Modularity of biological regulation: How can we model the interplay between gene regulation and metabolic control?

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Outline

1st hour:

- Introduction on biological regulation
- Elements of Metabolic Control Theory
- Treatment of modularity in Metabolic Control Theory

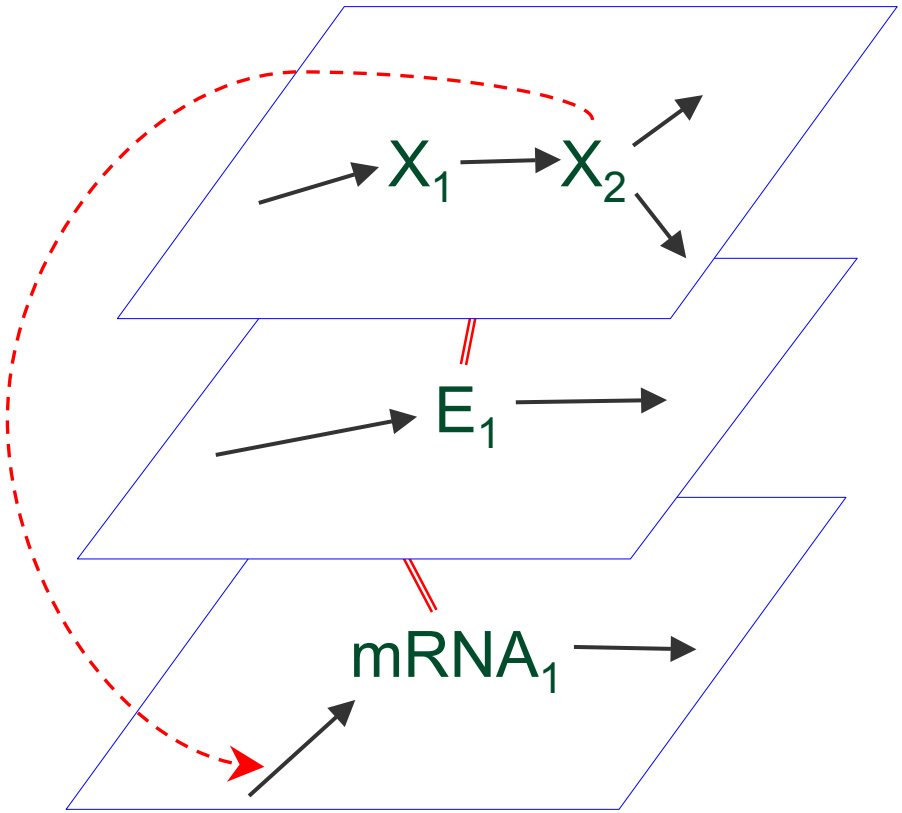
2nd hour:

- Approaches for studying the interplay between gene regulation and metabolic regulation
- The MetaGenoReg project

General question of biological regulation

- Cellular regulation involves several levels, including:
  - Gene regulatory networks
  - Metabolic regulation
- These levels interact:
  - Gene expression impacts metabolism through changes in enzyme concentrations
  - Conversely metabolism influences gene expression
- What is the rationale articulating both types of regulation?
  - Are they interchangeable ?
  - How much are they constrained?
  - What is the relative importance of gene and metabolic regulation?

‘Hierarchical’ analysis



module 1
metabolism

module 2
protein level

module 3
RNA level

Example of modular regulation #1

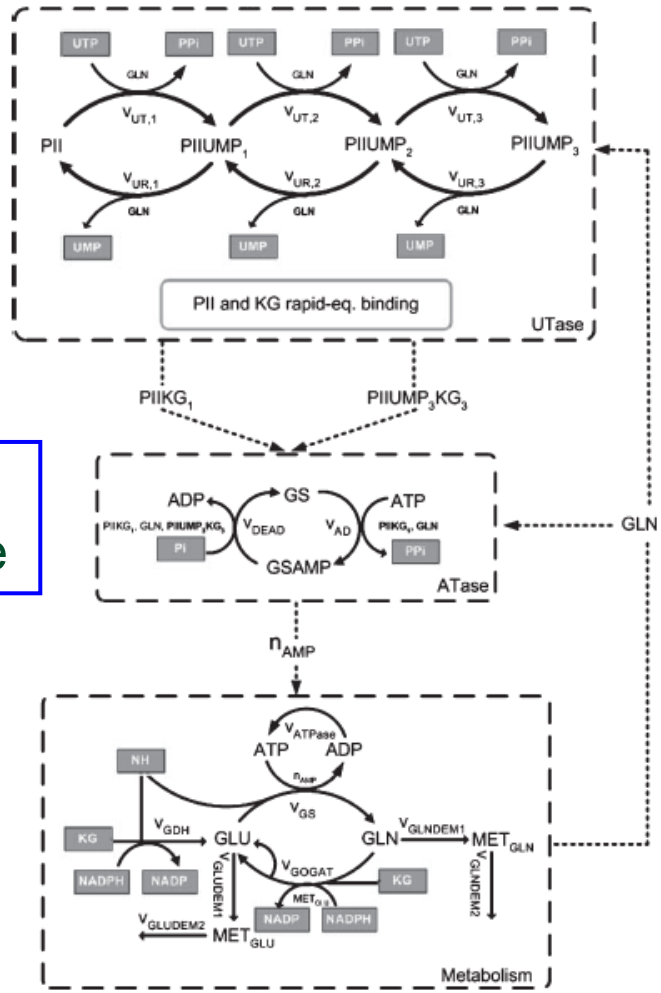
Cascade regulating nitrogen assimilation in *E. coli*

Bruggeman *et al*. (2005) *FEBS J.* 272, 1965

P_{II} interconversion

Adenylylation of glutamine synthetase

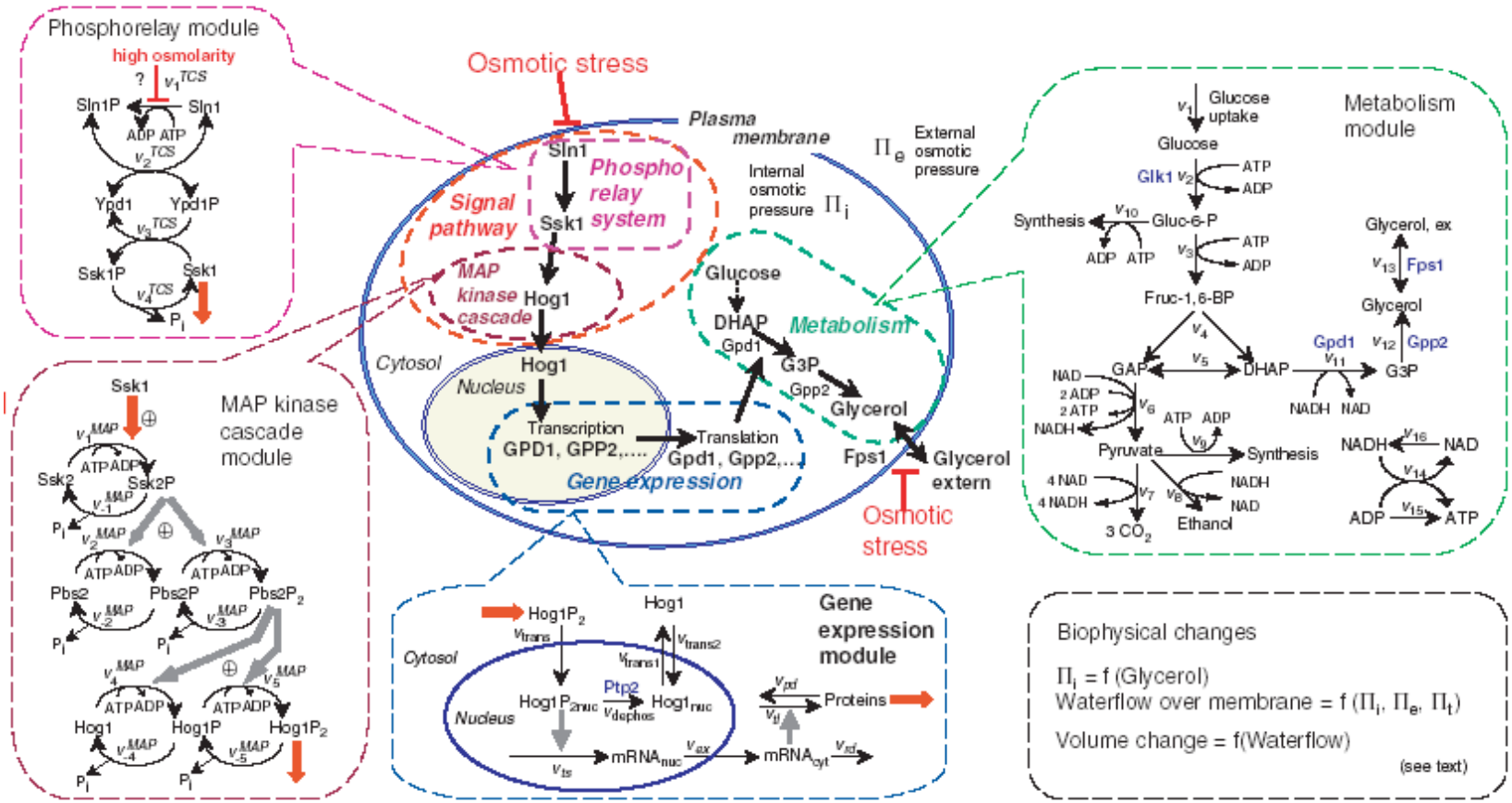
Glutamate and glutamine synthesis



Example of modular regulation #2

Yeast osmoregulation

Klipp *et al.* (2005) *Nature Biotech* 23,975-82



1. Elements of Metabolic Control Theory

It is possible to derive a very general treatment of metabolic control theory for metabolic systems of arbitrary complexity.
C. Reder (1988) *J. Theoret. Biol.* 135:175-201

General definitions:

$\mathbf{x} = \mathbf{x}(t,\mathbf{p})$ Molarity vector

$\mathbf{X} = \mathbf{X}(\mathbf{p})$ *Steady-state* molarity vector: $d\mathbf{x} / dt = 0$

$\mathbf{v} = \mathbf{v}(\mathbf{x},\mathbf{p})$ Rate vector

$\mathbf{J} = \mathbf{J}(\mathbf{p})$ *Steady-state* flux vector

$= \mathbf{v}[\mathbf{X}(\mathbf{p}),\mathbf{p}]$

System definition

Metabolic Control Theory addresses observable steady-states of metabolism. Therefore:

- The system must be open in order to reach a non-trivial steady-state (*i.e.*, with non-zero fluxes)
- The steady-state must be stable
- Most reactions should be sensitive to both substrate and product concentrations, allowing for the balancing of metabolite production and consumption rates

Control matrices

Concentration control matrix Γ :

$$d\mathbf{X} / d\mathbf{p} = \Gamma \, d\mathbf{v}/d\mathbf{p}$$

$\mathbf{p}$ being a parameter vector affecting the rates $\mathbf{v}$.

Flux control matrix Φ :

$$d\mathbf{J} / d\mathbf{p} = \Phi \, d\mathbf{v}/d\mathbf{p}$$

Control coefficients reflect the sensitivity of the system to changes in reaction rates.

The stoichiometry matrix

- Reactions in the network are expressed in the *stoichiometry matrix* $\mathbf{N}$, whose columns contain the stoichiometric coefficients for each reaction
- This matrix reflects the system's structure
- The stoichiometry matrix $\mathbf{N}$ is of maximal rank if and only if there is no conservation relationship constraining the different concentrations, which we will assume here for simplicity

Example: C metabolism in *E. coli*

Example taken from JWS online at http://jjj.biochem.sun.ac.za/

Olivier & Snoep (2004) *Bioinformatics* 20, 2143

Model of central carbon metabolism by:

Chassagnole *et al.* (2002) *Biotech. Bioeng.* 79, 53-73

System evolution

The evolution of the system's concentration vector $\mathbf{x}$ is a simple function of the reaction rate vector $\mathbf{v}$:

$$d\mathbf{x} / dt = \mathbf{N} \cdot \mathbf{v}(\mathbf{x},\mathbf{p})$$

where $\mathbf{p}$ is a parameter vector, and the Jacobian is :

$$\mathfrak{J} = \mathbf{N} \cdot d\mathbf{v}/d\mathbf{x}$$

dv_i/dx_j are non-normalized 'elasticities'.

Steady-state flux constraints

- We are interested in analysing the steady-state of the system:

$$\mathrm{d}\mathbf{x} / \mathrm{d}t = \mathbf{N} \cdot \mathbf{v}(\mathbf{X},\mathbf{p}) = \mathbf{0}$$

  where $\mathbf{X}$ is the vector of steady-state concentrations

- The steady-state introduces linear dependencies between fluxes:

$$\mathbf{N} \cdot \mathbf{J}(\mathbf{p}) = \mathbf{0}$$

- Therefore the flux vector $\mathbf{J}$ can be expressed in a basis of $\mathrm{Ker}(\mathbf{N})$ (often termed $\mathbf{K}$)

Expressing systemic control

Differentiating the steady-state equation with respect to $\mathbf{p}$:

$$\mathbf{N} \cdot d\mathbf{v}/d\mathbf{X} \cdot d\mathbf{X}/d\mathbf{p} + \mathbf{N} \cdot d\mathbf{v}/d\mathbf{p} = \mathbf{0}$$

$$d\mathbf{X}/d\mathbf{p} = - \mathfrak{J}^{-1} \cdot \mathbf{N} \cdot d\mathbf{v}/d\mathbf{p}$$

- This equation relates systemic changes in steady-state concentrations $\mathbf{X}$ to changes in rates $\mathbf{v}$.
- The matrix $\Gamma = - \mathfrak{J}^{-1} \cdot \mathbf{N}$ contains all concentration control coefficients.

Flux control

- Let us calculate the resulting steady-state flux:

$$\mathbf{J} = \mathbf{v}(\mathbf{X},\mathbf{p})$$

and differentiate it with respect to $\mathbf{p}$:

$$d\mathbf{J}/d\mathbf{p} = d\mathbf{v}/d\mathbf{x} \cdot d\mathbf{X}/d\mathbf{p} + d\mathbf{v}/d\mathbf{p}$$
$$= (d\mathbf{v}/d\mathbf{x} \cdot \Gamma + \mathbf{I}) \cdot d\mathbf{v}/d\mathbf{p}$$

- This equation relates systemic changes in steady-state fluxes $\mathbf{J}$ to changes in rates $\mathbf{v}$.
- The matrix $\Phi = \mathbf{I} + d\mathbf{v}/d\mathbf{x} \cdot \Gamma$ contains all flux control coefficients.

2. Modular Analysis in MCT

Kahn & Westerhoff (1991) *J. Theoret. Biol.* 153:255-285

- Block matrix decomposition
- The absence of bridging reactions between the modules makes the stoichiometry matrix block-diagonal

$$\mathbf{N} = \begin{pmatrix} \mathbf{N}_1 & & \mathbf{0} \\ & \mathbf{N}_2 & \\ \mathbf{0} & & \mathbf{N}_3 \end{pmatrix}$$

- This allows to explicitly calculate the effect of intermodule interactions upon global control
- For instance one can calculate the effect of metabolic feed-back upon the sensitivity ('control') of gene expression

Two-module control analysis

- We study MODULE 1 with all concentrations of MODULE 2 ***clamped*** at their values attained in the global steady-state and study the steady-state of MODULE 1 (assumed to exist), with respect to the rates of the entire system and obtain INTRINSIC control matrices Γ_1 and Φ_1.
- Similarly we study MODULE 2 and obtain the control matrices Γ_2 and Φ_2
- The problem we wish to solve is :
  How can we calculate the control matrices of the entire system as a function of intrinsic control matrices of modules 1 and 2 ?

Quantifying feed-back

$$\Gamma_{11}^{*} = (\mathbf{I} - \Gamma_1 \, d\mathbf{v}_1/d\mathbf{x}_2 \, \Gamma_2 \, d\mathbf{v}_2/d\mathbf{x}_1)^{-1} \; \Gamma_1$$

$$\Gamma_{12}^{*} = (\mathbf{I} - \Gamma_1 \, d\mathbf{v}_1/d\mathbf{x}_2 \, \Gamma_2 \, d\mathbf{v}_2/d\mathbf{x}_1)^{-1} \; \Gamma_1 \, d\mathbf{v}_1/d\mathbf{x}_2 \, \Gamma_2$$

$$\Gamma_{21}^{*} = (\mathbf{I} - \Gamma_2 \, d\mathbf{v}_2/d\mathbf{x}_1 \, \Gamma_1 \, d\mathbf{v}_1/d\mathbf{x}_2)^{-1} \; \Gamma_2 \, d\mathbf{v}_2/d\mathbf{x}_1 \, \Gamma_1$$

$$\Gamma_{21}^{*} = (\mathbf{I} - \Gamma_2 \, d\mathbf{v}_2/d\mathbf{x}_1 \, \Gamma_1 \, d\mathbf{v}_1/d\mathbf{x}_2)^{-1} \; \Gamma_2$$

These relations show how feed-back affects control non-linearly, both within a module and between modules.

The 'strength' of the feed-back is embodied in the *cyclic regulatory matrices* :

$$\Gamma_i \, d\mathbf{v}_i/d\mathbf{x}_j \, \Gamma_j \, d\mathbf{v}_j/d\mathbf{x}_i$$

MCT and regulation analysis

MCT is a first order theory:

- Sensitivities are defined locally

  → this provides only for local behaviour
  in the vicinity of a steady-state

- MCT is not well adapted to abrupt transitions
- Rigorously it cannot treat transients
  between remote steady-states
- Strong non-linearities in $\mathbf{v}(\mathbf{x},\mathbf{p})$ must be captured differently
- Therefore one needs a different approach to take into account strongly cooperative effects as found commonly in gene activation

Second hour

- Approaches for studying the interplay between gene regulation and metabolic regulation
- The MetaGenoReg project

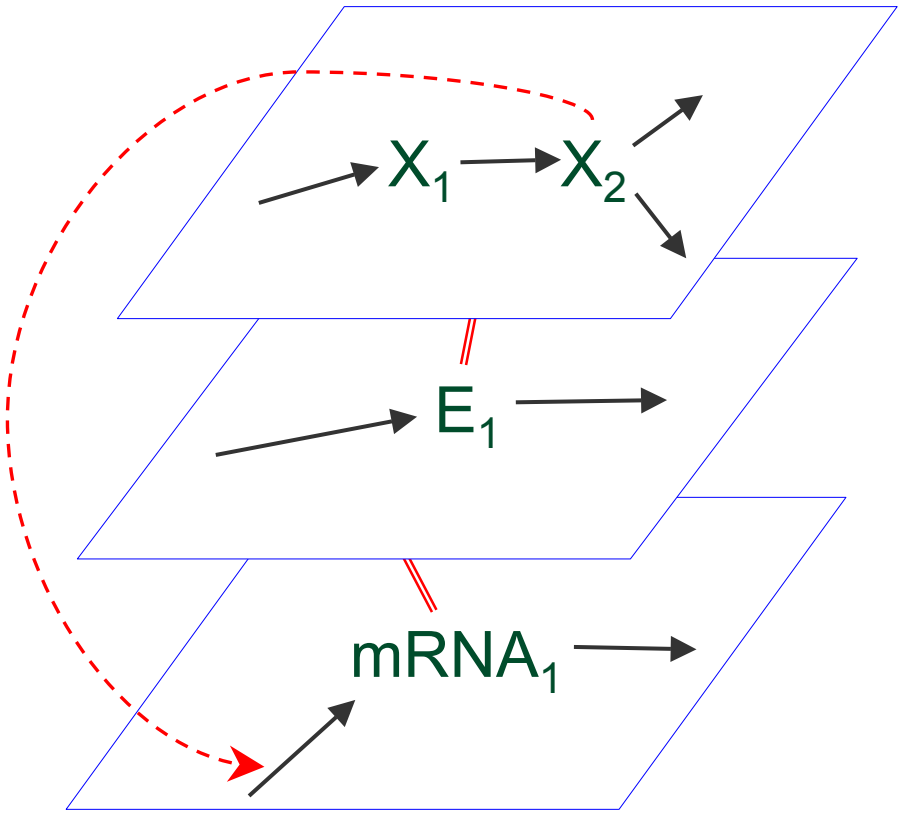
3.Integrating gene regulation & metabolism

How can we dissect the relative contributions of gene regulation and metabolic control in a biological response?

a. Measurement of regulation coefficients according to Rossell *et al.* (2006), *PNAS* 103:2166-2171

b. The MetaGenoReg project

‘Hierarchical’ analysis



module 1
metabolism

module 2
protein level

module 3
RNA level

Unraveling the complexity of flux regulation: A new method demonstrated for nutrient starvation in *Saccharomyces cerevisiae*

Rossell *et al.* (2006), *PNAS* 103:2166-2171

$$v = v(e, \mathbf{X}, \mathbf{K}) = f(\mathrm{e}) \cdot g(\mathbf{X}, \mathbf{K}).$$

$$1 = \frac{\Delta \log f(\mathrm{e})}{\Delta \log J} + \frac{\Delta \log g(\mathbf{X}, \mathbf{K})}{\Delta \log J} = \rho_h + \rho_m.$$

‘hierarchical’ ~ metabolic regulation coefficients

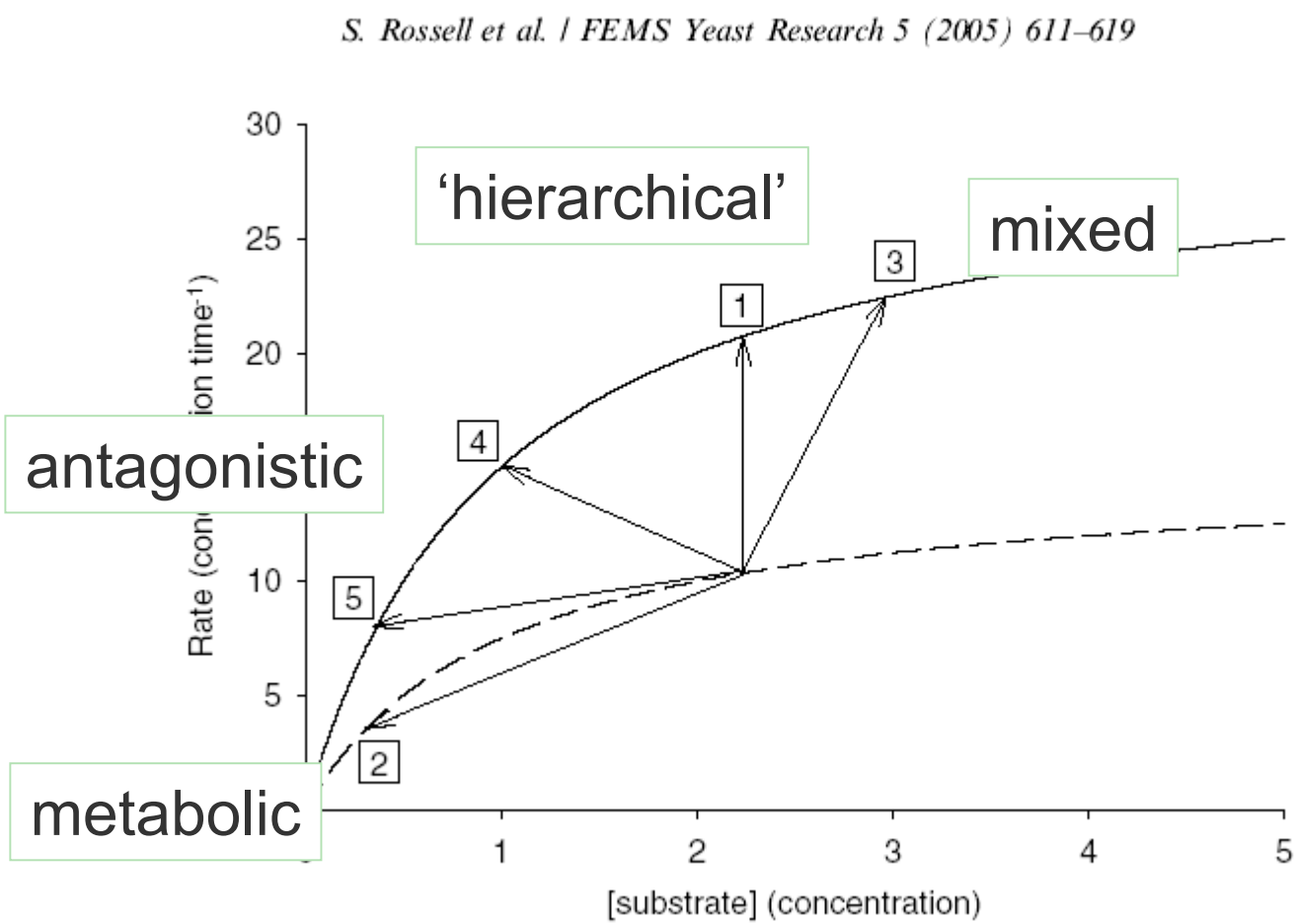
Experimental setup

- Yeast grown in synthetic medium + glucose
- N or C starvation for 24 h
- Fluxes measured over 30 min in the presence of glucose
  → $\Delta \log J$
- Enzyme assays
  → $\Delta \log E$

$$\rho_h = \frac{\Delta \log E}{\Delta \log J}$$

$$\rho_m = 1 - \rho_h$$

Different types of regulation



Mainly gene regulation

	Nitrogen starvation			Carbon starvation		
Enzyme	ρ_h	SEM	ρ_m	ρ_h	SEM	ρ_m
GLT	1.2	0.1	−0.2	0.4	0.1	0.6
HK	1.0	0.2	0.0	0.1	0.0	0.9
PGI	0.8	0.3	0.2	0.0	0.0	1.0
PFK	0.4	0.2	0.6	0.4	0.4	0.6
ALD	1.1	0.5	−0.1	0.0	0.2	1.0
TPI	0.1	0.9	0.9	−0.4	0.2	1.4
GAPDH	0.7	0.5	0.3	0.1	0.0	0.9
PGK	0.0	0.2	1.0	−0.3	0.1	1.3
PGM	1.0	0.4	0.0	0.0	0.0	1.0
ENO	0.4	0.5	0.6	0.3	0.1	0.7
PK	1.4	0.3	−0.4	0.1	0.0	0.9
PDC	2.3	0.6	−1.3	0.1	0.0	0.9
ADH	1.7	0.4	−0.7	−1.3	0.2	2.3

PDC, pyruvate decarboxylase; PFK, 6-phosphofructokinase; PGI, glucose-6-phosphate isomerase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; PK, pyruvate kinase; TPI, triose-phosphate isomerase.

Mainly metabolic regulation

	Nitrogen starvation			Carbon starvation		
Enzyme	ρ_h	SEM	ρ_m	ρ_h	SEM	ρ_m
GLT	1.2	0.1	−0.2	0.4	0.1	0.6
HK	1.0	0.2	0.0	0.1	0.0	0.9
PGI	0.8	0.3	0.2	0.0	0.0	1.0
PFK	0.4	0.2	0.6	0.4	0.4	0.6
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Mixed regulation

	Nitrogen starvation			Carbon starvation		
Enzyme	ρ_h	SEM	ρ_m	ρ_h	SEM	ρ_m
GLT	1.2	0.1	−0.2	0.4	0.1	0.6
HK	1.0	0.2	0.0	0.1	0.0	0.9
PGI	0.8	0.3	0.2	0.0	0.0	1.0
PFK	0.4	0.2	0.6	0.4	0.4	0.6
ALD	1.1	0.5	−0.1	0.0	0.2	1.0
TPI	0.1	0.9	0.9	−0.4	0.2	1.4
GAPDH	0.7	0.5	0.3	0.1	0.0	0.9
PGK	0.0	0.2	1.0	−0.3	0.1	1.3
PGM	1.0	0.4	0.0	0.0	0.0	1.0
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Antagonistic regulation

	Nitrogen starvation			Carbon starvation		
Enzyme	ρ_h	SEM	ρ_m	ρ_h	SEM	ρ_m
GLT	1.2	0.1	−0.2	0.4	0.1	0.6
HK	1.0	0.2	0.0	0.1	0.0	0.9
PGI	0.8	0.3	0.2	0.0	0.0	1.0
PFK	0.4	0.2	0.6	0.4	0.4	0.6
ALD	1.1	0.5	−0.1	0.0	0.2	1.0
TPI	0.1	0.9	0.9	−0.4	0.2	1.4
GAPDH	0.7	0.5	0.3	0.1	0.0	0.9
PGK	0.0	0.2	1.0	−0.3	0.1	1.3
PGM	1.0	0.4	0.0	0.0	0.0	1.0
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PK	1.4	0.3	−0.4	0.1	0.0	0.9
PDC	2.3	0.6	−1.3	0.1	0.0	0.9
ADH	1.7	0.4	−0.7	−1.3	0.2	2.3

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How to interpret this complex interplay?

- Different types of regulation can occur in the same pathway
- Flux through same reaction can be regulated differently, depending on physiological state
- This 'hierarchical' regulation coefficient does not take into account changes in expression of other enzymes
- The latter changes are accounted for indirectly through their effects on metabolism.
- Therefore the relative contributions of gene regulation and metabolic control are not fully separated

  → use of an alternative approach

4. The MetaGenoReg project

- Modelling combined metabolic and gene regulation
  - Reduce and simplify in order to understand the system's behaviour
  - Develop a method for joint modelling combining different approximations suited to both types of regulation
  - Measure their respective contribution
- Analyse the model's strengths and weaknesses from a systemic point of view
- Understand the biological rationale underlying the distribution of regulation between metabolism and gene expression

Which reduction, which approximations?

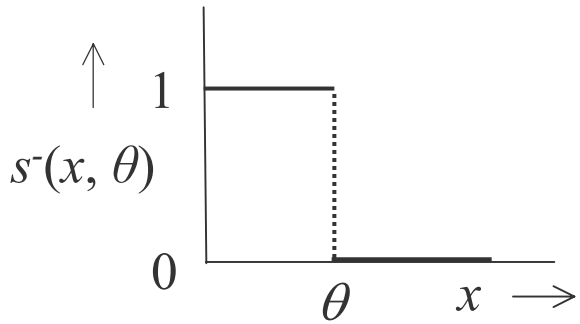
- Decompose the system into a slow (gene) and a fast (metabolic) component
  → fast algebraic subsystem (quasi steady-state hypothesis)
- Variable aggregation
- Strongly cooperative effects to be approximated by step functions
- Various types of linearization of metabolic responses

PL model for gene regulation

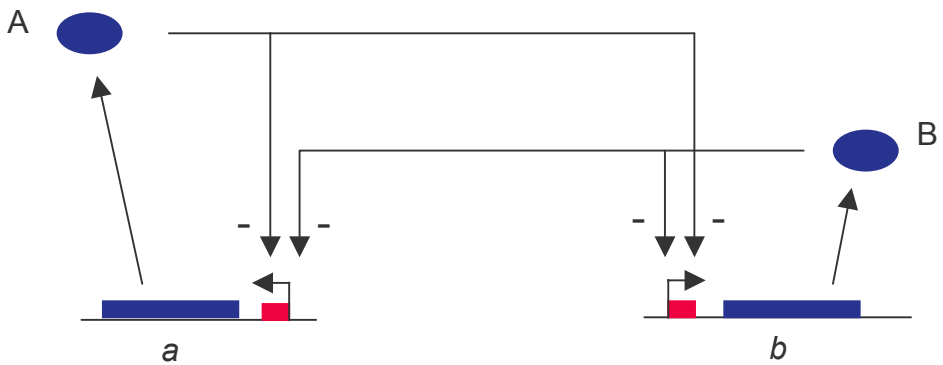
de Jong *et al.* (2003) *Bioinformatics* 19:336-344

- Approximate promoter responses by step-functions:

$$\dot{x}_a = \kappa_a \, s^-(x_a, \theta_{a2}) \, s^-(x_b, \theta_{b1}) - \gamma_a \, x_a$$
$$\dot{x}_b = \kappa_b \, s^-(x_a, \theta_{a1}) \, s^-(x_b, \theta_{b2}) - \gamma_b \, x_b$$



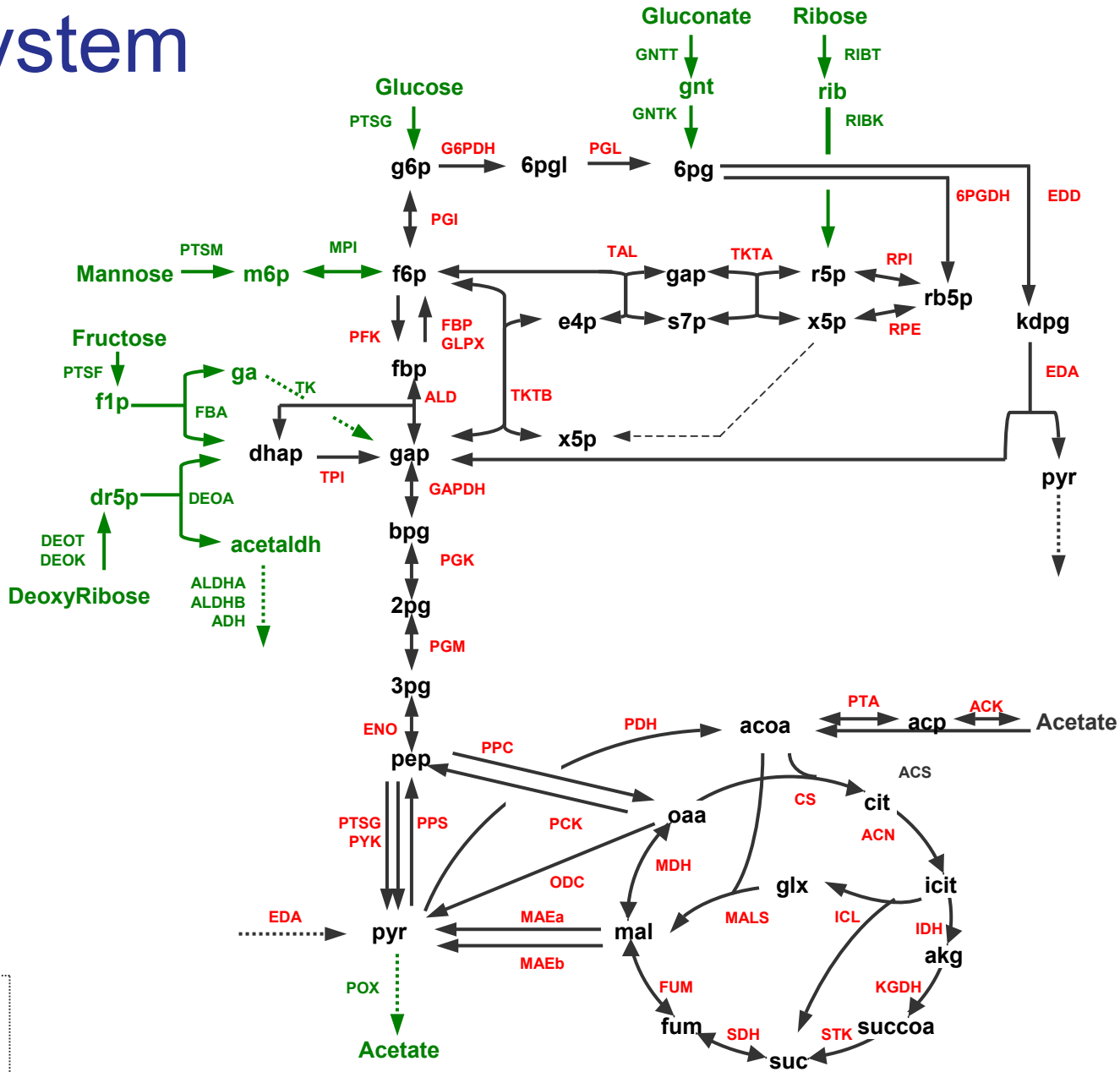
x : protein concentration
θ : threshold concentration
κ, γ : rate constants



➢ Piece-wise linear (PL) differential systems

Biological system

E. Coli carbon metabolism



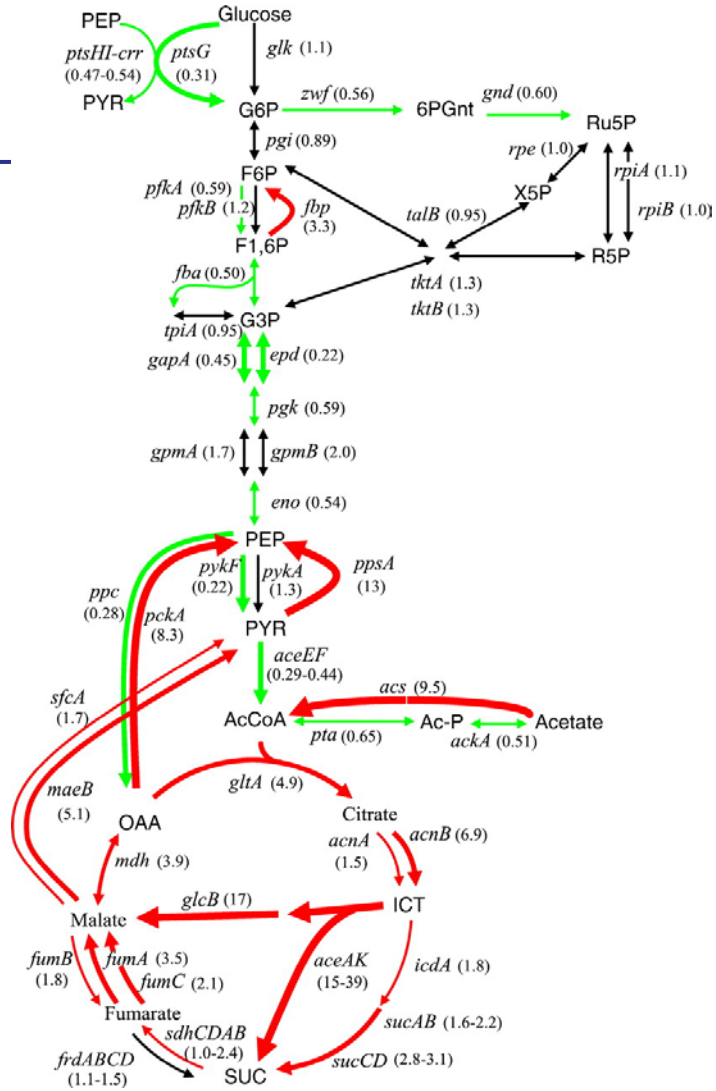
n1 (ADP+Pi) n1 ATP
NADH,H+ → NAD+ (RESP1)

n2 (ADP+Pi) n2 ATP
FADH2 → FAD (RESP2)

n2 (ADP+Pi) n2 ATP
Pyruvate → Acetate + CO2 (POX)

Glucose-acetate diauxie

- Well-characterised transition
- Involves major changes
  - at the metabolic level:
    Gluconeogenesis *vs.* glycolysis
  - at the gene expression level
- Strong interaction between metabolic and gene expression levels

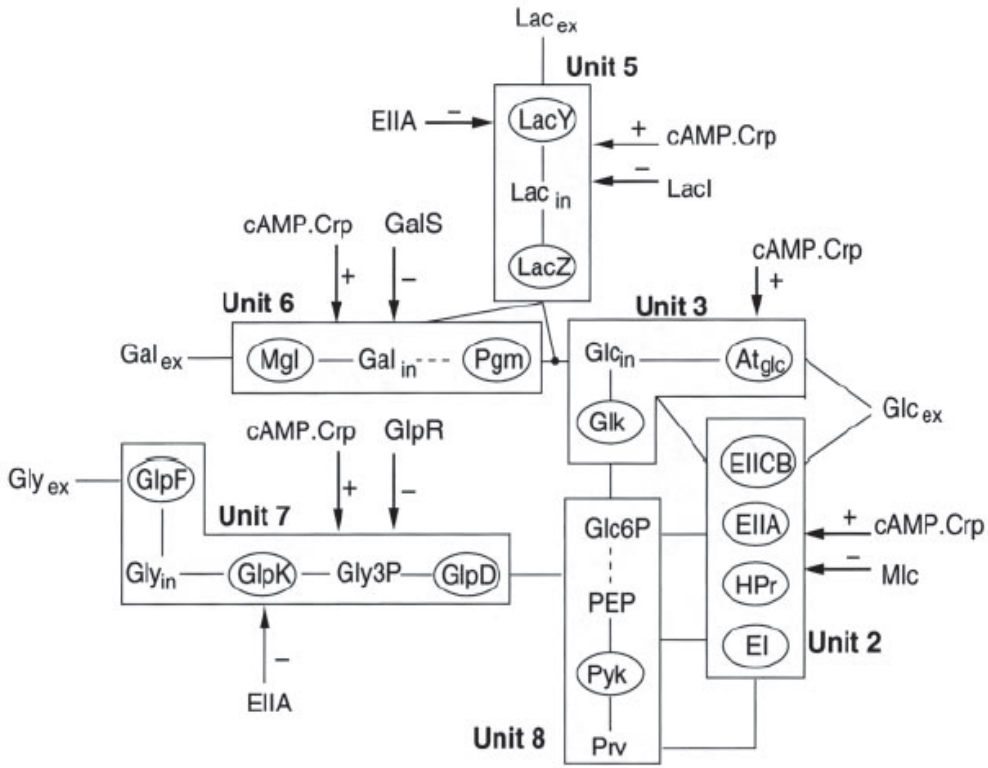


Oh *et al.* (2002), *J Biol Chem.* 277(15):13175-83.

ODE based model

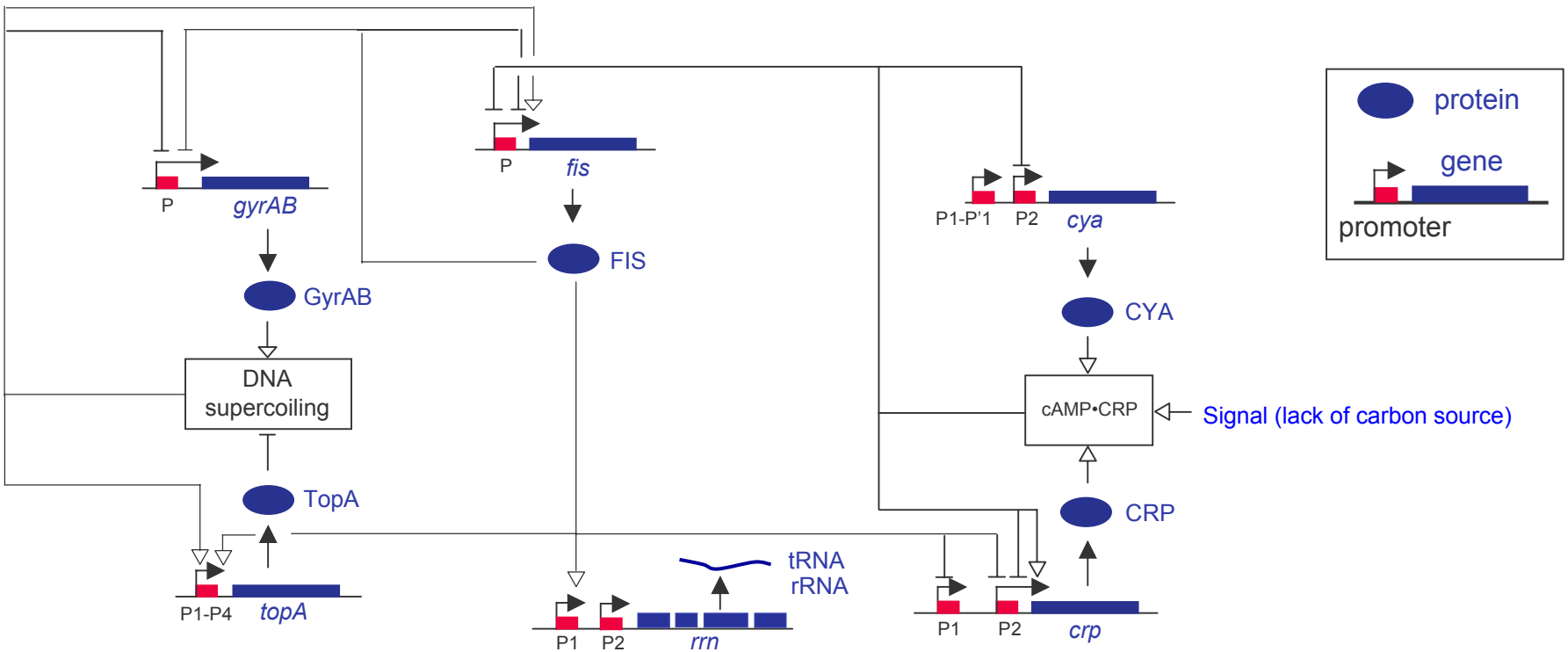
Bettenbrock *et al.* (2005), *J. Biol. Chem.*, 281: 2578-2584
Chassagnole *et al.* (2002) *Biotech. Bioeng. 79*: 53-73

Kinetic model with tens of equations and over 100 parameters



Gene regulation model

Ropers *et al.* (2006), *Biosystems* 84:124-152



Benchmark model

- Toy regulation model entirely specified with ODEs
- Combines metabolic and macromolecular variables
- Includes metabolic and gene regulation
- 'Experimental' object used to test the quality of various reductions and approximations by comparison of simplified models with complete ODE model

Which reduction, which approximations?

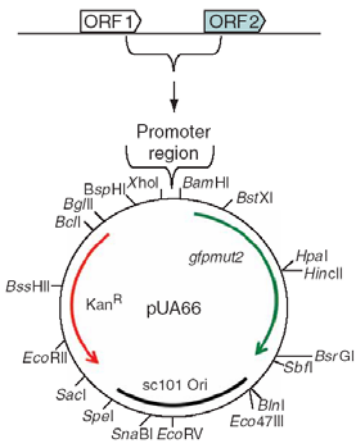
- Decompose the system into a slow (gene) and a fast (metabolic) component
  → fast algebraic subsystem (quasi steady-state hypothesis)
- Variable aggregation
- Strongly cooperative effects to be approximated by step-functions
- Various types of linearization of metabolic effects
- Compare reduced / approximated models with complete ODE-specified model

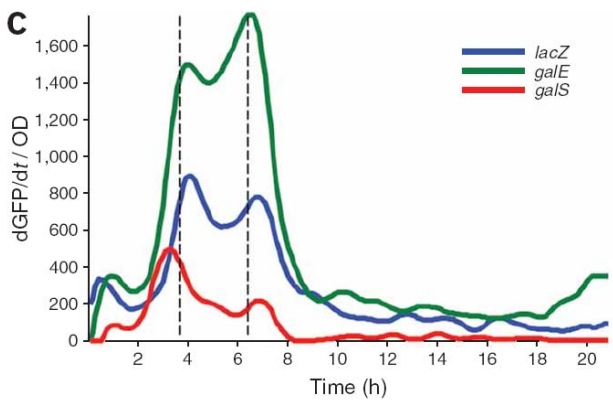
Modelling the glucose-acetate diauxie

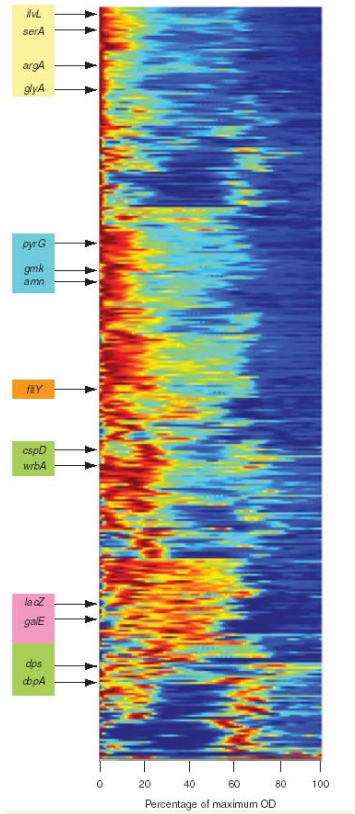
- Assessment of the simplified model by analysing its qualitative dynamics (model-checking)
- Assessing parameter identifiability
- Parameter estimation for the simplified model on the basis of experimental data generated in the project:
  - Metabolic (concentrations and fluxes: ^{13}C NMR, IC-MS)
    Jean-Charles Portais, Toulouse
  - Gene expression (enzyme activities, microarrays, reporter genes)
    Hans Geiselmann, Grenoble

Experimental validation

- The most interesting model predictions will be verified using appropriate mutated strains:
  - Metabolic measurements
  - Gene expression dynamics (fluorescent reporter proteins)







Zaslaver *et al.* (2006), *Nature Methods*, 3(8): 623-628

Roles of metabolic and gene regulation

- Study the metabolic response in the model when gene regulation is abolished
- Evaluate (quantify) the contribution of gene regulation to the metabolic response
- Conversely calculate the contribution of metabolic effects to gene reguation

Partners and support

- Hidde de Jong, Delphine Ropers, INRIA Grenoble
- Daniel Kahn, INRIA HELIX & Lyon 1 University
- Jean-Luc Gouzé, INRIA COMORE, Sophia-Antipolis
- Hans Geiselmann, CNRS-UJF Grenoble
- Jean-Charles Portais, INRA-INSA Toulouse

- Agence Nationale pour la Recherche
- EU

Fellowships available on MetaGenoReg

- Thesis fellowship, 2007-2010

  Application deadline: April 30

- Post-doctoral fellowships, 2007-2009

- Contact 

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