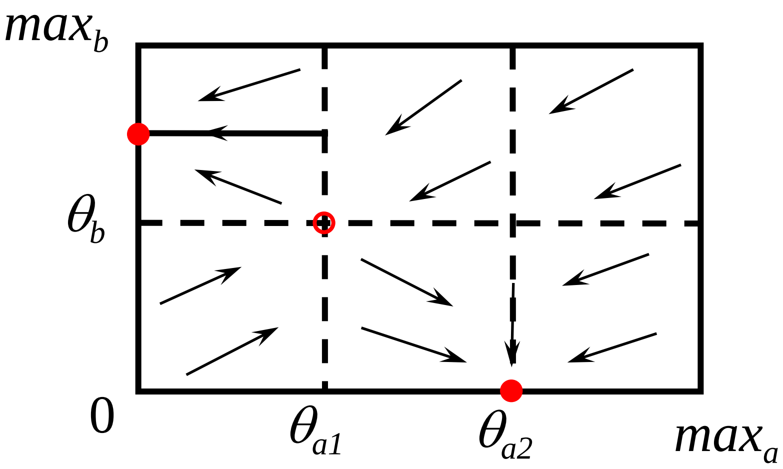
$$\dot{x}_b = \kappa_b \, s^-(x_a, \theta_{a1}) - \gamma_b \, x_b$$

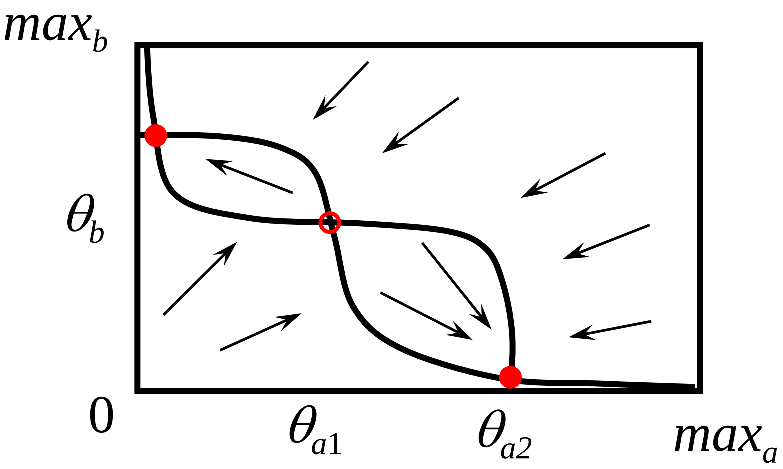
- Extension of PL differential **equations** to differential **inclusions** using Filippov approach

Gouzé and Sari (2002), *Dyn. Syst.*, 17(4):299-316

Qualitative analysis of PL models

- Analysis of global dynamics obtained by piecing together local dynamics in regions
  - PL approximation preserves bistability of cross-inhibition network

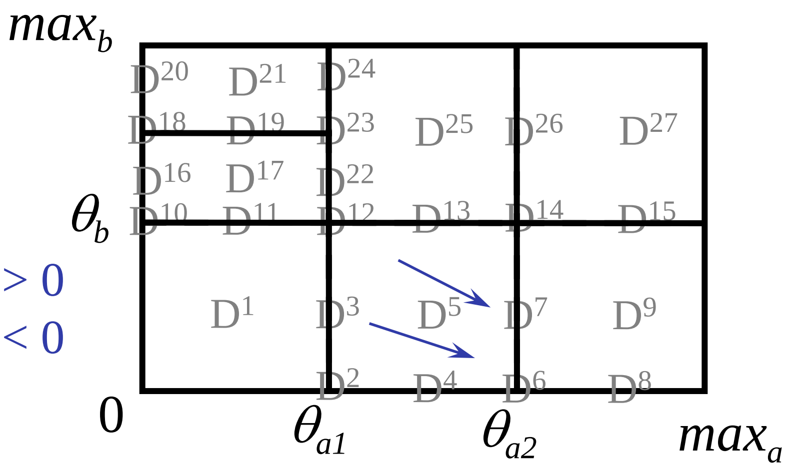




Qualitative analysis of PL models

- State space can be partitioned into regions with **unique** derivative sign pattern
- **Qualitative abstraction** yields state transition graph that provides discrete picture of continuous dynamics

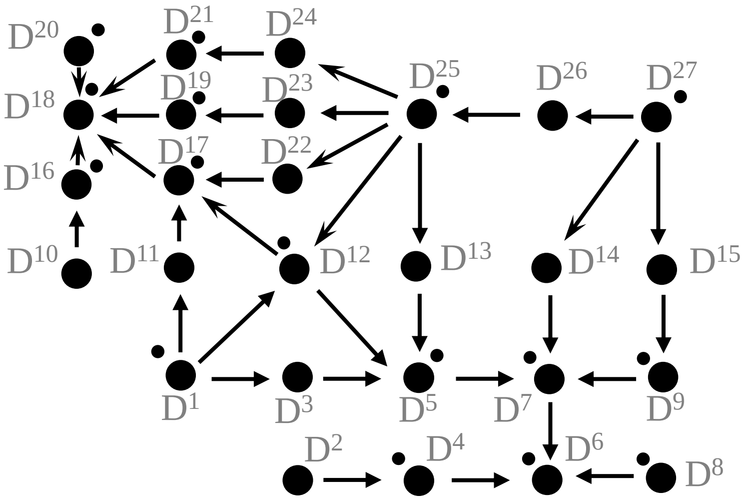
Alur *et al.* (2000), *Proc. IEEE*, 88(7):971-84



D5: $\dot{x}_a > 0$
$\dot{x}_b < 0$

de Jong *et al.* (2004), *Bull. Math. Biol.*, 66(2):301-40

Batt *et al.* (2008), *Automatica*, 44(4):982-9



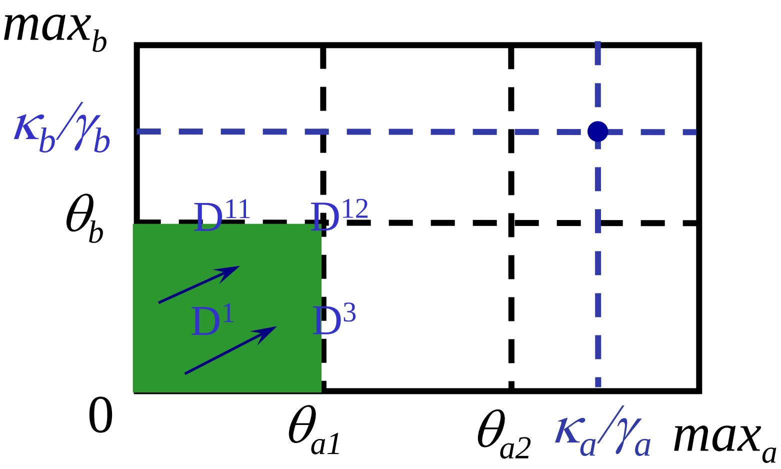
D1: $\dot{x}_a > 0$
$\dot{x}_b > 0$

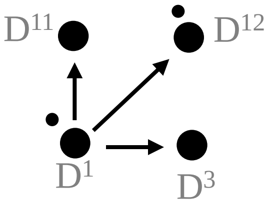
D5: $\dot{x}_a > 0$
$\dot{x}_b < 0$

D7: $\dot{x}_a = 0$
$\dot{x}_b < 0$

Qualitative analysis of PL models

- State transition graph gives **conservative approximation** of continuous dynamics
  - Every solution of PL model corresponds to path in state transition graph
  - Converse is not necessarily true!
- State transition graph is **invariant** for given inequality constraints on parameters





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

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

Batt *et al.* (2008), *Automatica*, 44(4):982-9

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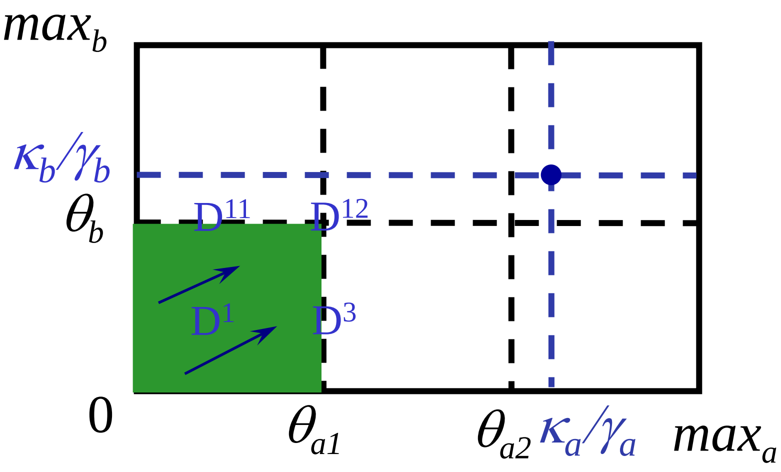

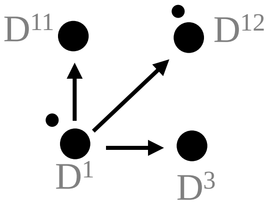

$$0 < \theta_{a1} < \theta_{a2} < \kappa_a/\gamma_a < max_a$$

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

Batt *et al.* (2008), *Automatica*, 44(4):982-9

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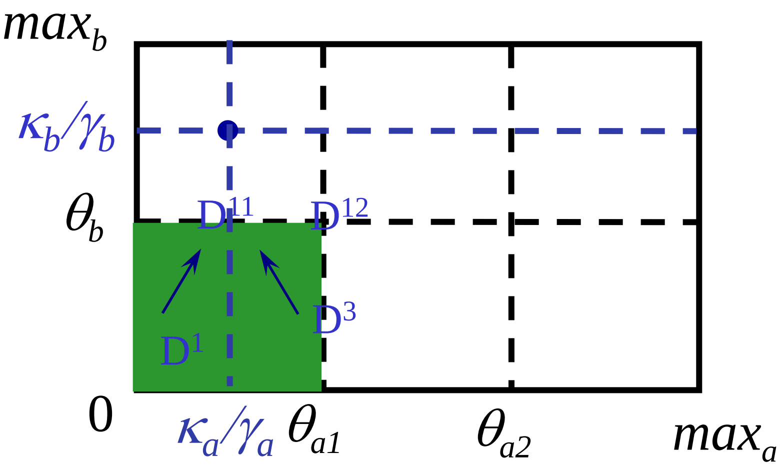
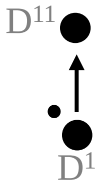




$$0 < \kappa_a/\gamma_a < \theta_{a1} < \theta_{a2} < max_a$$

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

Batt *et al.* (2008), *Automatica*, 44(4):982-9

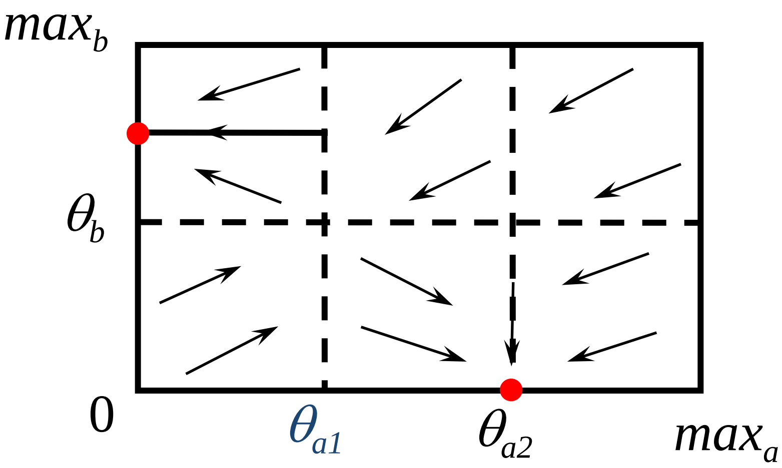
Use of state transition graph

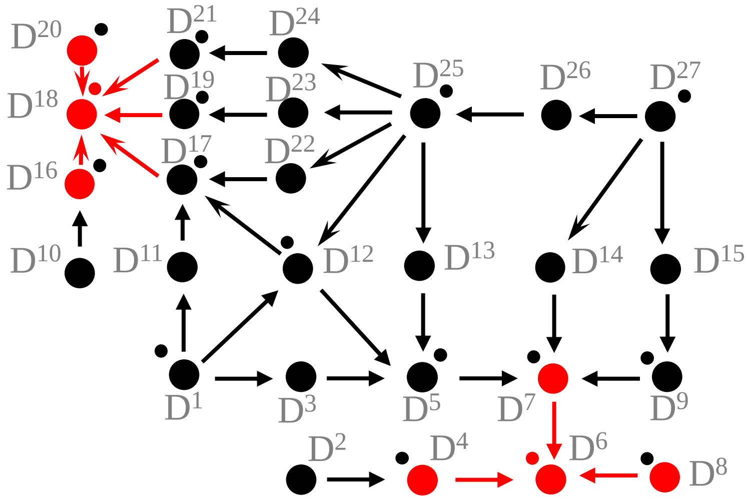
- Analysis of **steady states** and **limit cycles** of PL models
  - Attractor states in graph correspond (under certain conditions) to stable steady states of PL model
  - Attractor cycles in graph correspond (under certain conditions) to stable limit cycles of PL model

Casey *et al.* (2006), *J. Math Biol.*, 52(1):27-56

Glass and Pasternack (1978), *J. Math Biol.*, 6(2):207-23

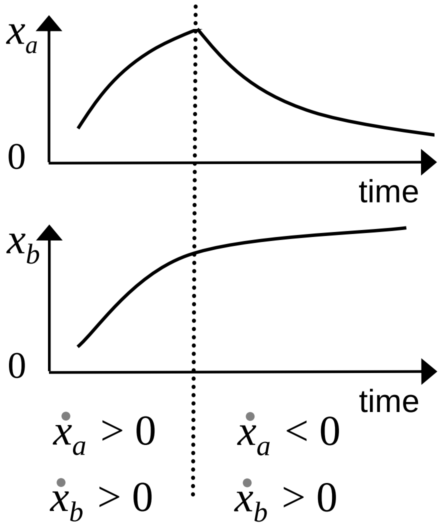
Edwards (2000), *Physica D*, 146(1-4):165-99





Use of state transition graph

- Paths in state transition graph represent predicted sequences of qualitative events
- **Model validation**: comparison of predicted and observed sequences of qualitative events

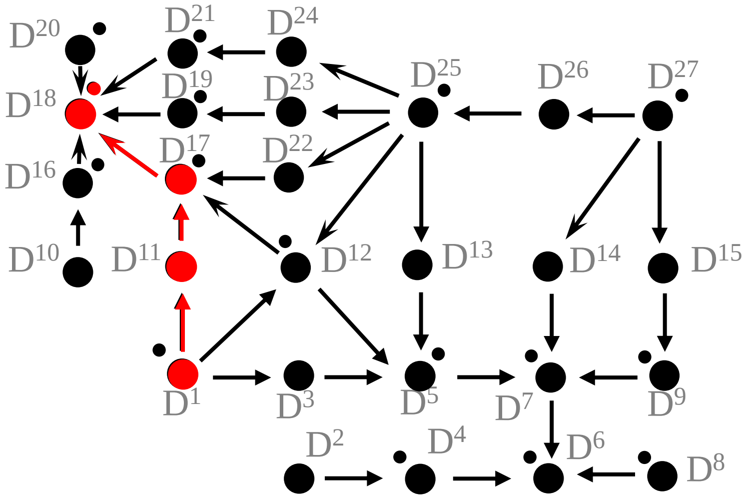


$\dot{x}_a > 0$ $\dot{x}_a < 0$

$\dot{x}_b > 0$ $\dot{x}_b > 0$

Concistency?

Yes



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

D^{17}: $\dot{x}_a < 0$, $\dot{x}_b > 0$

D^{18}: $\dot{x}_a = 0$, $\dot{x}_b = 0$

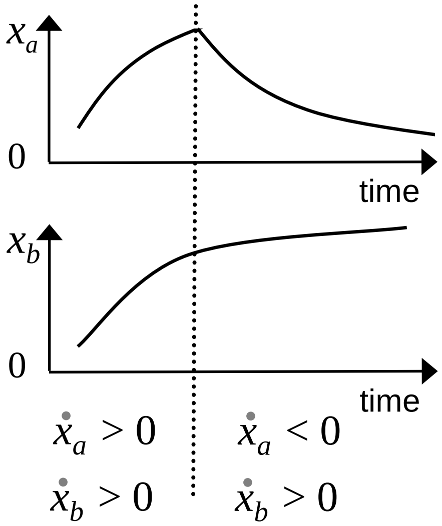
- Need for automated and efficient tools for model validation

Model validation by model checking

- Dynamic properties of system can be expressed in **temporal logic** (CTL)

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 $\dot{x}_b > 0$

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

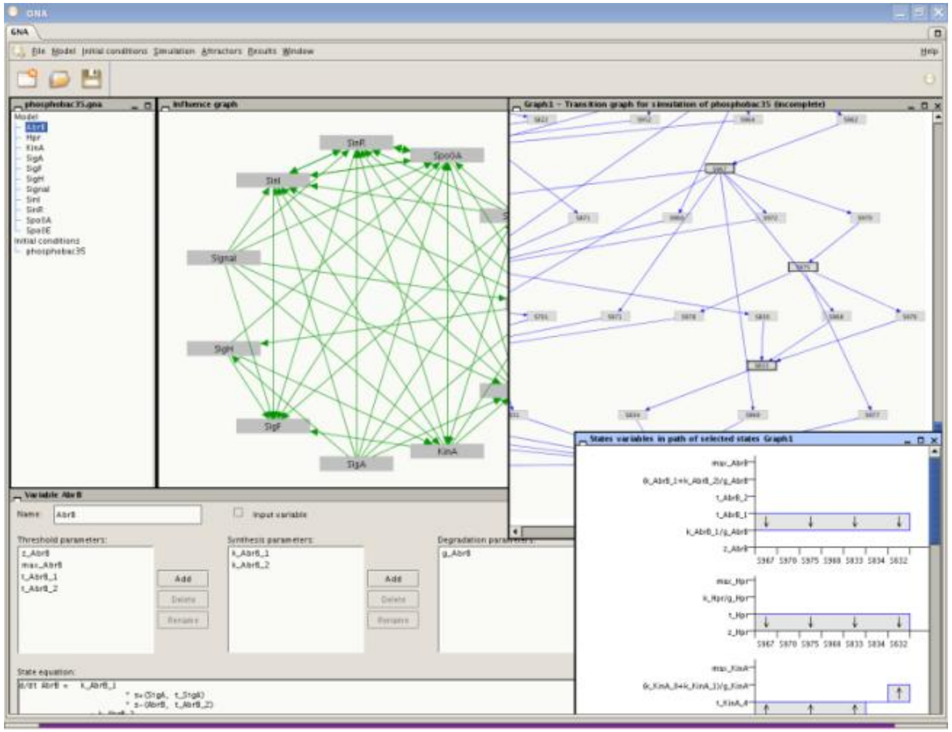


- **Model checking** is automated technique for verifying that state transition graph satisfies temporal-logic statements
  - Efficient computer tools available for model checking

Batt *et al.* (2005), *Bioinformatics*, 21(supp. 1): i19-i28

Genetic Network Analyzer (GNA)

- Qualitative analysis of PL models implemented in Java: **Genetic Network Analyzer (GNA)**



Genostar

bioinformatics solutions

de Jong *et al.* (2003), *Bioinformatics*, 19(3):336-44

http://www-helix.inrialpes.fr/gna

Inria

Genetic Network Analyzer (GNA)

- Model-checking technology made available to GNA user
  - Develop temporal logics tailored to biological questions

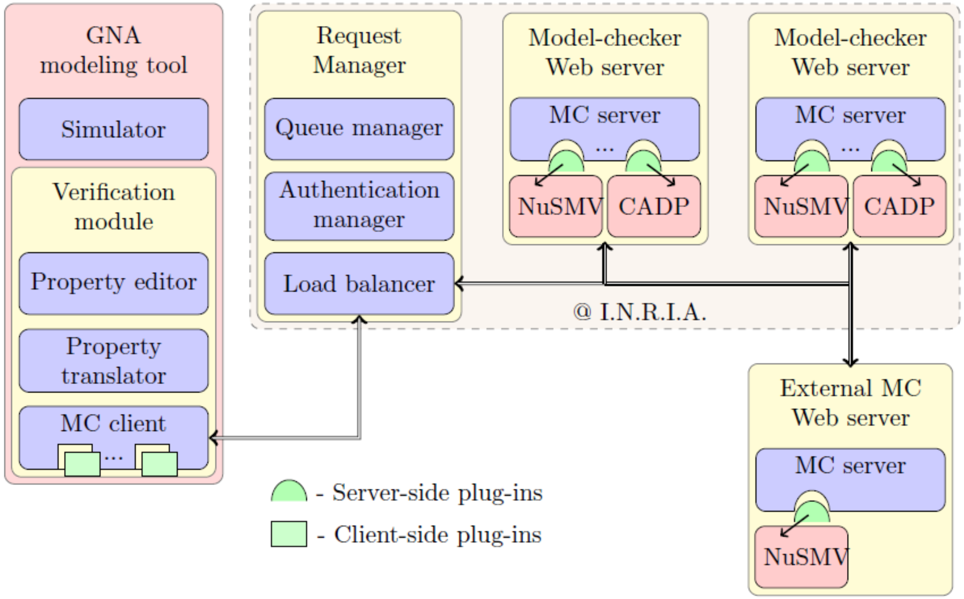
    Mateescu *et al.* (2011), *Theor. Comput. Sci.*, 412:2854-83

  - Develop temporal-logic patterns for frequently-asked modeling questions

    Monteiro *et al.* (2008), *Bioinformatics*, 24(16):i227-33

  - Connect GNA to standard model checkers through a web-server connection

    Monteiro *et al*., (2009), *BMC Bioinform.*, 10:450



Analysis of bacterial regulatory networks

- Applications of qualitative simulation in bacteria:
  - Initiation of sporulation in *Bacillus subtilis*

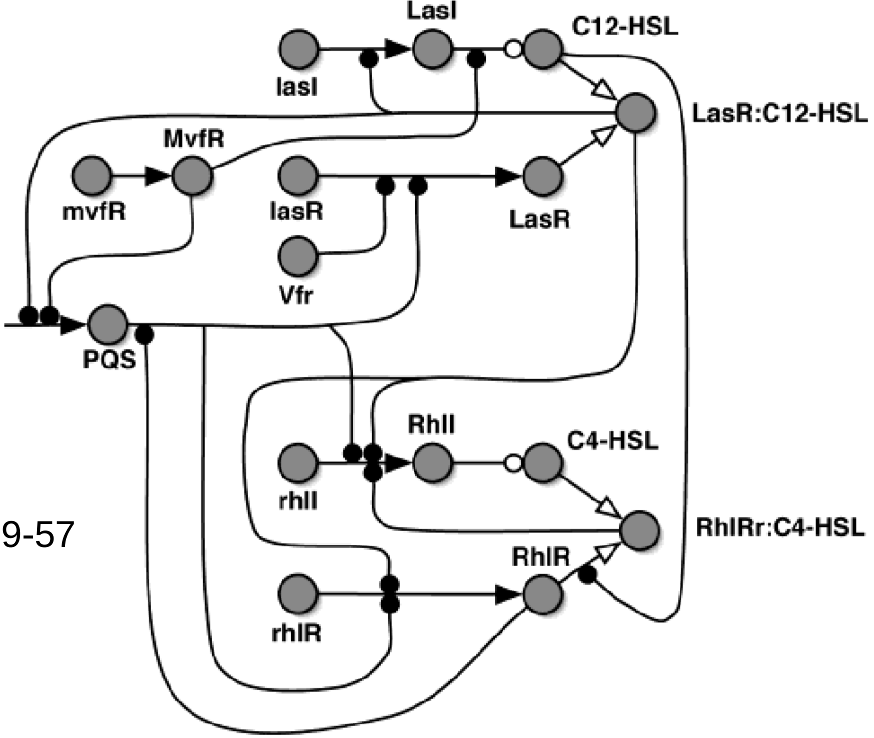
    de Jong, Geiselmann *et al.* (2004), *Bull. Math. Biol.*, 66(2):261-300

  - Quorum sensing in *Pseudomonas aeruginosa*

    Viretta and Fussenegger (2004), *Biotechnol. Prog.*, 20(3):670-8

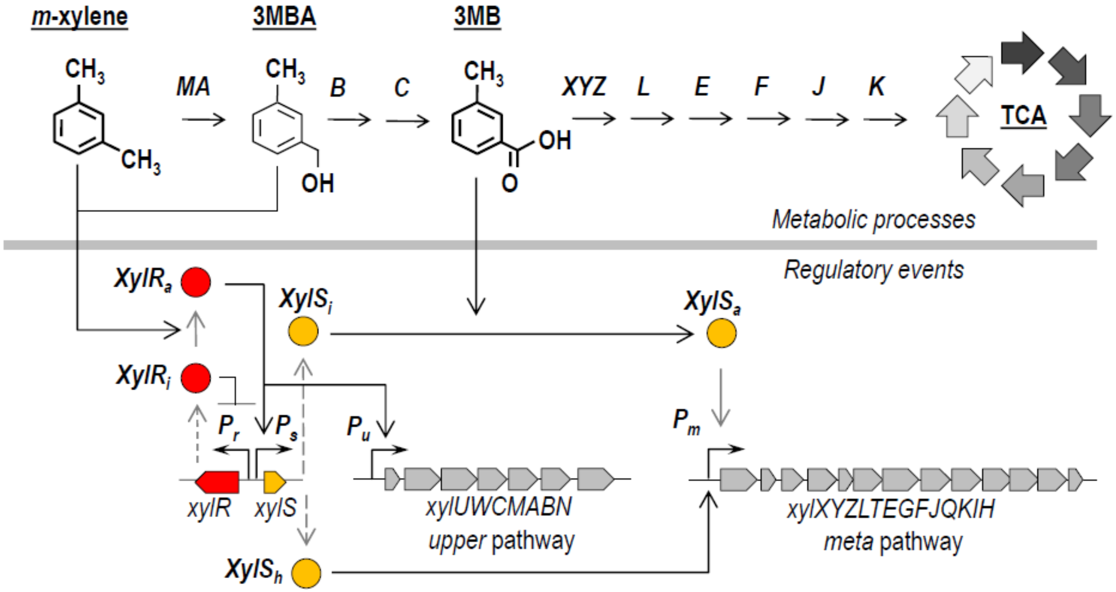
  - Onset of virulence in *Erwinia chrysanthemi*

    Sepulchre *et al.* (2007), *J. Theor. Biol.*, 244(2):239-57



Biodegradation of polluants by *P. putida*

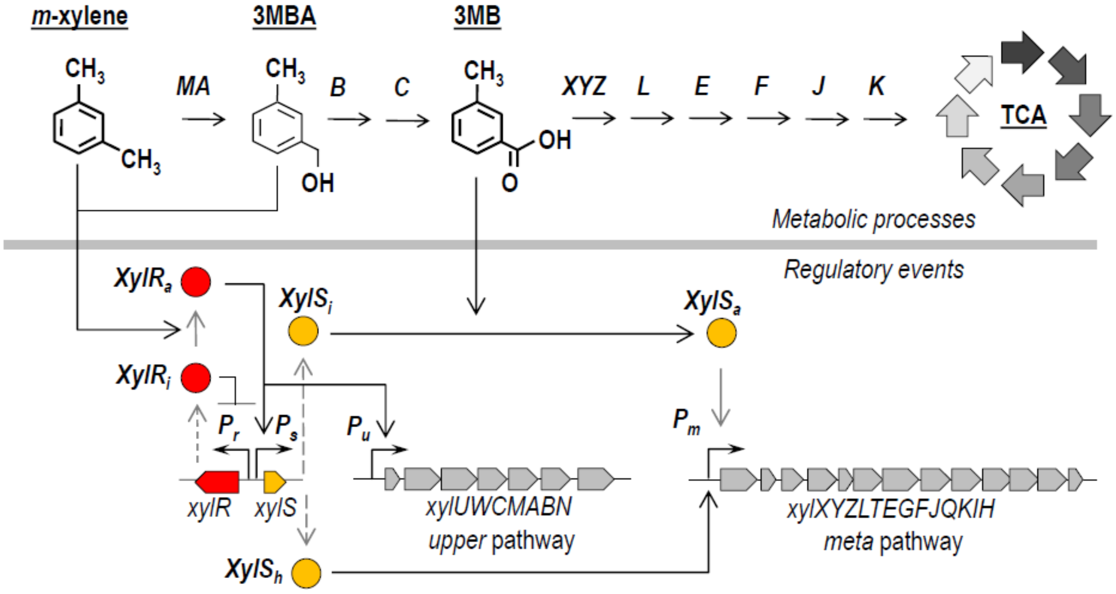
- Soil bacterium *Pseudomonas putida* mt-2 is archetypal model for environmental biodegradation of aromatic pollutants
    - TOL network involved in degradation of *m*-xylene to intermediates for central carbon metabolism



Rocha-Silva *et al.* (2011), *Environ. Microbiol.*, 13(9):2389-402

Role of regulators of TOL network

- **Question**: what is the role of the central, plasmid-encoded regulators XylR and XylS?

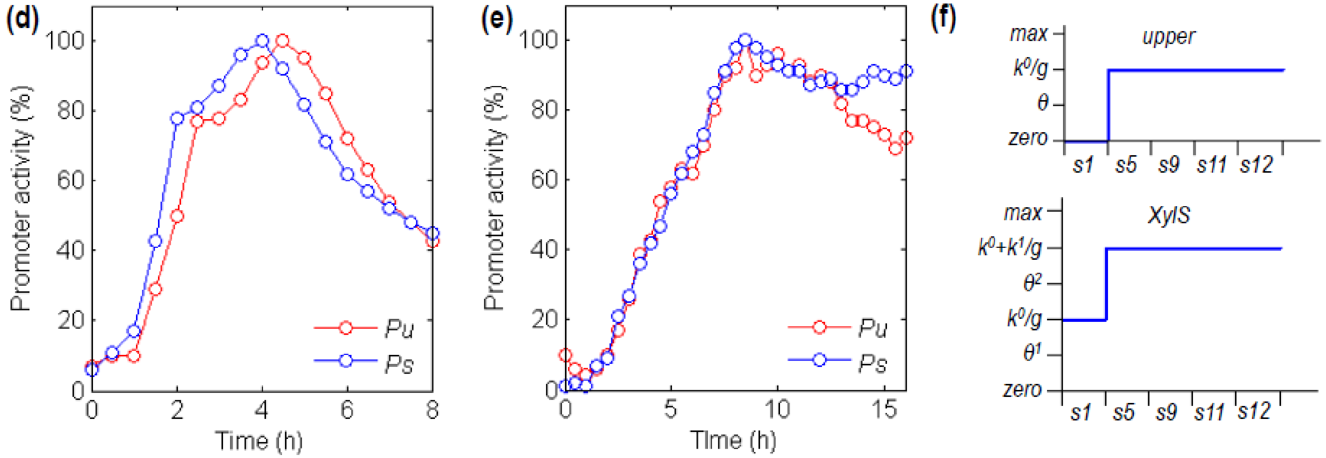


- Development of PL model of TOL network
  - Translation of network diagram into regulatory logic and PL model

Rocha-Silva *et al.* (2011), *BMC Syst. Biol.*, 5:191

Role of regulators of TOL network

- Validation of model by testing predictions under different perturbation conditions (mutants, metabolic inducers, …)

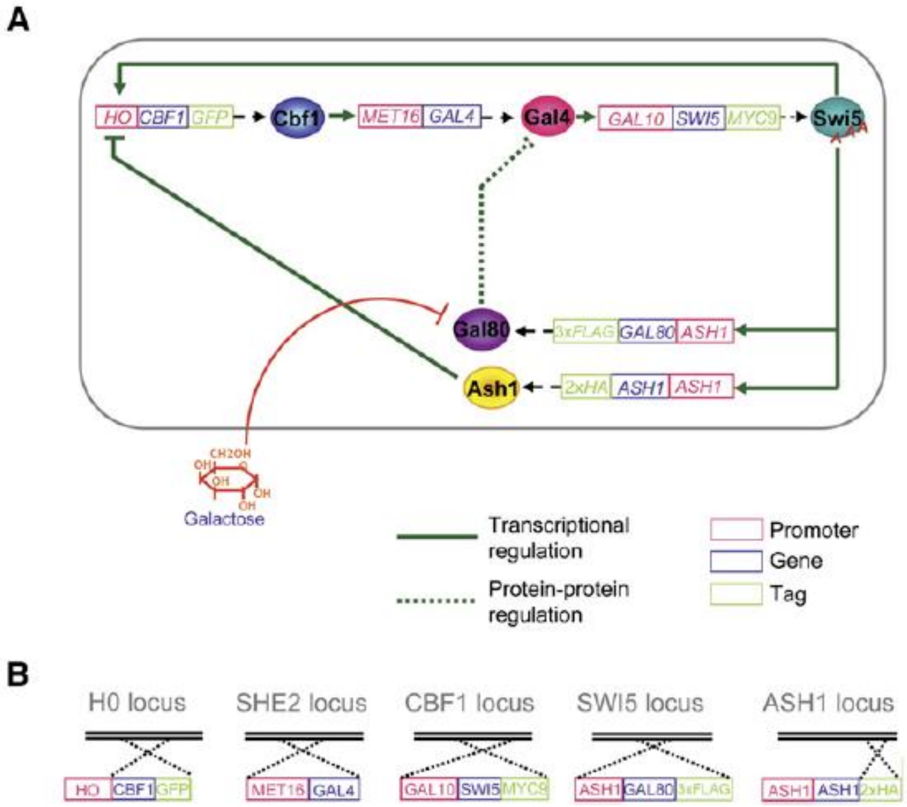


- Plasmid-encoded regulators of TOL network act as **regulatory firewall**
  - Prevent toxic *m*-xylene and its biodegradation intermediates from intervening with indigenous metabolic pathways

Rocha-Silva *et al.* (2011), *BMC Syst. Biol.*, 5:191

IRMA: synthetic network in yeast

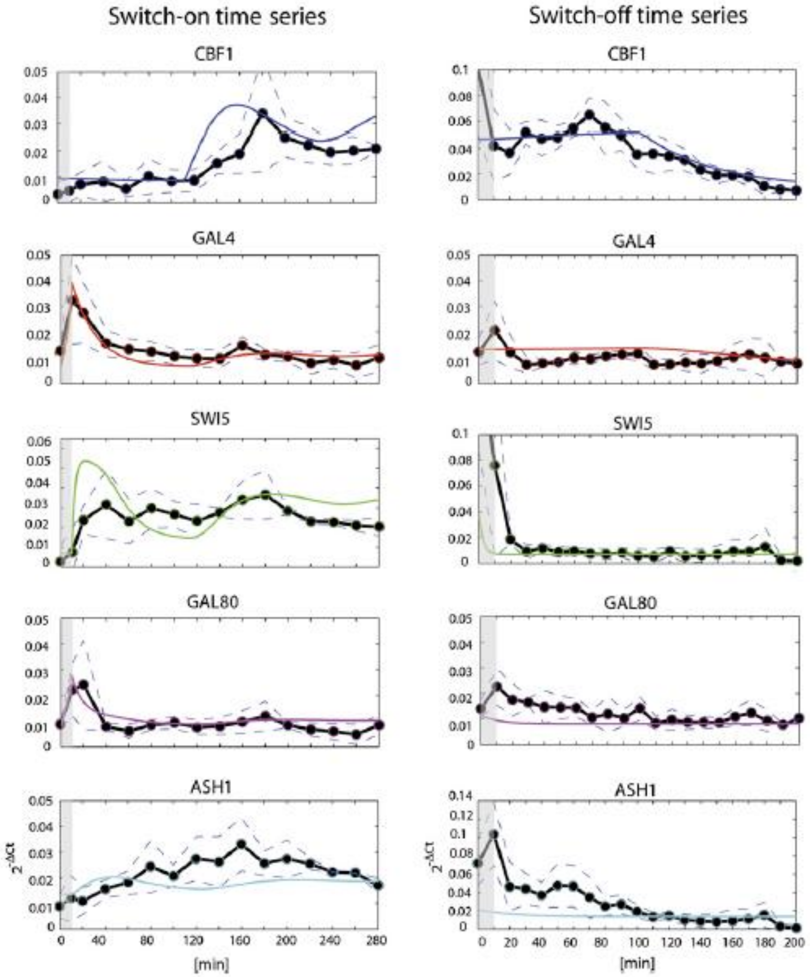
- **IRMA**: synthetic network in yeast consisting of interlocked positive and negative feedback loops
  - Networks functions independently from host cell
- Network can be externally controlled by growing cells in glucose or galactose



Cantone *et al.* (2009), *Cell*, 137(1):172-81

IRMA: synthetic network in yeast

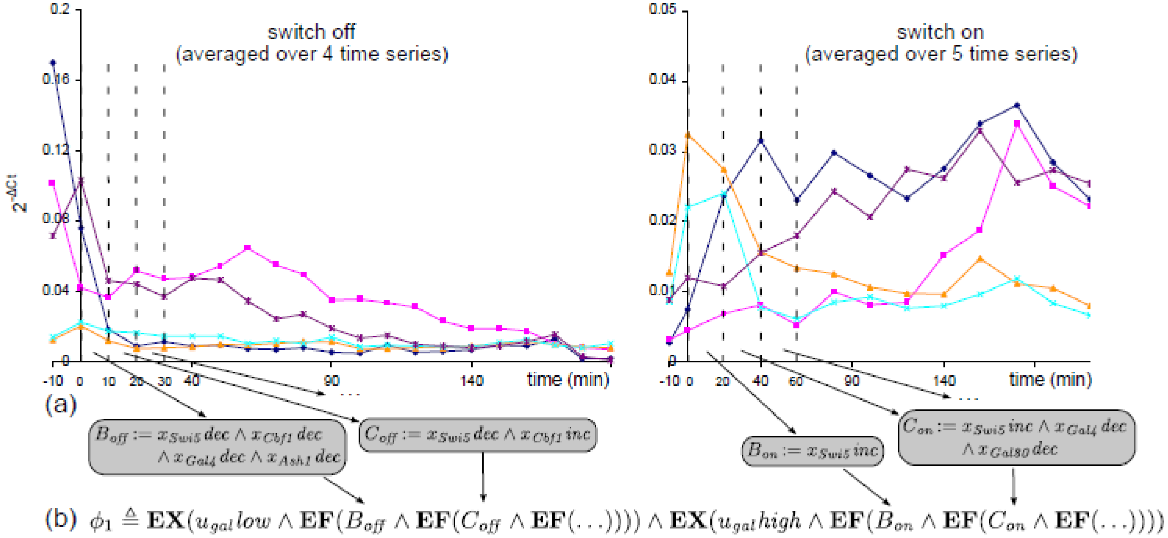
- IRMA proposed as a benchmark for modeling and identification approaches
- IRMA dynamics measured over time in galactose (switch-on) and glucose (switch-off)
  - Quantitative RT-PCR
- **Question**: are measured dynamics consistent with constructed network structure?



Cantone *et al.* (2009), *Cell*, 137(1):172-81

Test of consistency structure-dynamics

- Development of (unparametrized) PL model representing network structure
- Approach to test consistency between network structure and data based on automated parameter constraint search:
  - Generate temporal logic formulae encoding observed network dynamics



$B_{off} := x_{Swi5}\, dec \wedge x_{Cbf1}\, dec \wedge x_{Gal4}\, dec \wedge x_{Ash1}\, dec$

$C_{off} := x_{Swi5}\, dec \wedge x_{Cbf1}\, inc$

$B_{on} := x_{Swi5}\, inc$

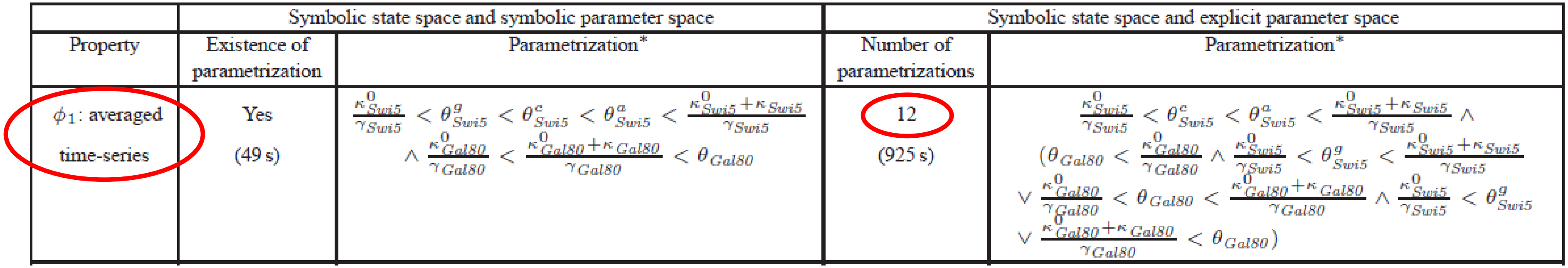
$C_{on} := x_{Swi5}\, inc \wedge x_{Gal4}\, dec \wedge x_{Gal80}\, dec$

(b) $\phi_1 \triangleq \mathbf{EX}(u_{gal}\, low \wedge \mathbf{EF}(B_{off} \wedge \mathbf{EF}(C_{off} \wedge \mathbf{EF}(\ldots)))) \wedge \mathbf{EX}(u_{gal}\, high \wedge \mathbf{EF}(B_{on} \wedge \mathbf{EF}(C_{on} \wedge \mathbf{EF}(\ldots))))$

Batt *et al.* (2010), *Bioinformatics*, 26(18):i603-10

Test of consistency structure-dynamics

- Development of (unparametrized) PL model representing network structure
- Approach to test consistency between network structure and data based on automated parameter constraint search:
  - Generate temporal logic formulae encoding observed network dynamics
  - Test if there are any parametrizations of PL model satisfy temporal logic formulae



	Symbolic state space and symbolic parameter space		Symbolic state space and explicit parameter space	
Property	Existence of parametrization	Parametrization*	Number of parametrizations	Parametrization*
ϕ_1: averaged time-series	Yes (49 s)	$\frac{\kappa^0_{Swi5}}{\gamma_{Swi5}} < \theta^g_{Swi5} < \theta^c_{Swi5} < \theta^a_{Swi5} < \frac{\kappa^0_{Swi5}+\kappa_{Swi5}}{\gamma_{Swi5}} \wedge \frac{\kappa^0_{Gal80}}{\gamma_{Gal80}} < \frac{\kappa^0_{Gal80}+\kappa_{Gal80}}{\gamma_{Gal80}} < \theta_{Gal80}$	12 (925 s)	$\frac{\kappa^0_{Swi5}}{\gamma_{Swi5}} < \theta^c_{Swi5} < \theta^a_{Swi5} < \frac{\kappa^0_{Swi5}+\kappa_{Swi5}}{\gamma_{Swi5}} \wedge$ $(\theta_{Gal80} < \frac{\kappa^0_{Gal80}}{\gamma_{Gal80}} \wedge \frac{\kappa^0_{Swi5}}{\gamma_{Swi5}} < \theta^g_{Swi5} < \frac{\kappa^0_{Swi5}+\kappa_{Swi5}}{\gamma_{Swi5}}$ $\vee \frac{\kappa^0_{Gal80}}{\gamma_{Gal80}} < \theta_{Gal80} < \frac{\kappa^0_{Gal80}+\kappa_{Gal80}}{\gamma_{Gal80}} \wedge \frac{\kappa^0_{Swi5}}{\gamma_{Swi5}} < \theta^g_{Swi5}$ $\vee \frac{\kappa^0_{Gal80}+\kappa_{Gal80}}{\gamma_{Gal80}} < \theta_{Gal80})$

* All parametrizations additionally include $\kappa^1_{Cbf1}/\gamma_{Cbf1} < \theta_{Cbf1} < (\kappa^1_{Cbf1} + \kappa^2_{Cbf1})/\gamma_{Cbf1} \wedge \kappa^0_{Gal4}/\gamma_{Gal4} < \theta_{Gal4} < (\kappa^0_{Gal4} + \kappa_{Gal4})/\gamma_{Gal4} \wedge \kappa^0_{Ash1}/\gamma_{Ash1} < \theta_{Ash1} < (\kappa^0_{Ash1} + \kappa_{Ash1})/\gamma_{Ash1}$.

Test of consistency structure-dynamics

- Development of (unparametrized) PL model representing network structure
- Approach to test consistency between network structure and data based on automated parameter constraint search:
  - Generate temporal logic formulae encoding observed network dynamics
  - Test if there are any parametrizations of PL model satisfy temporal logic formulae
  - Analyze parametrizations for biological plausibility

« Activation threshold of CBF1 by Swi5 higher than activation threshold of ASH1 »: confirmed by independent experimental data

Batt *et al.* (2010), *Bioinformatics*, 26(18):i603-10

Test of consistency structure-dynamics

- Development of (unparametrized) PL model representing network structure
- Approach to test consistency between network structure and data based on automated parameter constraint search:
  - Generate temporal logic formulae encoding observed network dynamics
  - Test if there are any parametrizations of PL model satisfy temporal logic formulae
  - Analyze parametrizations for biological plausibility
- Automated approach for testing consistency based on model-checking techniques

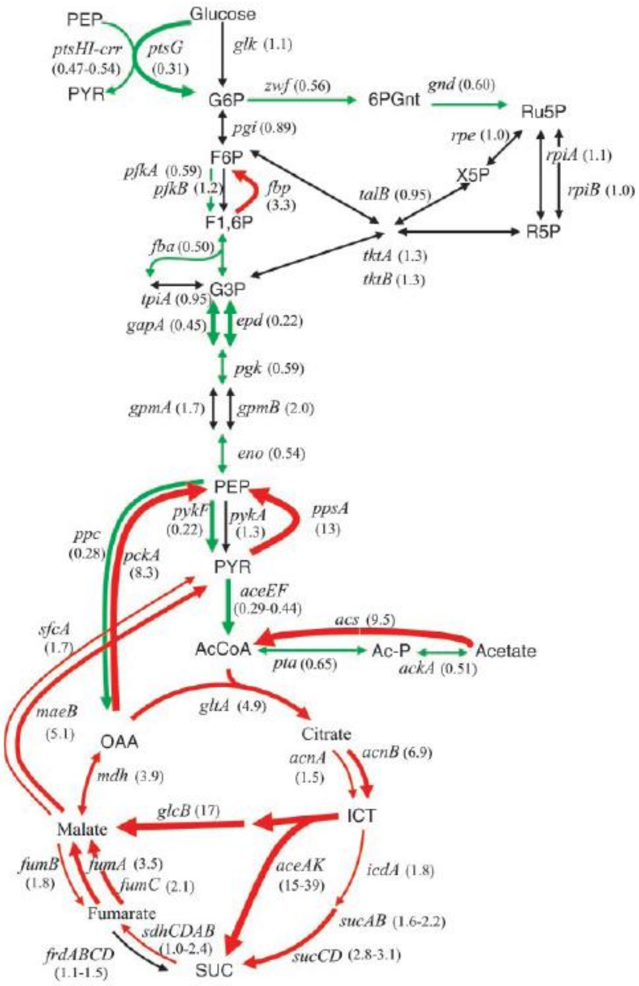
  Symbolic encoding of model, dynamics and properties to make problem feasible

Bacterial growth and adaptation

- 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

❖ **Question**: how does cell coordinate changes in enzyme concentrations (and other proteins)?



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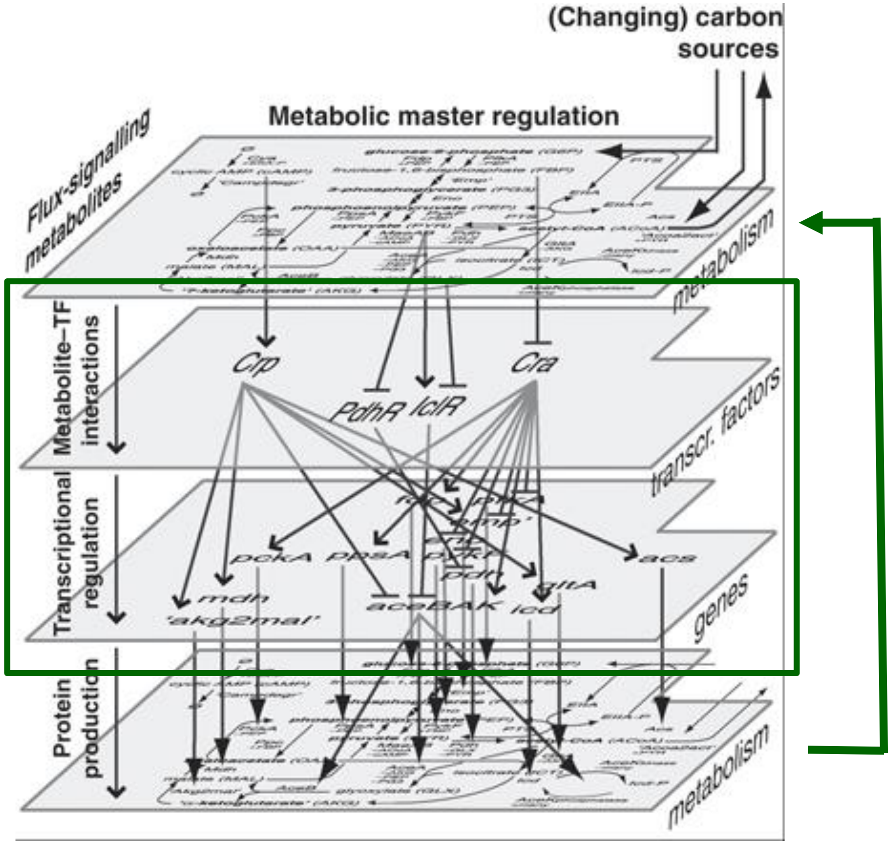
Broader view on gene regulatory networks

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

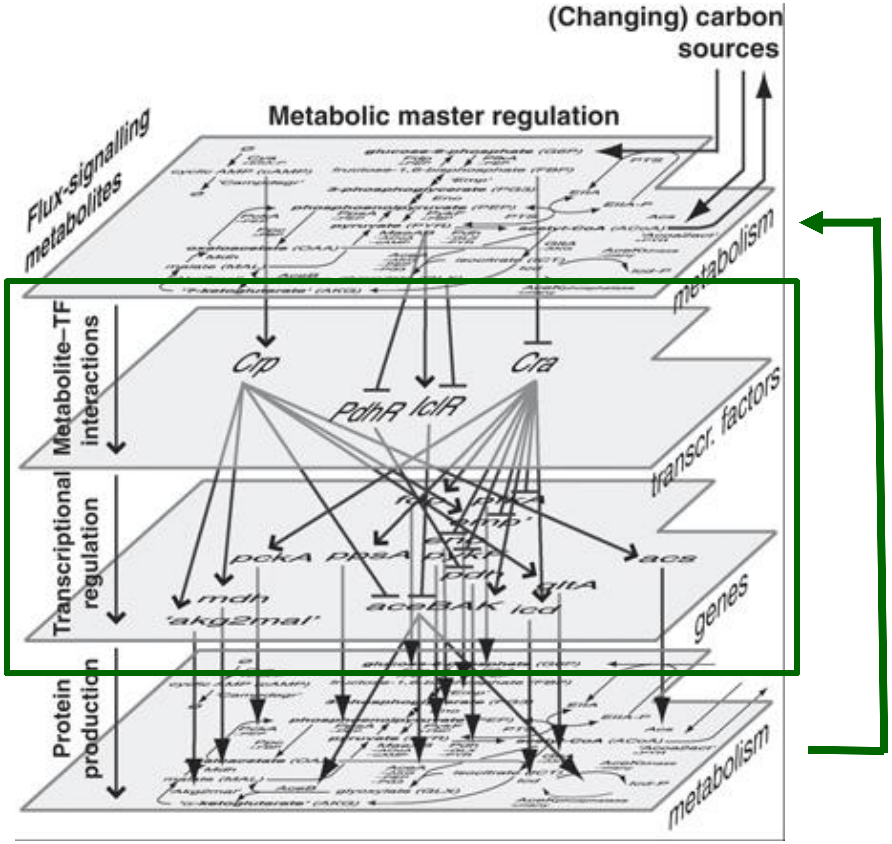


- **But**: adaptation of gene expression leads to changes in metabolism which feed back into regulatory network
- Gene regulatory networks are intertwined with metabolic and signaling networks

  Complex, heterogeneous systems evolving on different time-scales

Broader view on gene regulatory networks

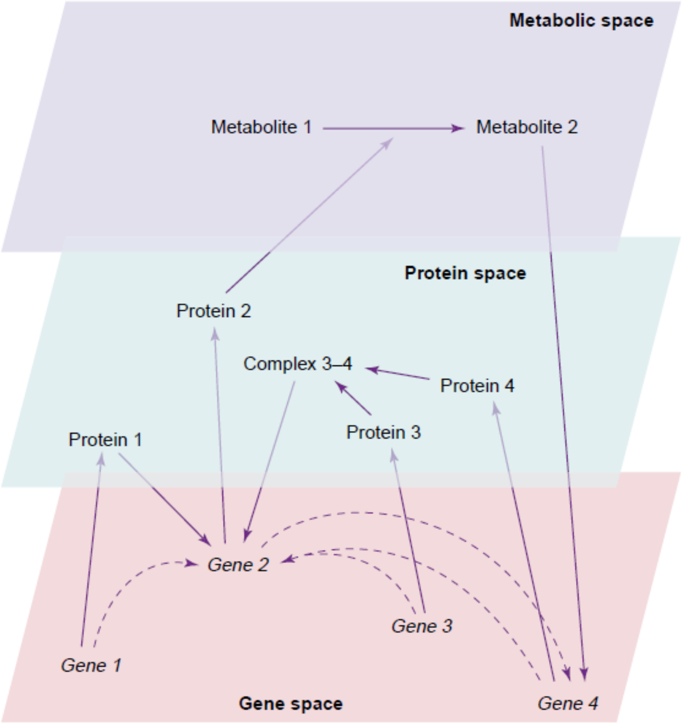
- Gene regulatory networks control changes in expression levels in response to environmental perturbations



- Feedback through metabolism leads to indirect regulatory interactions: metabolic coupling
  - Regulatory effects of enzymes on gene expression

Broader view on gene regulatory networks

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

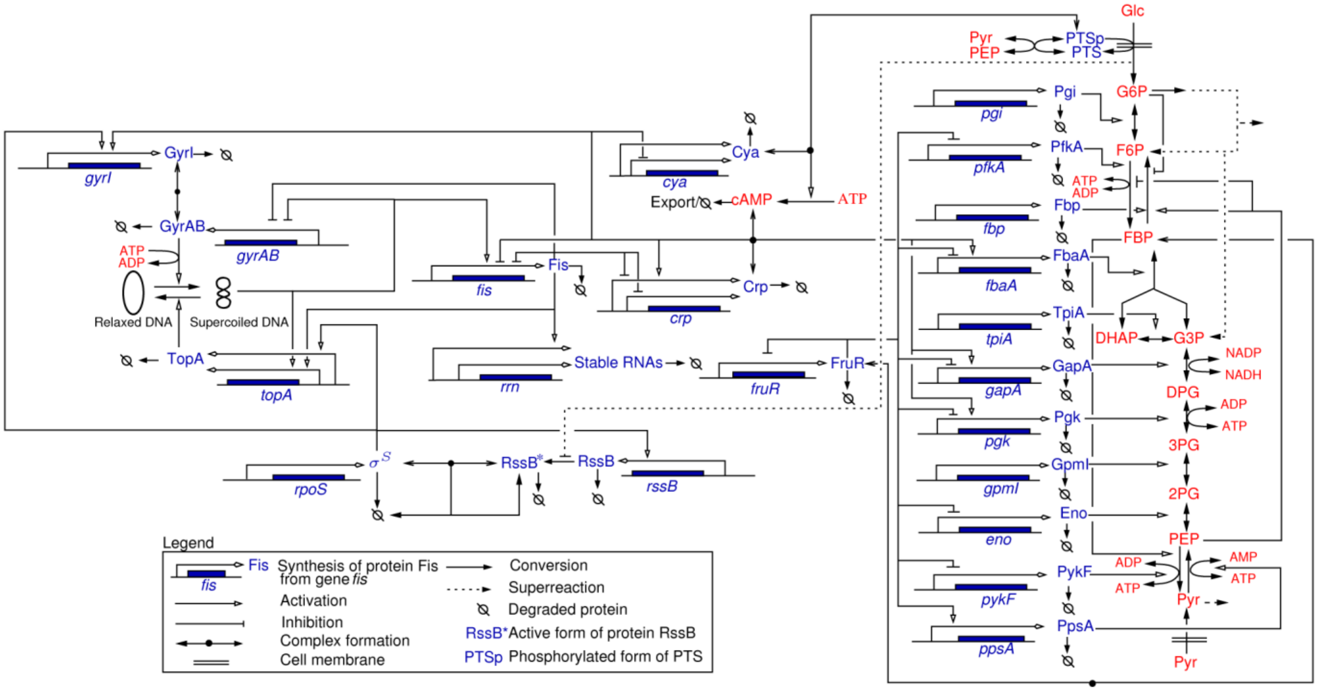


- Feedback through metabolism leads to indirect regulatory interactions: metabolic coupling
  - Regulatory effects of enzymes on gene expression

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

Analysis of metabolic coupling

- Complex regulatory network controlling response of *E. coli* to change of carbon source
  - Metabolism, signal transduction, gene expression



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

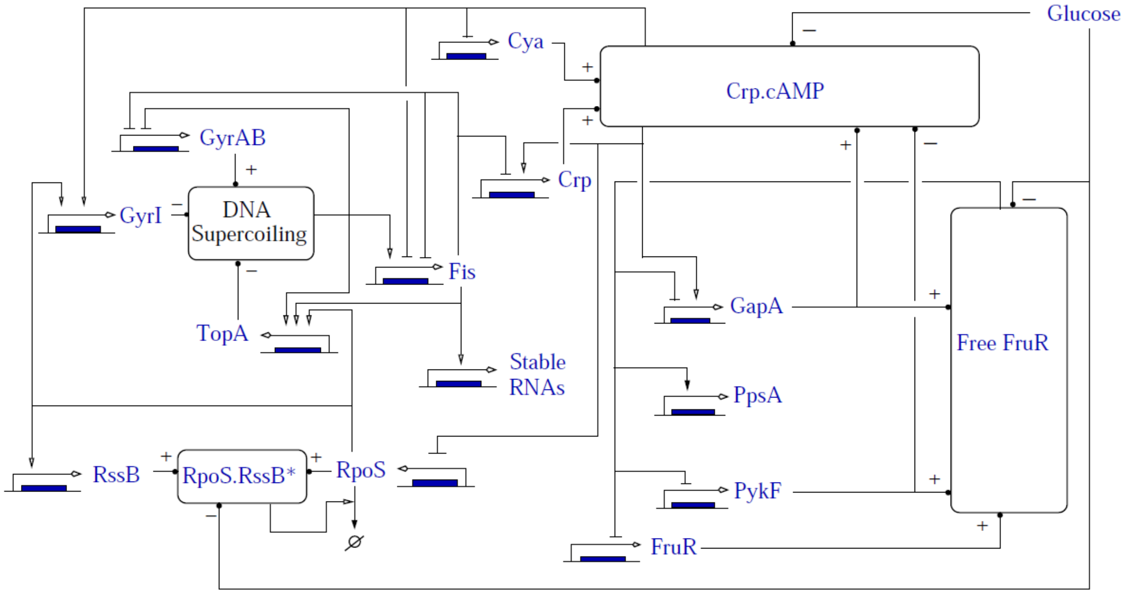
Analysis of metabolic coupling

- Derivation of gene regulatory network including indirect interactions due to metabolic coupling
- Approach based on reduction of stoichiometric model of system of biochemical reactions, making following weak assumptions:
  - Distinct time-scale hierarchies between metabolism and gene expression: model reduction using **quasi-steady-state approximation**
  - Stability of fast subsystem: use of **control** and **elasticity coefficients** from metabolic control analysis

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

Analysis of metabolic coupling

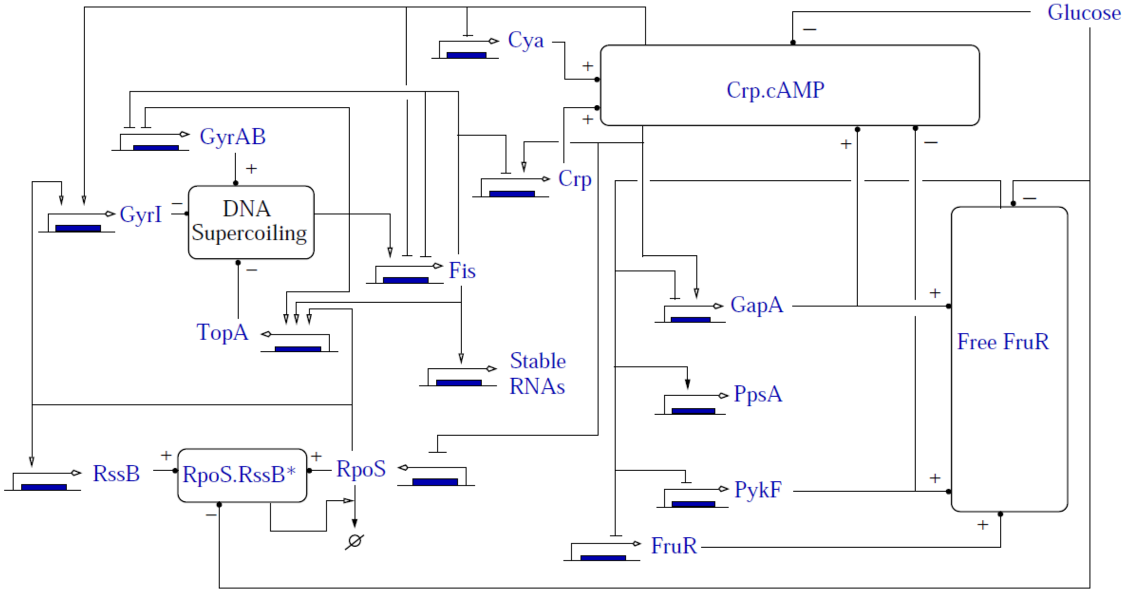
- Derivation of gene regulatory network including indirect interactions due to metabolic coupling



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

Formulation of PL models

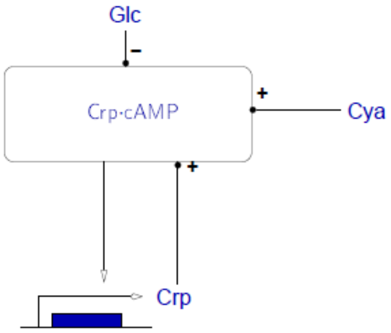
- Can PL models help understanding role of metabolic coupling in adjustment of gene expression during glucose-acetate shift?
- Translation of network diagram into PL models

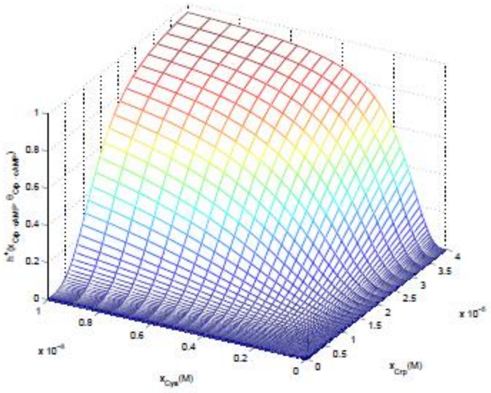


Baldazzi *et al.* (2012), *J. Theor. Biol.*, 295:100-15

Formulation of PL models

- Can PL models help understanding role of metabolic coupling in adjustment of gene expression during glucose-acetate shift?
- Translation of network diagram into PL models
  - Straightforward for direct interactions…
  - … but also possible for indirect interactions





$$v_1(x_{Crp\cdot cAMP}) = \kappa_{crp}\, h^+(x_{Crp\cdot cAMP}, \theta_{Crp\cdot cAMP}, n_1)$$

$$x_{Crp\cdot cAMP} = g(x_{Crp}, x_{Cya}, u_{Glc}) = \frac{h^-(u_{Glc}, \theta_{Glc}, n_2)\, x_{Cya}}{h^-(u_{Glc}, \theta_{Glc}, n_2)\, x_{Cya} + K}\, x_{Crp}$$

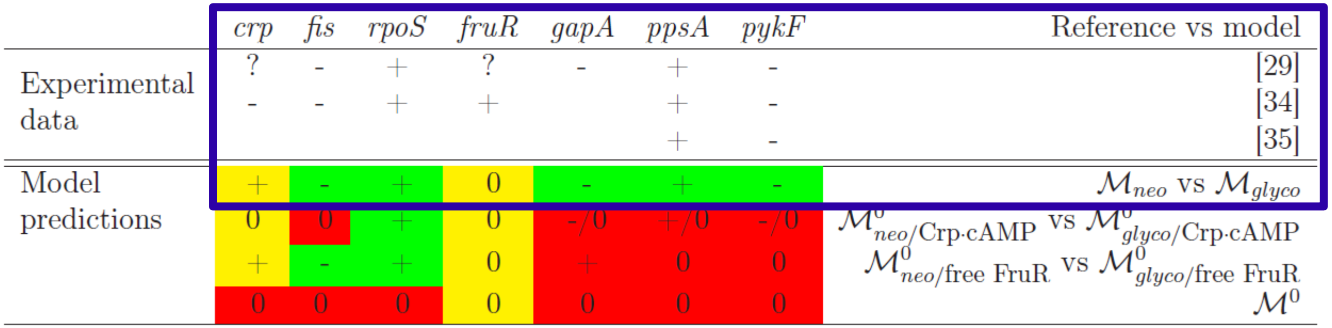
$$v_1(x_{Crp}, x_{Cya}, u_{Glc}) = \kappa_{crp}\, h^-(u_{Glc}, \theta_{Glc}, n_2)\, h^+(x_{Crp}, \theta_{Crp}, n_3)\, h^+(x_{Cya}, \theta_{Cya}, n_4)$$

Baldazzi *et al.* (2012), *J. Theor. Biol.*, 295:100-15

Dynamic analysis of metabolic coupling

- Can PL models help understanding role of metabolic coupling in adjustment of gene expression during glucose-acetate shift?
- Comparison of model predictions with published data sets: indirect interactions induced by metabolic coupling are **essential for reproducing gene expression dynamics**

  Steady-state mRNA concentration levels and initial transcriptional response of metabolic and regulatory genes



	crp	*fis*	*rpoS*	*fruR*	*gapA*	*ppsA*	*pykF*	Reference vs model
Experimental data	?	-	+	?	-	+	-	[29]
	-	-	+	+		+	-	[34]
						+	-	[35]
Model predictions	+	-	+	0	-	+	-	$\mathcal{M}_{neo}$ vs $\mathcal{M}_{glyco}$
	0	0	+	0	-/0	+/0	-/0	$\mathcal{M}^0_{neo/\text{Crp·cAMP}}$ vs $\mathcal{M}^0_{glyco/\text{Crp·cAMP}}$
	+	-	+	0	+	0	0	$\mathcal{M}^0_{neo/\text{free FruR}}$ vs $\mathcal{M}^0_{glyco/\text{free FruR}}$
	0	0	0	0	0	0	0	$\mathcal{M}^0$

Baldazzi *et al.* (2012), *J. Theor. Biol.*, 295:100-15

Conclusions

- Modeling of genetic regulatory networks in bacteria often hampered by lack of information on parameter values
- Use of coarse-grained PL models that provide reasonable approximation of dynamics
- Mathematical methods and computer tools for analysis of qualitative dynamics of PL models
  - Weak information on parameter values (inequality constraints)
- Use of PL models may gain insight into functioning of large and complex networks
- PL models provide first idea of qualitative dynamics that may guide modeling by means of quantitative models

Contributors and sponsors

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