Modeling and simulation of gene regulatory networks 5

Hidde de Jong
IBIS
INRIA Grenoble – Rhône-Alpes
Hidde.de-Jong@inria.fr

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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**

   Towards integrated models of the cell: metabolism, gene expression, signalling

Towards integrated models of the cell

- Introduction and motivation: what are integrated models of the cell and why are they necessary?

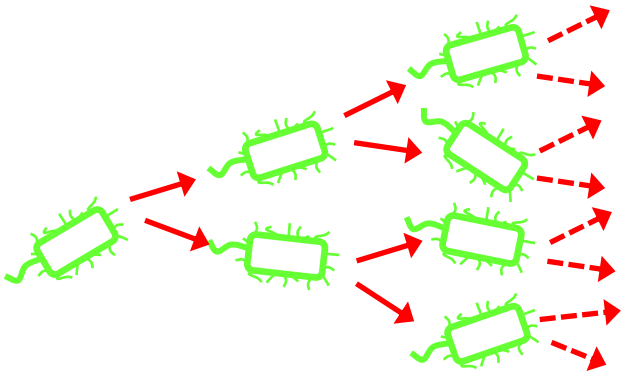
- Examples of integrated models of the cell
  - Flux balance models
  - Kinetic models of cellular functions: towards whole-cell models
  - Resource allocation models

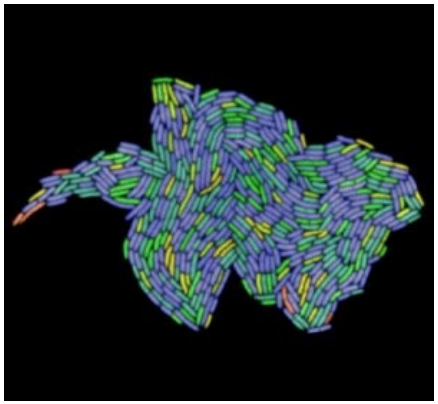
- Conclusions and perspectives

Bacterial growth and metabolism

- **Bacteria** are unicellular organisms geared towards growth and division

  Escherichia coli cells have doubling times up to 20 min





Stewart *et al.* (2005), *PLoS Biol.*, 3(2): e45

- **Metabolism** fuels growth by production of energy and building blocks for macromolecules, using nutriments from environment

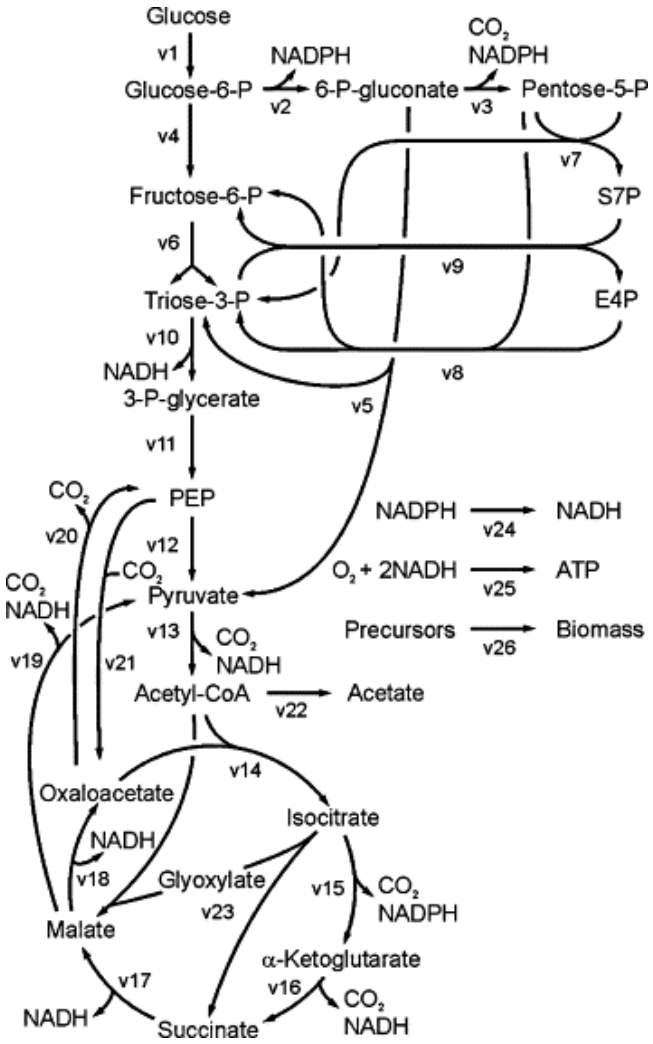
  ATP, amino acids, nucleotides, …

Bacterial growth and metabolism

- Central **carbon metabolism** breaks down carbon sources for energy production and macromolecular synthesis

  Glucose, acetate, lactose, …

- Reactions are catalyzed by enzymes

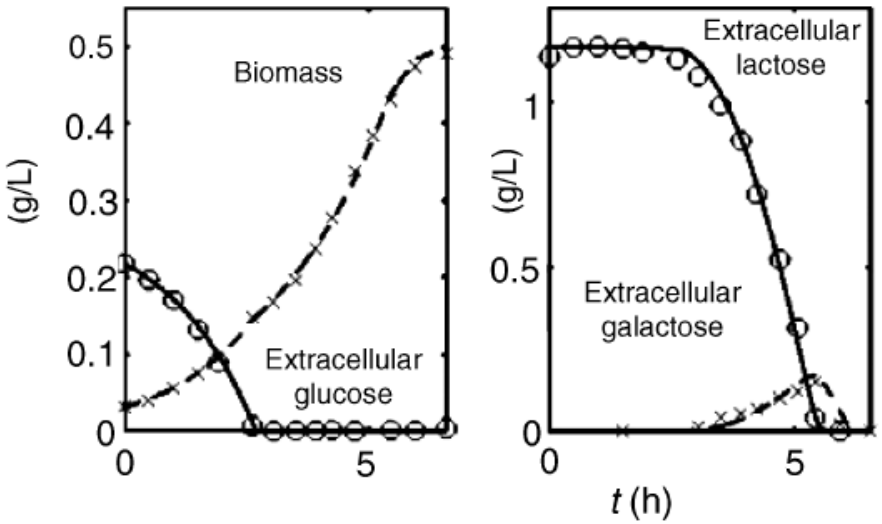


Fischer *et al.* (2004), *Anal. Biochem.*, 325(2):308–16

Bacterial growth and metabolism

- Bacterial metabolism is **flexible**, allowing cells to grow on different carbon sources

  Preferential utilisation: **diauxic growth** on glucose and lactose

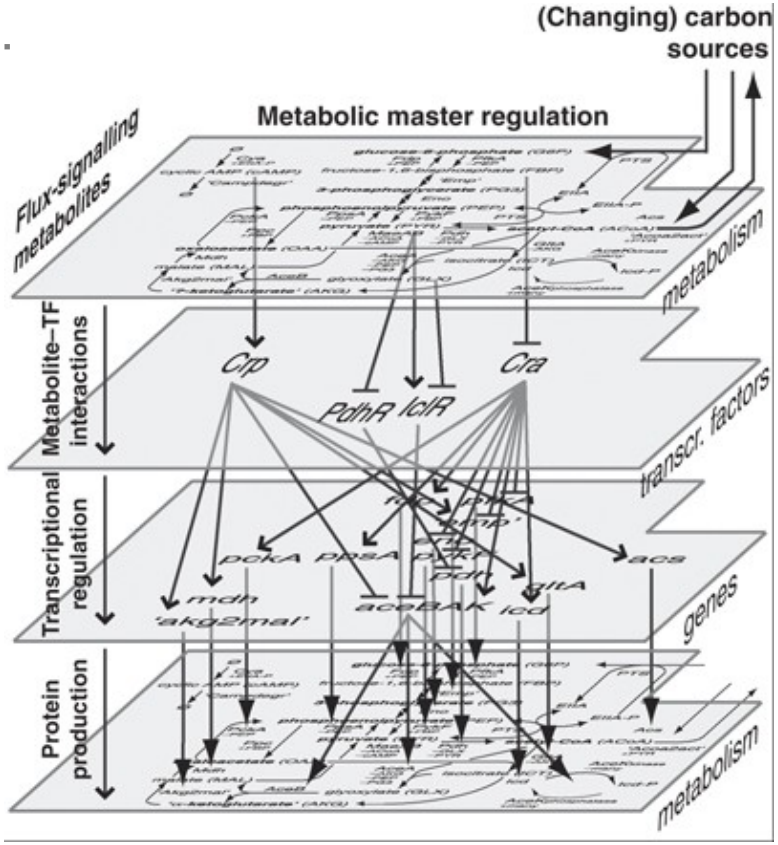


Bettenbrock *et al.* (2006), *J. Biol. Chem.*, 281(5):2578-84

- Adaptation of bacterial physiology to different carbon sources

Coordination of adaptative responses

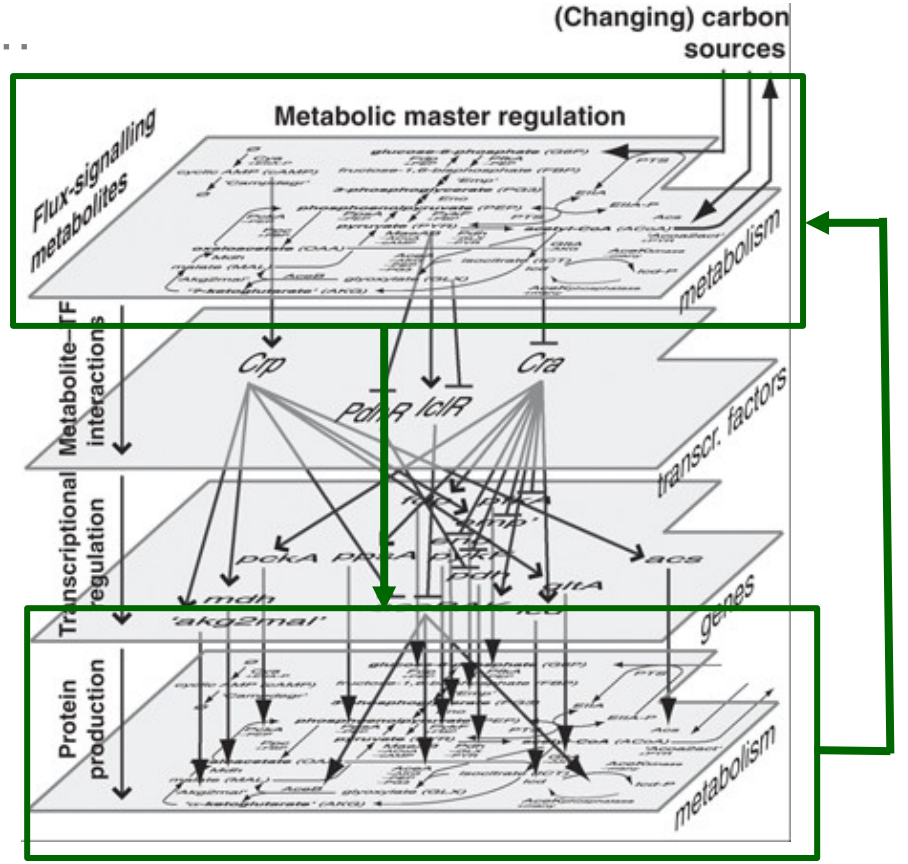
- Coordination of adaptative responses of bacterial cell achieved by **large and complex regulatory networks**
  - Variety of molecular mechanisms…
  - … operating on different time-scales…



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

Coordination of adaptative responses

- Coordination of adaptative responses of bacterial cell achieved by **large and complex regulatory networks**
  - Variety of molecular mechanisms…
  - … operating on different time-scales…
  - … involving numerous feedback loops across levels



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

Towards integrated models of cell

- Systems biology has addressed a huge variety of problems, using a large number of methods and formalisms
- However, most studies focus on isolated, relatively small subsystems
- Increasing awareness that for answering many interesting questions, one needs to consider **integrated models of the cell**:
  - Multiple levels of regulation: metabolism, gene expression, signal transduction,…
  - Multiple functions: motility, growth, replication, …
  - Explicit modelling of interactions with environment and ecosystem
  - …

Towards integrated models of the cell

- Integrated models of the cell are emerging, but some interesting precursors exist
  - Coarse-grained model of an *E. coli* cell

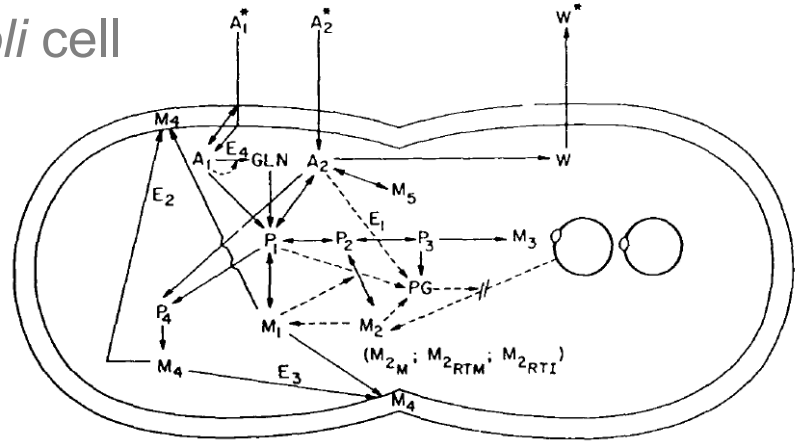


FIGURE 7 An idealized sketch of the model of *E. coli B/rA* growing in a glucose-ammonium salts medium with glucose or ammonia as the limiting nutrient. At the time shown the cell has just completed a round of DNA replication and initiated cross-wall formation and a new round of DNA replication. Solid lines indicate the flow of material, while dashed lines indicate flow of information. Reproduced with permission from Shuler and Domach, 1983.

A_1 = ammonium ion
A_2 = glucose (and associated compounds in the cell)
W = waste products (CO_2, H_2O, and acetate) formed from energy metabolism during aerobic growth
P_1 = amino acids
P_2 = ribonucleotides
P_3 = deoxyribonucleotides
P_4 = cell envelope precursors
M_1 = protein (both cytoplasmic and envelope)
$M_{2_{RI}}$ = immature "stable" RNA
$M_{2_{RM}}$ = mature "stable" RNA (*r*-RNA and *r*-RNA—assume 85% *r*-RNA throughout)
M_{2_M} = messenger RNA
M_3 = DNA
M_4 = non-protein part of cell envelope (assume 16.7% peptidoglycan, 47.6% lipid, and 35.7% polysaccharide)
M_5 = glycogen
PG = *ppGpp*
E_2, E_3 = molecules involved in directing cross-wall formation and cell envelope synthesis—the approach used in the prototype model was used here but more recent experimental support is available
GLN = glutamine
E_1 = glutamine synthetase
*—the material is present in the external environment.

Domach *et al.* (1984), *Biotechnol. Bioeng.*, 26(3):203-16

Towards integrated models of the cell

- Integrated models of the cell are emerging, but some interesting precursors exist
- Several approaches for building integrated models of the cell:
  - Flux balance models
  - Kinetic models of cellular functions: towards whole-cell models
  - Resource allocation models

Kinetic modelling of metabolism

- Kinetic models of form $\dot{x} = N\, v(x)$
  - Concentration variables $x \in \mathbb{R}^n_+$
  - Reaction rates $v : \mathbb{R}^n_+ \to \mathbb{R}^q$
  - Stoichiometry matrix $N \in \mathbb{Z}^{n \times q}$

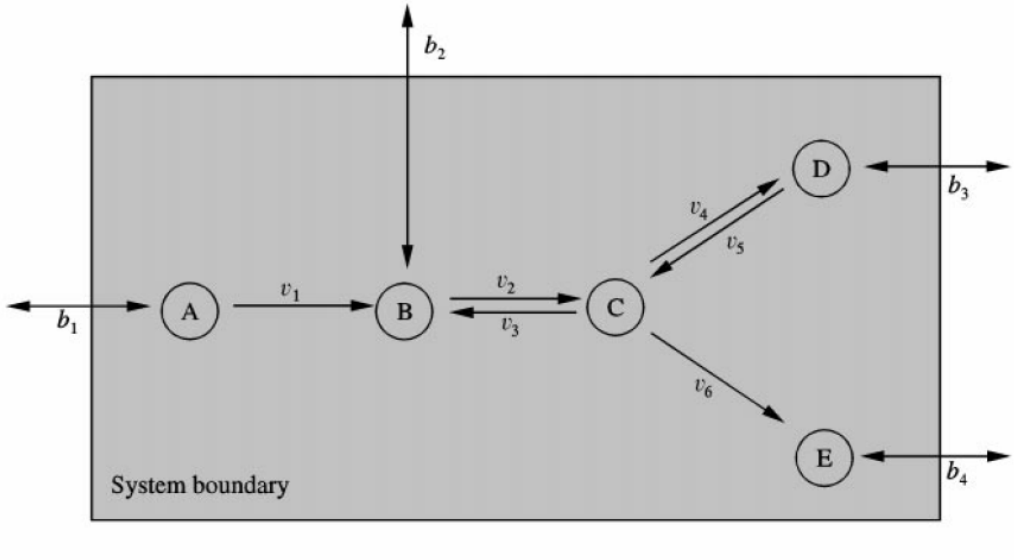
Heinrich and Schuster (1996), *The Regulation of Cellular Systems*, Chapman & Hall

- Stochiometry matrix N describes structure of reaction network
- Reaction rate v depends on concentrations of other cellular components

Kinetic modelling of metabolism

- Stochiometry matrix N describes structure of reaction network

  Internal reactions and exchange reactions, reversible and irreversible



$$N = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \end{bmatrix} \quad v = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \\ b_1 \\ b_2 \\ b_3 \\ b_4 \end{bmatrix}$$

Schilling *et al.* (2000), *J. Theor. Biol.*, 203(3):229-48

Flux balance analysis (FBA)

- Steady-state dynamics of metabolic network

$$N\,v = 0$$

  Steady-state reaction rates are called **fluxes**

- **Constraints** on fluxes: upper and lower bounds

$$v^l \leq v \leq v^u$$

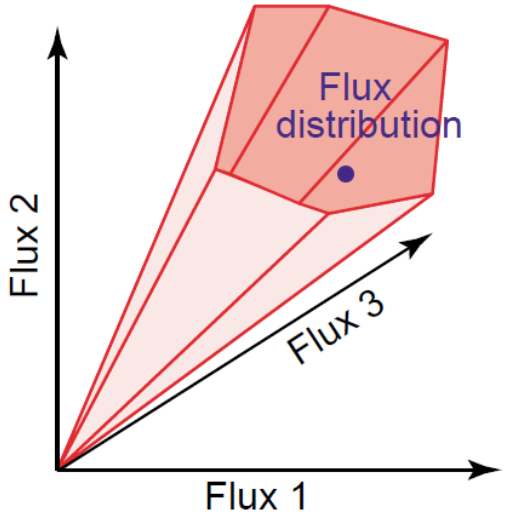
  - Bounds on fluxes derived from available information in literature, bounds may be infinite
  - For mathematical convenience, all fluxes must be positive $v \geq 0$
  - Reversible reaction modeled as pair of irreversible, positive fluxes

Flux balance analysis (FBA)

- Steady-state dynamics of metabolic network

$$N\,v = 0$$

- Stoichiometry matrix and constraints define convex space of possible solutions: **steady-state flux cone**
  - System of steady-state equations underdetermined: more reactions than concentrations variables.
  - Flux cone represents **metabolic capabilities** of network (possible flux distributions)



Stelling (2004), *Curr. Opin. Microbiol.*, 7:513-8

Flux balance analysis (FBA)

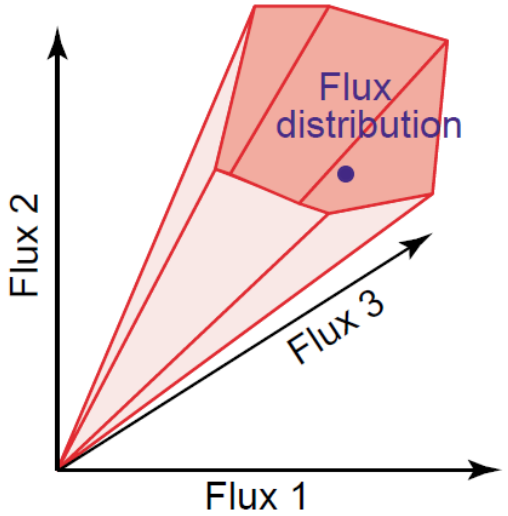
- Steady-state dynamics of metabolic network

$$N\,v = 0$$

- Stoichiometry matrix and constraints define convex space of possible solutions: **steady-state flux cone**
- FBA aims at finding solutions(s) maximising or minimising linear combination of fluxes: **objective function**

$$Z = c^T\,v \qquad\qquad c \in \mathbb{R}^n$$

- Typical objective functions: biomass production, ATP production, …



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Flux balance analysis (FBA)

- Steady-state dynamics of metabolic network

$$N\,v = 0$$

- Stoichiometry matrix and constraints define convex space of possible solutions: **steady-state flux cone**
- FBA aims at finding solutions(s) maximising or minimising linear combination of fluxes: **objective function**
- Constrained optimisation problem in mathematics
  - Use of LP (linear programming) for solving optimisation problem
  - COBRA toolbox for building and analysing FBA models

Palsson (2006), *Systems Biology: Properties of Reconstructed Networks*, Cambridge University Press

Orth *et al.* (2010), *Nat. Biotechnol.*, 28(3):245-8

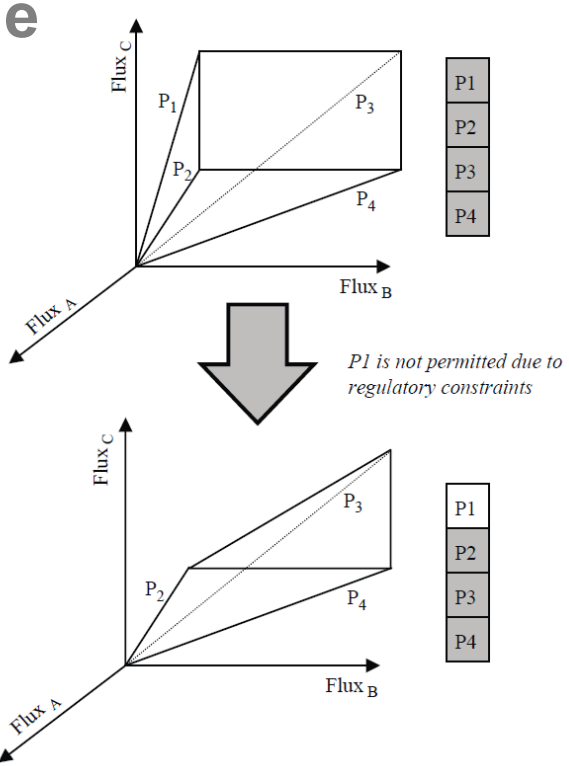
Flux balance analysis (FBA)

- Steady-state dynamics of metabolic network

$$N\,v = 0$$

- Stoichiometry matrix and constraints define convex space of possible solutions: **steady-state flux cone**
- Refinement of flux cone using additional constraints

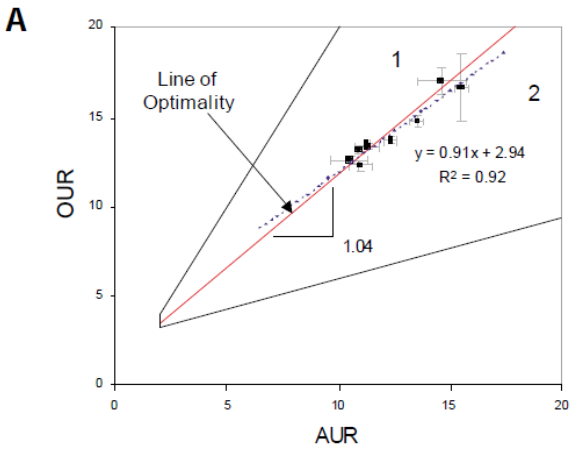
  Thermodynamics, regulation of enzyme activity or expression, …

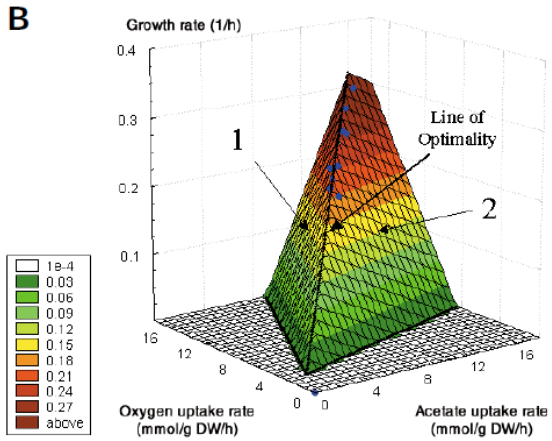


Covert *et al.* (2003), *J. Theor. Biol.*, 221(3):309-25

Genome-scale models of *E. coli* metabolism

- Genome-scale reconstruction of *E. coli* metabolism
- FBA predictions of flux distributions maximising growth rate with acetate as carbon source
  - Projection of flux cone on acetate and oxygen uptake rates
  - Line of optimality indicates combinations of acetate and oxygen uptake rates yielding maximal growth rate
  - Experimental test of predicted line of optimality: experimental control of acetate uptake rate and measurement of oxygen uptake rate



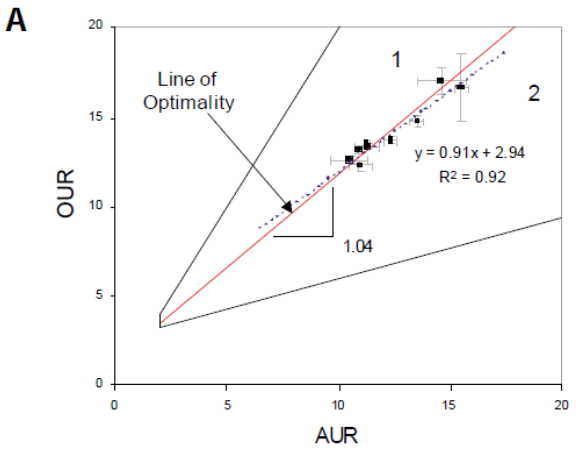


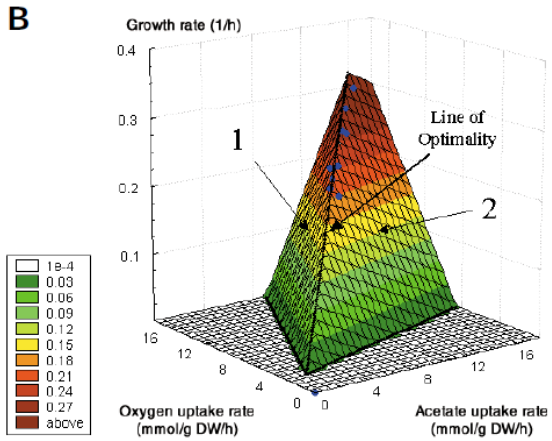
Edwards *et al.* (2001), *Nat. Biotechnol*, 19(2):125-30

Genome-scale models of *E. coli* metabolism

- Genome-scale reconstruction of *E. coli* metabolism
- FBA predictions of flux distributions maximising growth rate with acetate as carbon source
- Good correspondence of FBA predictions and experimental data suggests that *E. coli* metabolic network is optimised to maximise growth rate on acetate

  Idem succinate

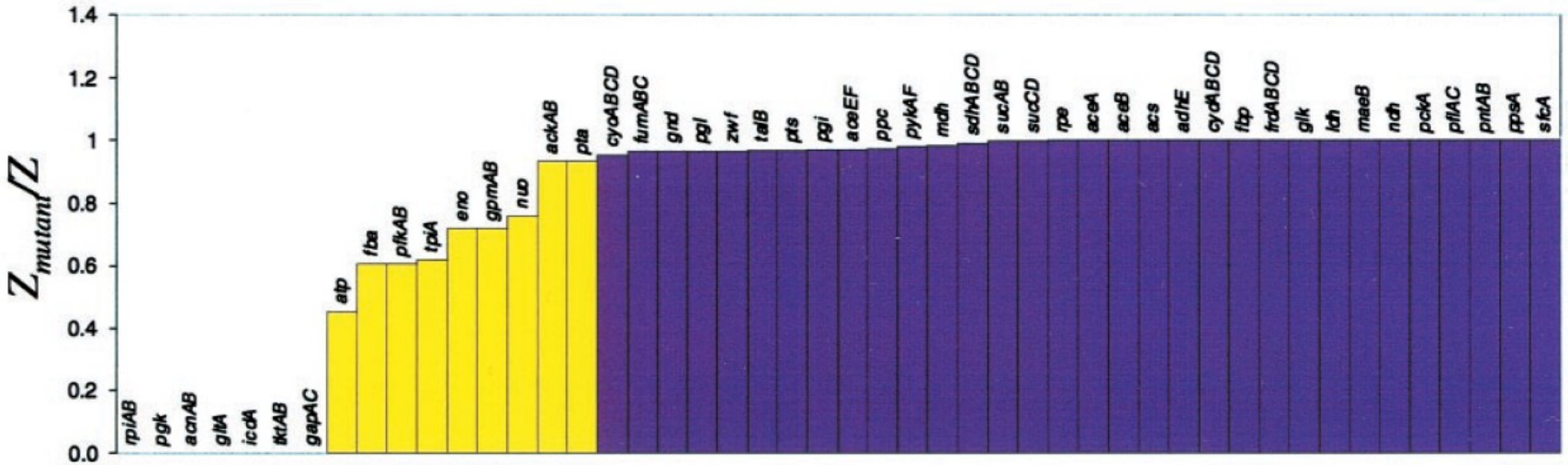




Edwards *et al.* (2001), *Nat. Biotechnol*, 19(2):125-30

Genome-scale models of *E. coli* metabolism

- Genome-scale reconstruction of *E. coli* metabolism
- FBA predictions of flux distributions maximising growth rate with glucose as carbon source and fixed oxygen uptake rate
- Effect on growth rate when deleting genes in central carbon metabolism



Edwards *et al.* (2000), *Proc. Natl. Acad. Sci. USA*, 97(10):5528-33

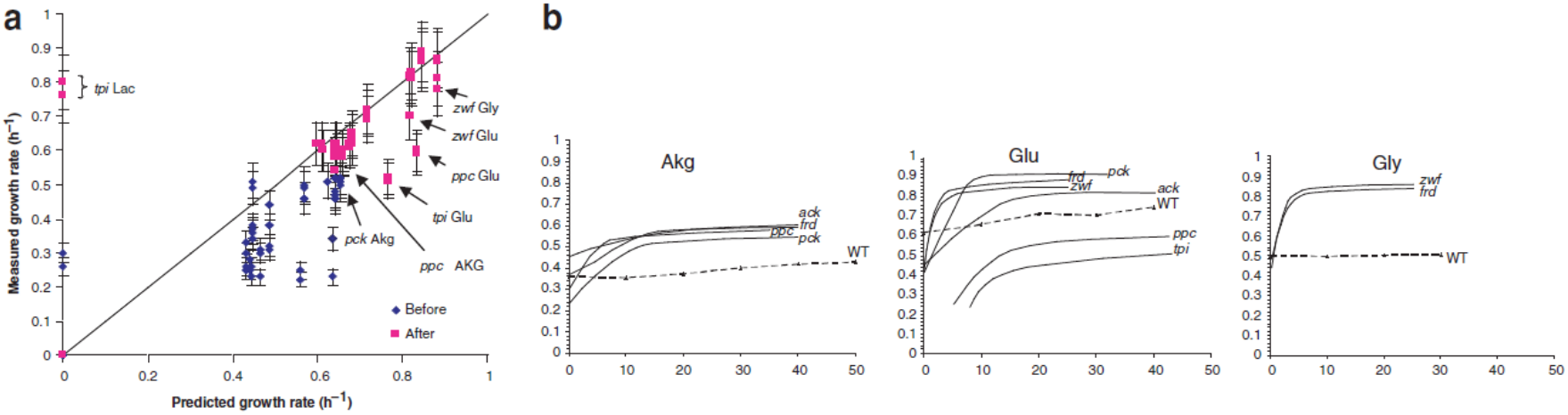
Genome-scale models of *E. coli* metabolism

- Genome-scale reconstruction of *E. coli* metabolism
- FBA predictions of flux distributions maximising growth rate with glucose as carbon source and fixed oxygen uptake rate
- Good correspondence with data for gene deletions examined (86%), but less so for broader range of conditions (60%)
  - Observed growth rate lower than predicted growth rate
- Not surprising: **regulatory structure** of wild-type cells may not be optimal in mutant backgrounds!
  - Regulatory structure selects wild-type flux distribution from possible flux distributions in flux cone
- However, experiments show that *E. coli* undergoes **adaptive evolution** to achieve predicted optimal growth rate by FBA

Ibarra *et al.* (2002), *Nature*, 420(6912):186-9

Genome-scale models of *E. coli* metabolism

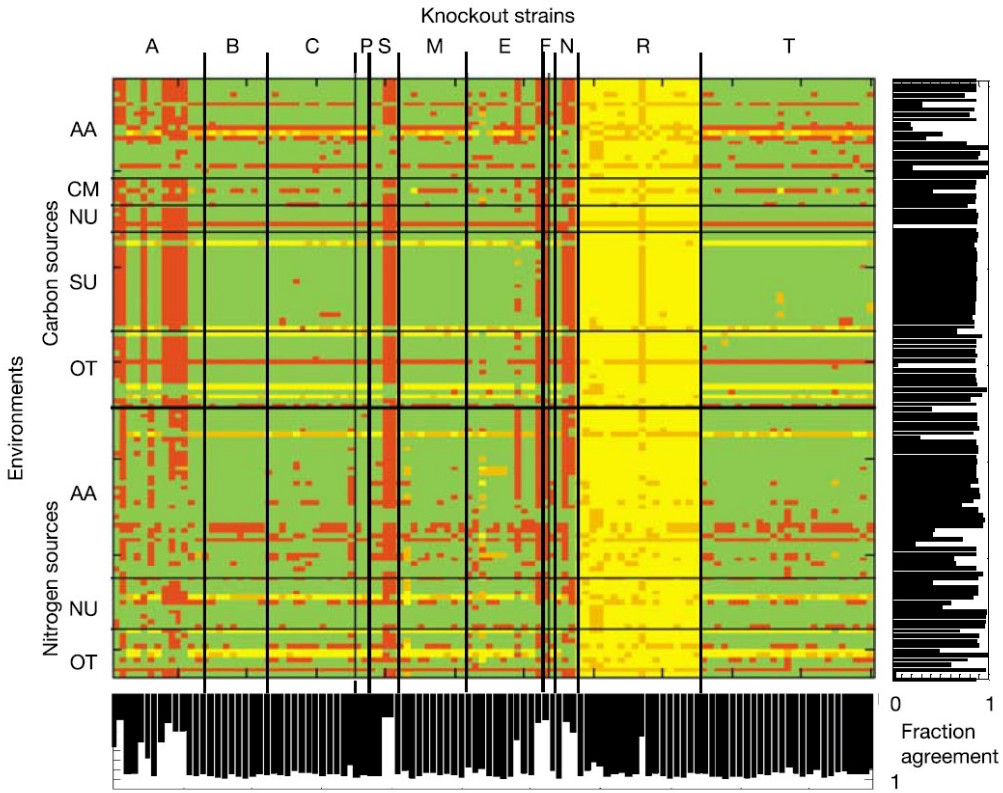
- However, experiments show that *E. coli* undergoes **adaptive evolution** to achieve predicted optimal growth rate by FBA
  - Growth on glucose, glycerol, and α-ketoglutarate in various mutants
  - Measured substrate and oxygen uptake rates as input for computational predictions



Fong *et al.* (2004), *Nat. Genet.*, 36(10):1056-8

Genome-scale models of *E. coli* metabolism

- Regulatory structure of wild-type cells may not be optimal in mutant backgrounds
- How do predictions change when **including regulatory structure**?
- Genome-scale model of *E. coli* metabolism, including regulation of enzymatic genes
  - Boolean models relating expression of enzymatic genes to growth conditions

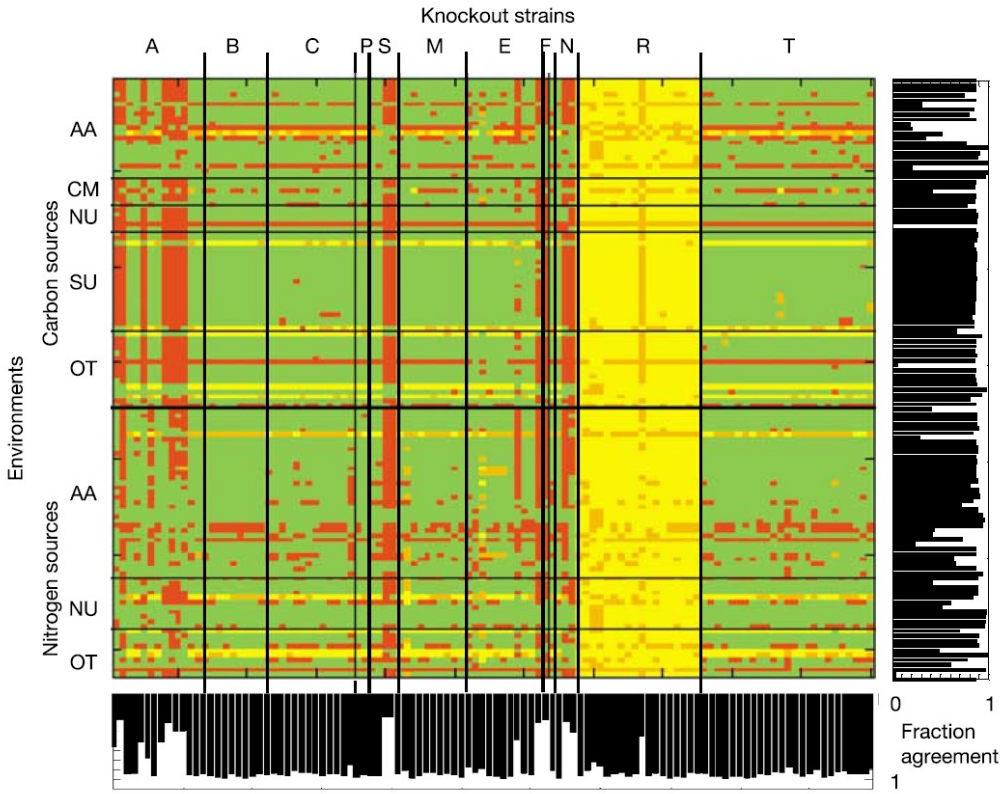


Covert *et al.* (2004), *Nature*, 429(6987):92-6

Genome-scale models of *E. coli* metabolism

- Regulatory structure of wild-type cells may not be optimal in mutant backgrounds
- Genome-scale model of *E. coli* metabolism, including regulation of enzymatic genes
- Prediction of growth rate in different mutants and growth conditions improved

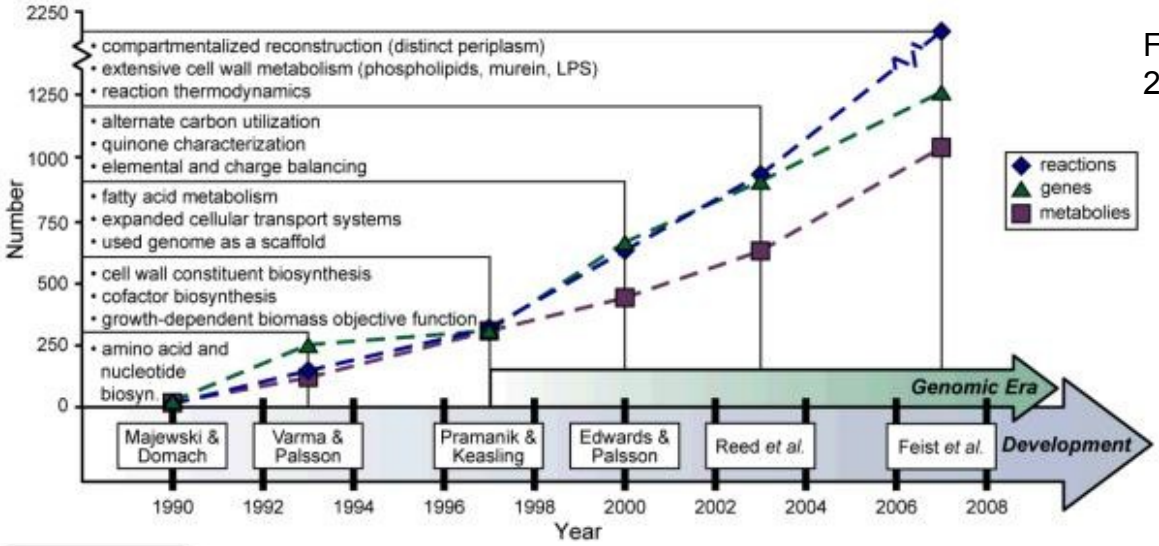
  60% vs 78%



Covert *et al.* (2004), *Nature*, 429(6987):92-6

Conclusion FBA

- FBA models provide genome-scale picture of metabolism and yield experimentally-testable predictions
  - Predictions of flux distributions in different growth conditions and genetic backgrounds
  - Tool for metabolic engineering
  - In *E. coli* and other (less well-characterised) organisms



Feist and Palsson (2008), *Nat. Biotechnol.*, 26(6):659-67

Conclusion FBA

- But FBA has problems as well!
  - Practical question: which **objective function** works best for problem considered?
  - Fundamental question: what do microorganisms optimise?

    Schuetz *et al.* (2007), *Mol. Syst. Biol.*, 3:119
  - Integration of **regulatory mechanisms** on metabolic and genetic level is not easy to achieve in FBA formalism
  - No predictions on **dynamics** of system

Kinetic modelling

- Kinetic models of form $\dot{x} = N\,v(x)$
  - Concentration variables $x \in \mathbb{R}^n_+$
  - Reaction rates $v : \mathbb{R}^n_+ \rightarrow \mathbb{R}^q$
  - Stoichiometry matrix $N \in \mathbb{Z}^{n \times q}$

Heinrich and Schuster (1996), *The Regulation of Cellular Systems*, Chapman & Hall

- Stochiometry matrix N describes structure of reaction network
- Reaction rate v depends on concentrations of other cellular components

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- Stochiometry matrix N describes structure of reaction network
- Reaction rate v depends on concentrations of other cellular components
  - Mass-action
  - Michaelis-Menten (reversible/irreversible)

$$v = \frac{k^+ \frac{S}{K_s} - k^- \frac{P}{K_p}}{1 + \frac{S}{K_s} + \frac{P}{K_p}}$$

Kinetic modelling

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  - Mass-action
  - Michaelis-Menten (reversible/irreversible)
  - Hill
  - Monod-Wyman-Changeux

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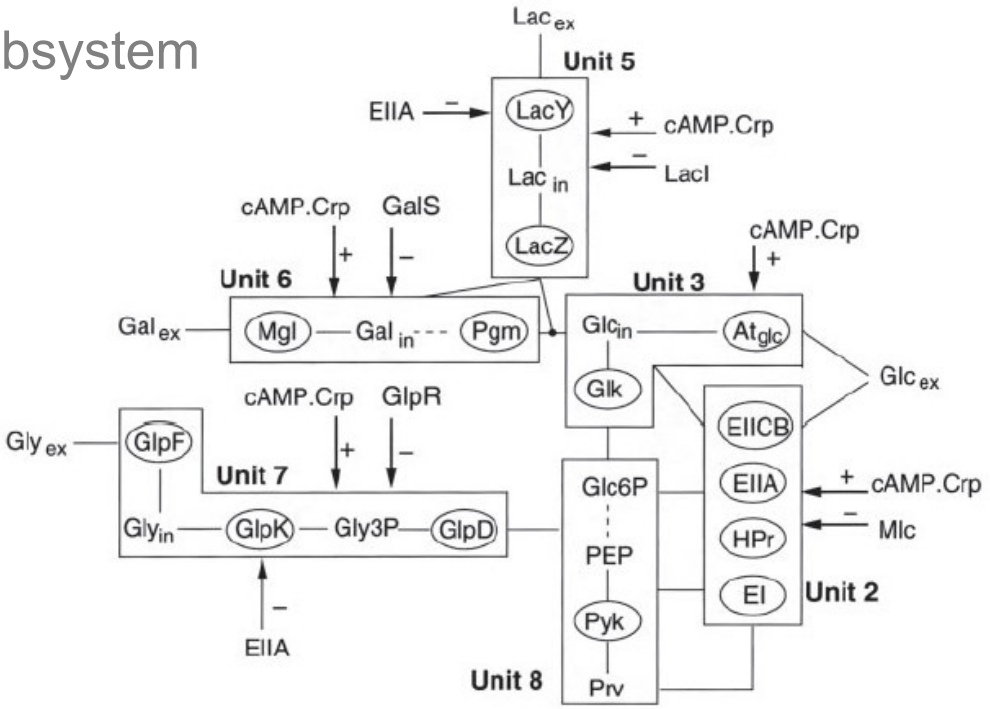
Heinrich and Schuster (1996), *The Regulation of Cellular Systems*, Chapman & Hall

- Stochiometry matrix N describes structure of reaction network
- Reaction rate v depends on concentrations of other cellular components
- In general, reaction rate functions are **nonlinear** and have **many parameters**, **difficult to measure** directly *in vivo*
- Nevertheless, some examples of well-calibrated models!

Kinetic modelling of *E. coli* metabolism

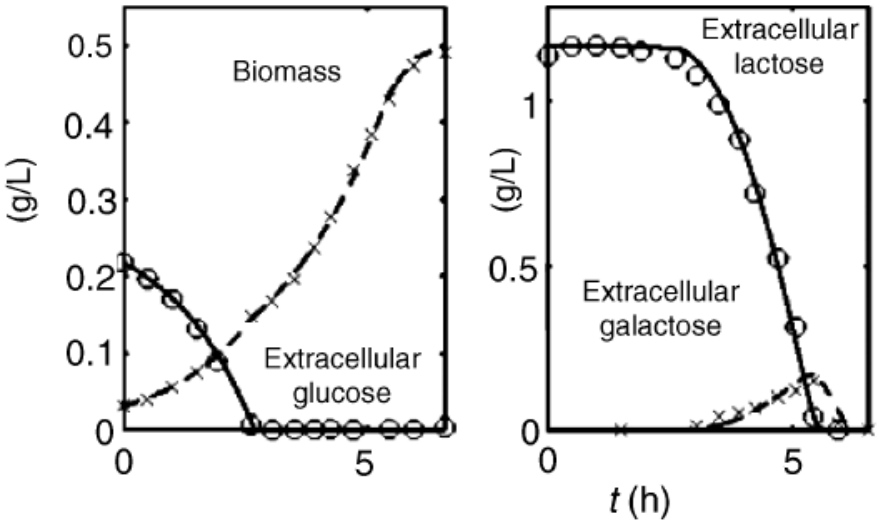
- Model of uptake of carbon sources (glucose, lactose, glycerol, …) by *E. coli*
  - Several dozens of equations and more than a hundred parameters, many of them unknown or unreliable
  - Mosty metabolic subsystem



Bettenbrock *et al.* (2005), *J. Biol. Chem.*, 281(5): 2578-2584

Kinetic modelling of *E. coli* metabolism

- Estimation of parameter values from time-series data on metabolite concentrations in wild-type and mutant strains
- Model has **good predictive capability**: growth kinetics well explained in variety of conditions

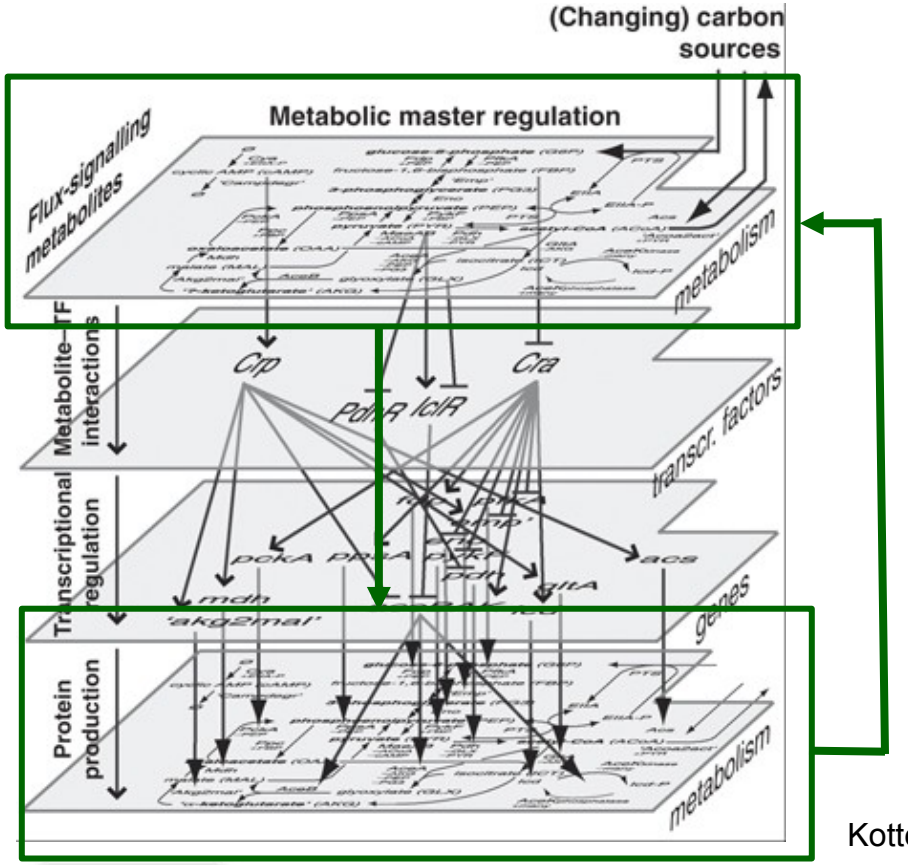


Bettenbrock *et al.* (2005), *J. Biol. Chem.*, 281(5): 2578-2584

Kinetic modelling of multi-scale networks

- Metabolic networks are integrated with gene regulatory networks and signalling networks

  Complex multi-level system with feedback across different time-scales



- **Fast response:** adaptation of metabolic fluxes and metabolite pools (metabolic network)
- **Slow response:** adaptation of enzyme and TF concentrations (gene regulatory network)
- **Feedback** across genetic and metabolic levels: complex system on different time-scales

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

Kinetic modelling of multi-scale networks

- Kinetic model of form $\dot{x} = N\,v(x)$
  - Concentration variables $x \in \mathbb{R}^n_+$
  - Reaction rates $v : \mathbb{R}^n_+ \to \mathbb{R}^q$
  - Stoichiometry matrix $N \in \mathbb{Z}^{n \times q}$
- Time-scale hierarchy motivates distinction between **fast** reaction rates $v^f \in \mathbb{R}^{q-p}$ and **slow** reaction rates $v^s \in \mathbb{R}^p$, such that

$$v = [v^s \; v^f]'$$

Typically, **enzymatic and complex formation** reactions are fast, **protein synthesis and degradation** are slow

Kinetic modelling of multi-scale networks

- Separation of fast and slow reactions motivates a linear transformation $T \in \mathbb{Z}^n \times \mathbb{Z}^n$ of the variables

$$\begin{bmatrix} x^s \\ x^f \end{bmatrix} = T\,x \qquad \begin{bmatrix} N^s & 0 \\ N^{s'} & N^f \end{bmatrix} = T\,N$$

- We call $x^s \in \mathbb{R}_+^m$ **slow variables** and $x^f \in \mathbb{R}_+^{n-m}$ **fast variables**
- Separation of fast and slow variables allows $\dot{x} = N\,v(x)$ to be rewritten as coupled slow (genetic) and fast (metabolic) subsystems

$$\dot{x}^s = N^s\,v^s(x^s, x^f)$$
$$\dot{x}^f = N^{s'}\,v^s(x^s, x^f) + N^f\,v^f(x^s, x^f) \approx N^f\,v^f(x^s, x^f)$$

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  Slow variables are typically **total protein concentrations**, fast variables **metabolites and biochemical complexes**

Kinetic modelling of multi-scale networks

- Separation of fast and slow variables allows original model to be rewritten as coupled slow (genetic) and fast (metabolic) subsystems

$$\dot{x}^s = N^s\, v^s(x^s, x^f)$$

$$\dot{x}^f = N^{s'}\, v^s(x^s, x^f) + N^f\, v^f(x^s, x^f) \approx N^f\, v^f(x^s, x^f)$$

- Under **quasi-steady-state approximation (QSSA)**, fast variables are assumed to instantly adapt to slow dynamics

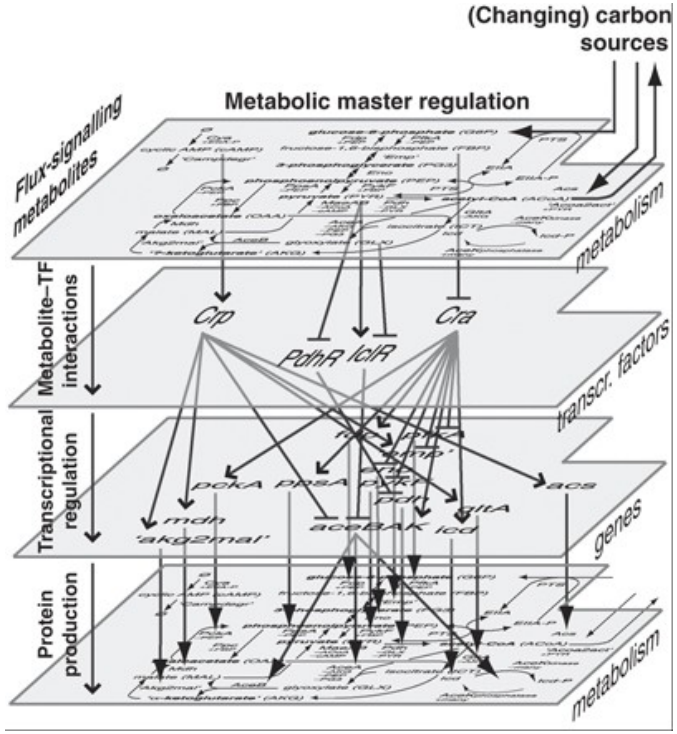
$$\dot{x}^f = 0 \;\Rightarrow\; N^f\, v^f(x^s, x^f) = 0$$

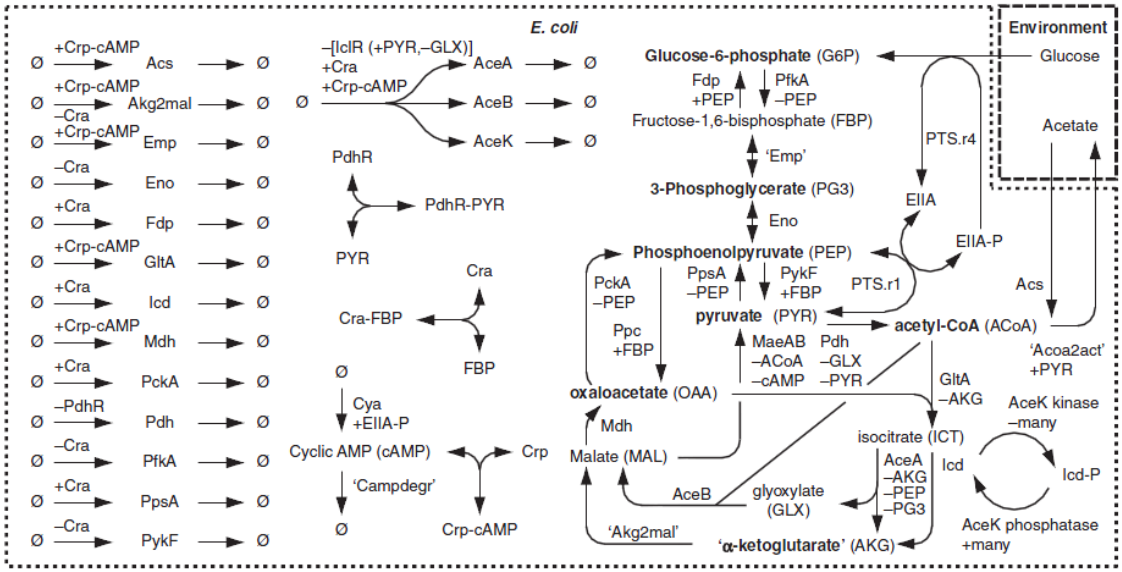
Heinrich and Schuster (1996), *The Regulation of Cellular Systems*, Chapman & Hall

Multi-scale network of *E. coli* metabolism

- Coupling of gene expression and metabolism into a single integrated model

$$\dot{x}^s = N^s \, v^s(x^s, x^f)$$
$$N^f \, v^f(x^s, x^f) = 0$$

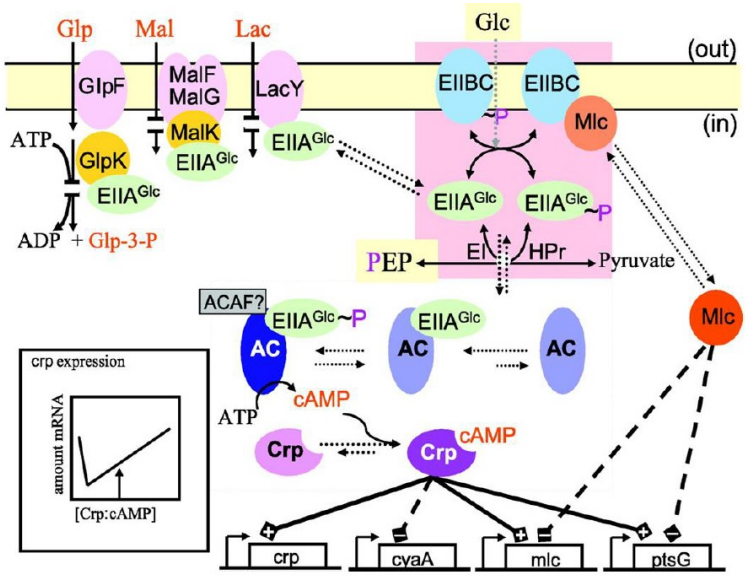




Kotte *et al.* (2010), *Mol. Syst. Biol.*, 6: 355

Multi-scale network of *E. coli* metabolism

- Kinetic model with 47 variables and 193 parameters
  - Parameters estimated from published experimental steady-state data sets for balanced growth on either glucose or acetate
- How does cell sense depletion of carbon source, in order to adapt to uptake and assimilation of another carbon source?
- Cell equiped with **flux sensors**
  - Phosphorylation level of PTS is sensor of glucose uptake flux



Deutscher *et al.* (2006), *Microbiol. Mol. Biol. Rev.*, 70(4):939-1031

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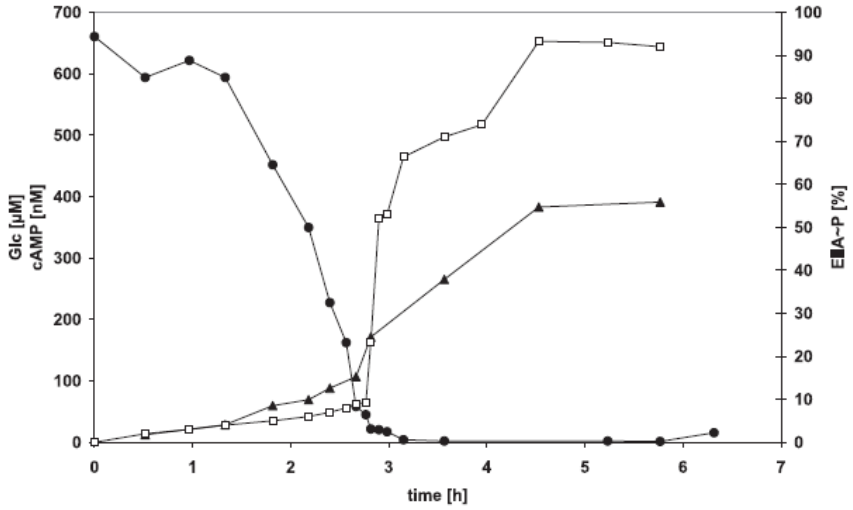
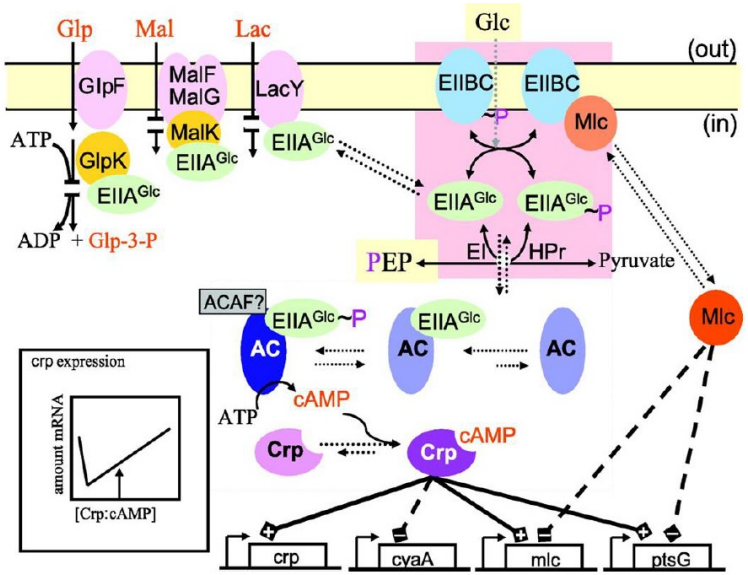


Bettenbrock *et al.* (2007), *J. Bacteriol.*, 189(19):6891-6900



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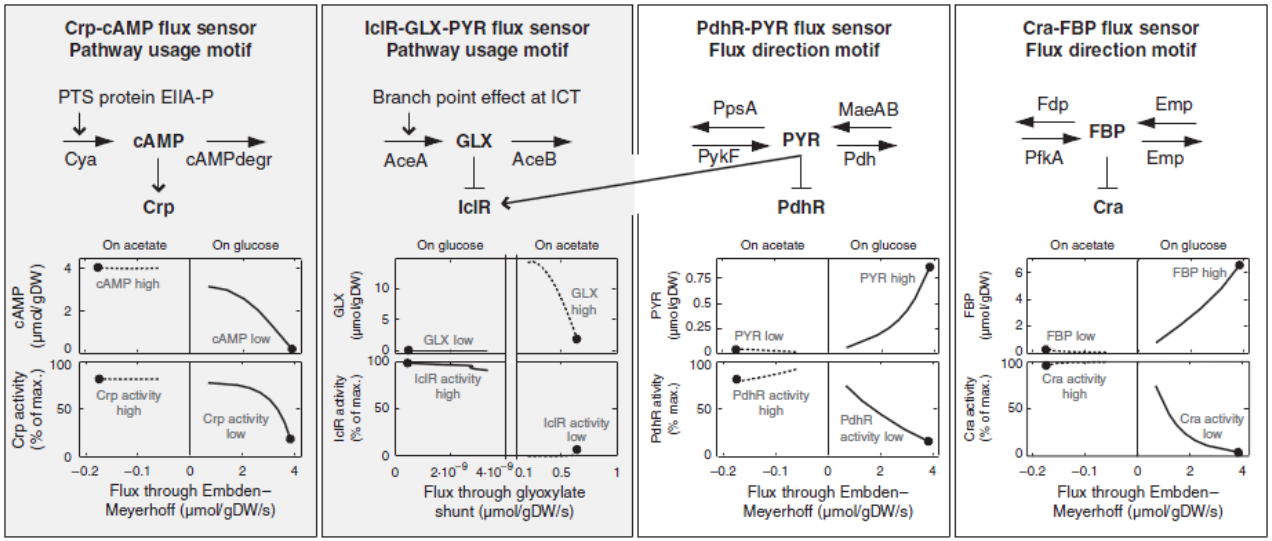
Multi-scale network of *E. coli* metabolism

- Kinetic model with 47 variables and 193 parameters
  - Parameters estimated from published experimental steady-state data sets for balanced growth on either glucose or acetate
- Model analysis shows that adaptation to change in carbon source is achieved by **distributed sensing of intracellular fluxes**



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

Gene expression and global physiology

- Adjustment of gene expression during growth transition involves specific flux sensors…
- … but also global physiological effects
  - Physiological parameters with effect on transcription and translation
  - Availability of RNA polymerase and ribosome, size of metabolic pools, gene copy number, …

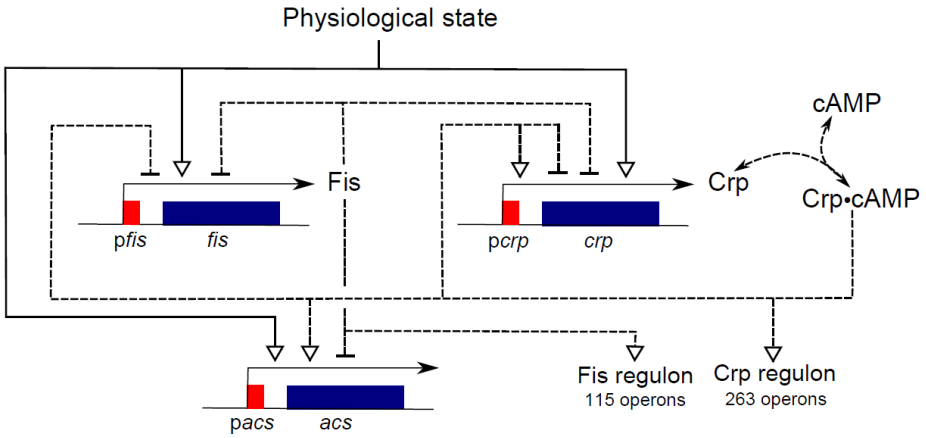
TABLE 3 Parameters pertaining to the macromolecular synthesis rates in exponentially growing *E. coli* B/r as a function of growth rate at 37°C

Parameter	Symbol	Units	At τ (min) and μ (doublings per h): τ, 100 μ, 0.6	τ, 60 μ, 1.0	τ, 40 μ, 1.5	τ, 30 μ, 2.0	τ, 24 μ, 2.5	Observed parameter(s)	Footnote
RNA polymerase protein/total protein	α_p	%	0.90	1.10	1.30	1.45	1.55	α_p	*a*
RNA polymerase molecules/cell	N_p	10^3 RNAP/cell	1.5	2.8	5.0	8.0	11.4	α_p, P_C	*b*
RNA polymerase activity	β_p	%	17	20	21	24	30	r_s, r_m, c_s, c_m, N_p	*c*
Active RNA polymerase per cell	N_{ap}	RNAP/cell	205	503	992	1,929	3,298		*c*
Stable RNA synthesized per total RNA synthesized	r_s/r_t	%	41	52	68	78	83	r_s/r_t	*d*
Active RNA polymerase synthesizing stable RNA	ψ_s	%	24	36	56	68	79	r_s/r_t c_s/c_m	*e*
rRNA chain elongation	c_s	Nucl./s	85	85	85	85	85	Indirect	*f*
mRNA chain elongation	c_m	Nucl./s	39	45	50	52	55	Indirect	*g*
Rate of stable RNA synthesis/cell	r_s	10^5 nucl./min/cell	3.0	9.9	29.0	66.4	132.5	R_C	*h*
Rate of mRNA synthesis/cell	r_m	10^5 nucl./min/cell	4.3	9.2	13.7	18.7	23.4	$r_s, r_s/r_t$	*i*
ppGpp concentration	ppGpp/*M*	pmol/OD_{460}	55	38	22	15	10	ppGpp/*M*	*j*
	ppGpp/*P*	pmol/10^{17} aa	8.5	6.6	4.2	2.9	2.0	P_M	*j*
r-Protein per total protein	α_r	%	9.0	11.4	14.8	17.5	21.1	P_M, R_M	*k*
			9	11	13.5	18.0	21.6	α_r	*l*
Ribosome activity	β_r	%	80	80	80	80	80	Indirect	*m*
Peptide chain elongation	c_p	aa residues/s	12	16	18	20	21	Indirect	*n*
Ribosomes/cell	N_r	10^3 ribosomes/cell	6.8	13.5	26.3	45.1	72.0	R_C, f_s, f_t	*o*
tRNA/cell	N_t	10^3 tRNA/cell	63	125	244	419	669	N_r, f_t	*p*
rrn genes/cell	N_{rrn}	Avg no./cell	12.4	15.1	20.0	26.9	35.9	C, D	*q*
rrn genes/genome	N_{rrn}/G	Avg no./genome	7.9	8.2	8.6	9.0	9.5	C	*r*
Initiation rate at *rrn* gene	i_{rrn}	Initiations/min/gene	4	10	23	39	58	N_t, N_{rrn}	*s*
Distance of ribosomes on mRNA	R_m/N_r	Nucl./ribosome	79	85	65	52	41	r_m, c_m, N_r	*t*

Bremer and Dennis (1996), *Escherichia Coli and Salmonella*, ASM Press, 1553-69

Gene expression and global physiology

- Changes in global physiology important for control of enzyme synthesis
  - Global effect of gene expression machinery may in some situations dominate effect of more specific regulators



Berthoumieux *et al.* (2013), *Mol. Syst. Biol.*, 9:634

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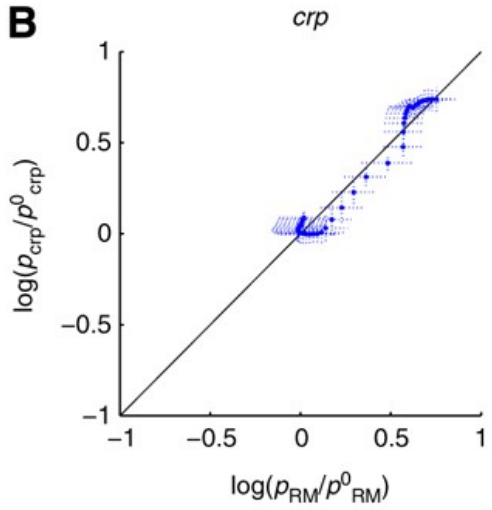
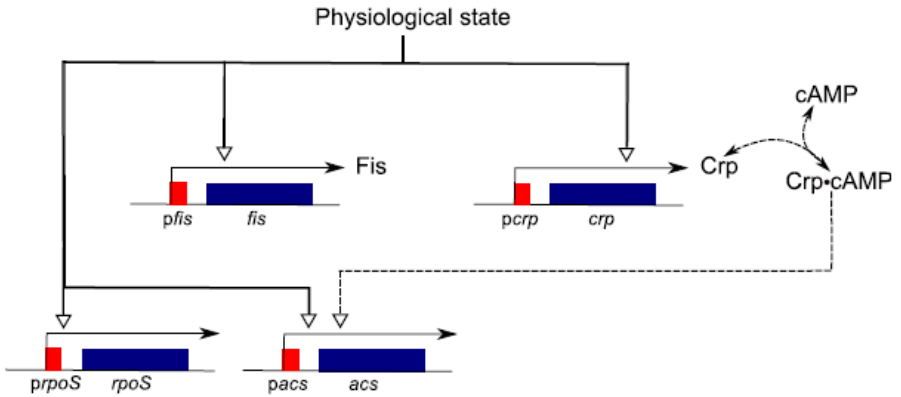
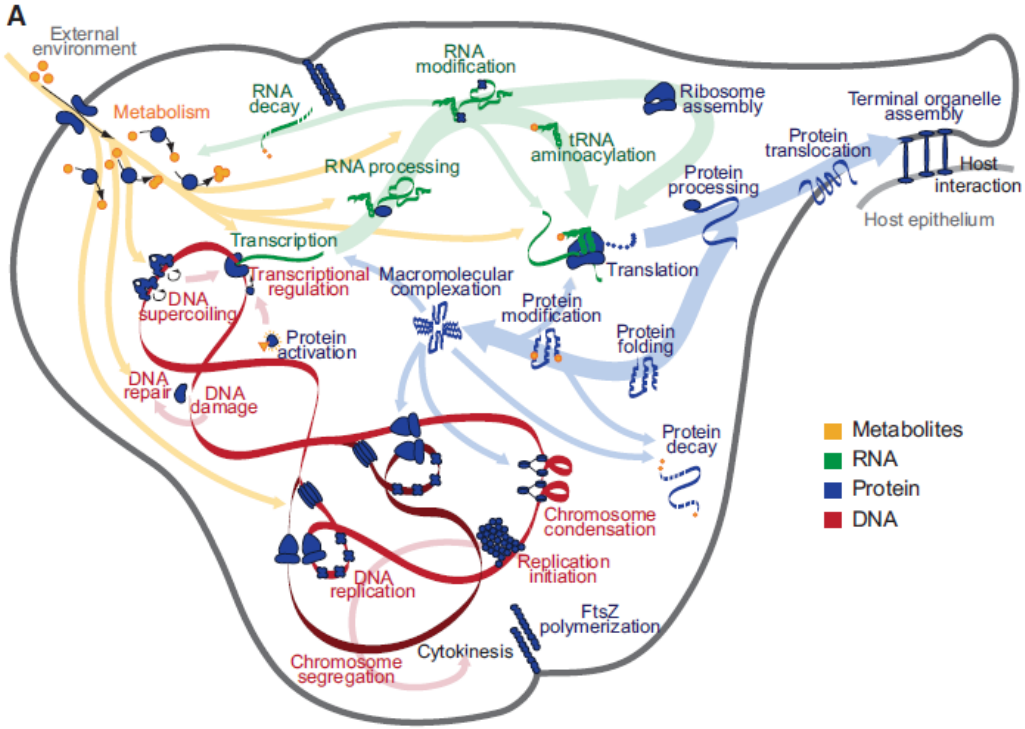




Berthoumieux *et al.* (2013), *Mol. Syst. Biol.*, 9:634

Whole-cell model *M. genitalium*

- Metabolic networks are integrated with gene networks and signalling networks
  - Complex multi-level system with feedback across different time-scales

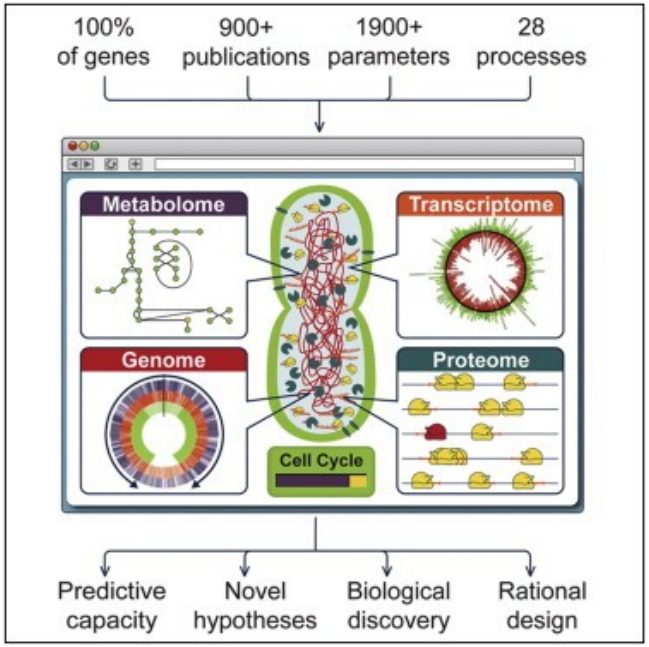


Whole-cell model of *Mycoplasma genitalium*

Karr *et al.* (2012), *Cell*, 150(2): 389-401

Whole-cell model *M. genitalium*

- Whole-cell model represents huge modelling effort:
  - Whole-genome model including **complete** known metabolic, gene, and signalling networks



Karr *et al.* (2012), *Cell*, 150(2): 389-401

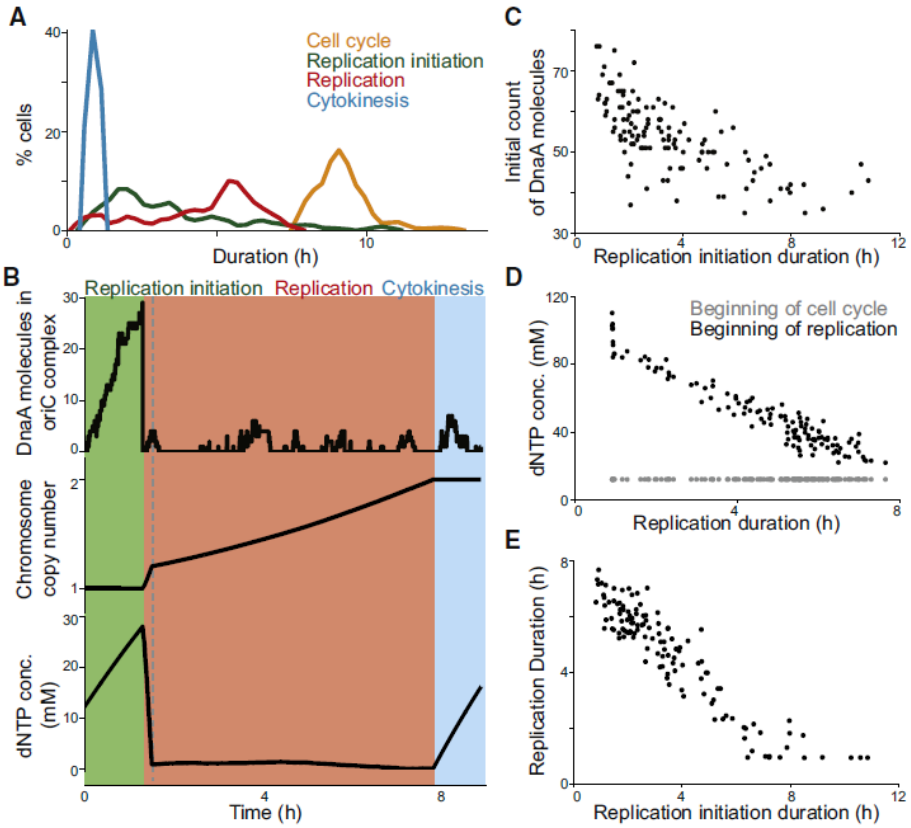
  - Variety of **formalisms** to model the 28 modules: FBA, kinetic ODE models, Boolean models, Markov chains, …
  - Cell cycle simulated for >100 cells, >30 mutants on 128-core machine

Whole-cell model *M. genitalium*

- Whole-cell simulation of *M. genitalium* cell cycle

Whole-cell model *M. genitalium*

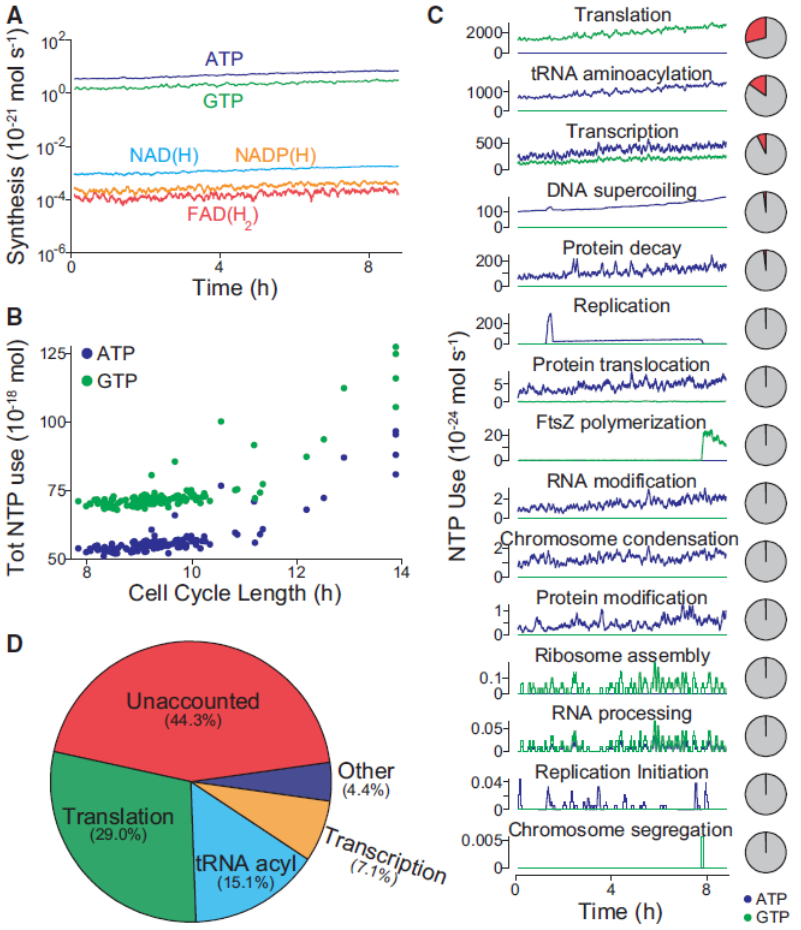
- Whole-cell simulations have provided new insights into **robustness of cell-cycle duration**
  - High variability of replication initiation buffered by dNTP-dependent duration of replication
  - This metabolic control of replication leads to decreased variability of cell-cycle length



Karr *et al.* (2012), *Cell*, 150(2): 389-401

Whole-cell model *M. genitalium*

- Whole-cell simulations have provided new insights into **global use and allocation of energy**
  - Transcription and translation most costly processes
  - Energy use largely independent of cell-cycle length
  - Usage of almost half of produced energy not accounted for!



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Conclusions kinetic multi-scale models

- Kinetic multi-scale models help analyse the dynamics of the interactions between multiple functions of the cell
  - Inclusion of different time-scales and different levels of regulation
  - Prediction of dynamics of complex nonlinear system with feedback loops across different levels
  - Models allow predictions to be confronted with experimental data and performance of thought experiments
  - Towards whole-cell models!

Conclusions kinetic multi-scale models

- But kinetic models have problems as well!
  - Models **difficult to construct**, to debug and to maintain
  - Huge **number of parameters**, many unknown: parameter estimation is a difficult problem requiring many data of high quality
  - How do we **extract fundamental insights** on cell functioning from large, mechanistic models?

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On Exactitude in Science

Jorge Luis Borges, *Collected Fictions*, translated by Andrew Hurley.

…In that Empire, the Art of Cartography attained such Perfection that the map of a single Province occupied the entirety of a City, and the map of the Empire, the entirety of a Province. In time, those Unconscionable Maps no longer satisfied, and the Cartographers Guilds struck a Map of the Empire whose size was that of the Empire, and which coincided point for point with it. The following Generations, who were not so fond of the Study of Cartography as their Forebears had been, saw that that vast Map was Useless, and not without some Pitilessness was it, that they delivered it up to the Inclemencies of Sun and Winters. In the Deserts of the West, still today, there are Tattered Ruins of that Map, inhabited by Animals and Beggars; in all the Land there is no other Relic of the Disciplines of Geography.

—Suarez Miranda, *Viajes de varones prudentes*, Libro IV, Cap. XLV, Lerida, 1658

Kinetic modelling of metabolism

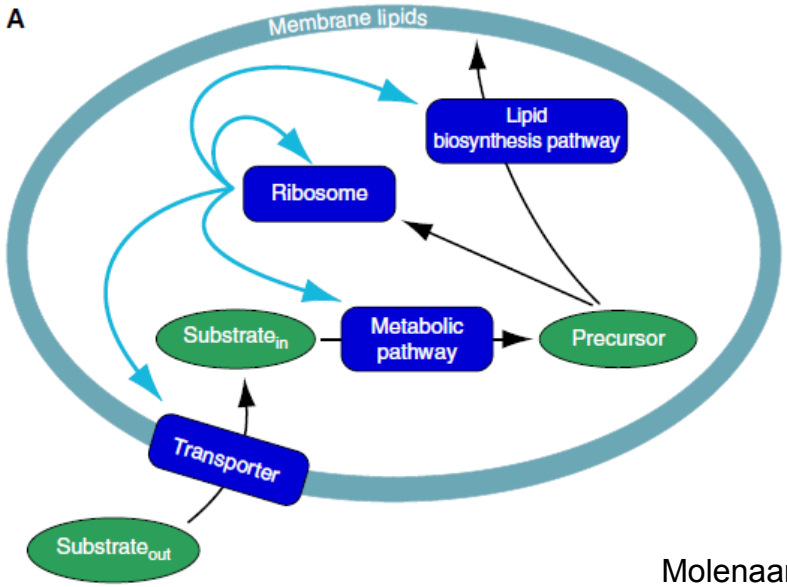
- Kinetic models of form $\dot{x} = N\,v(x)$
  - Concentration variables $x \in \mathbb{R}^n_+$
  - Reaction rates $v : \mathbb{R}^n_+ \rightarrow \mathbb{R}^q$
  - Stoichiometry matrix $N \in \mathbb{Z}^{n \times q}$

Heinrich and Schuster (1996), *The Regulation of Cellular Systems*, Chapman & Hall

- Stochiometry matrix N describes structure of reaction network
- Reaction rate v depends on concentrations of other cellular components

Coarse-grained model of the cell

- Minimal model of the cell based on **coarse-grained description** of growth-related processes
  - Molecular pools and macroreactions

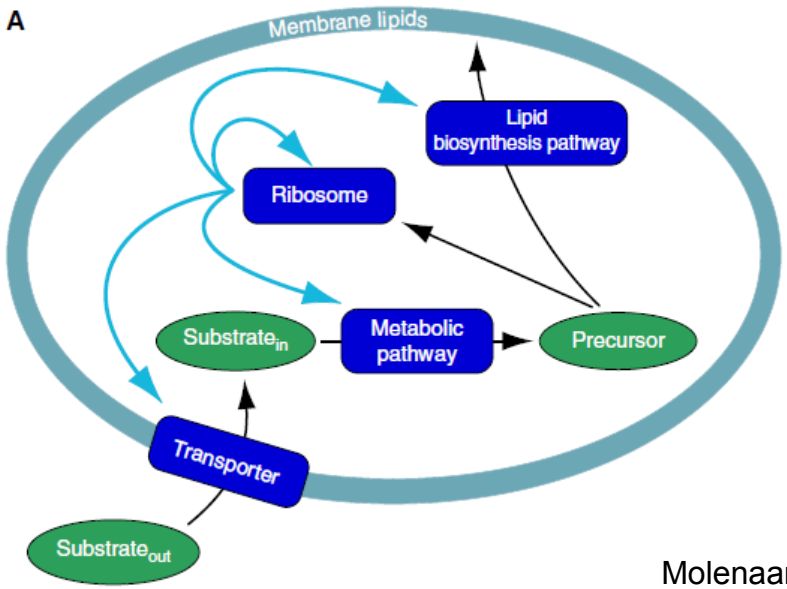


Molenaar *et al.* (2009), *Mol. Syst. Biol.*, 5:323

Coarse-grained models of the cell

- **Question**: how does cell allocate resources to different processes so as to optimize growth?

  Optimization problem similar to FBA



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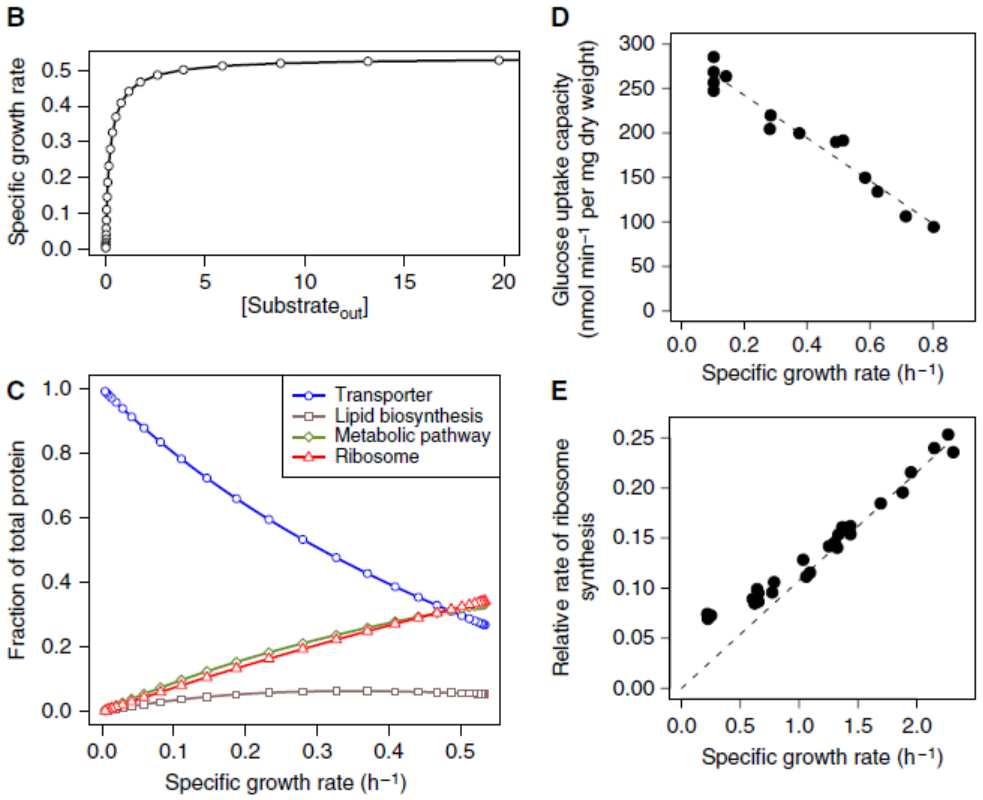

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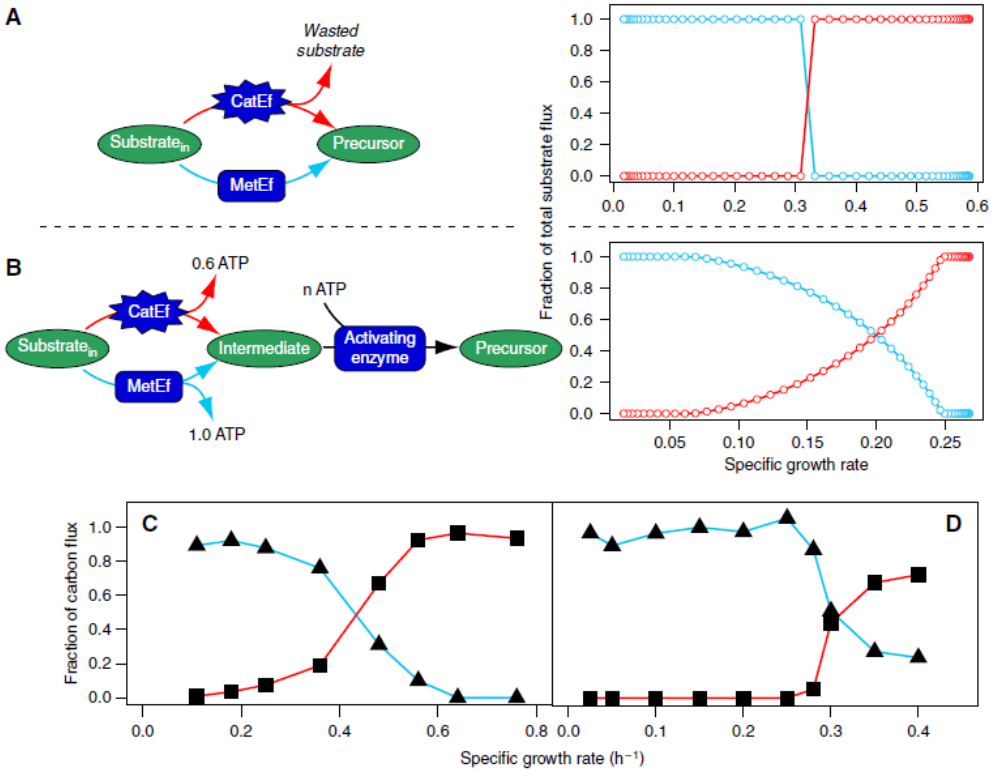
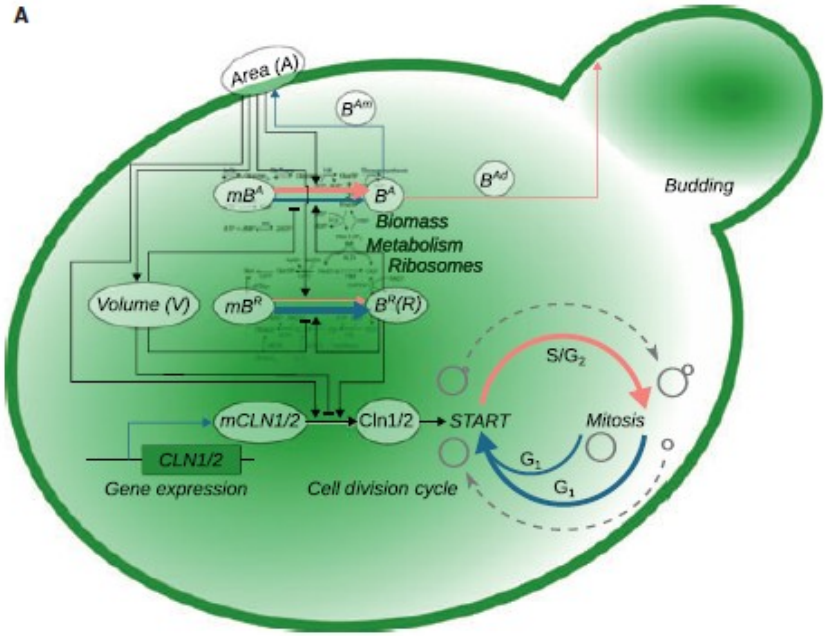


Molenaar *et al.* (2009), *Mol. Syst. Biol.*, 5:323

Coarse-grained models of the cell

- **Question**: how does cell recognize time to trigger cell division?

  Kinetic model connecting cell cycle, metabolism, and growth in yeast



Spiesser *et al.* (2012), *FEBS J.*, 279:4312-30

Table 2. List of model equations. The model consists of five differential equations (1)–(5), one stochastic function (8) and five algebraic equations (9)–(13).

$$\frac{dmCLN1/2}{dt} = -k_{d1} * f(mCLN1/2,\ t) \quad (1)$$

$$\frac{dCln1/2}{dt} = k_p * mCLN1/2 * B^R * \frac{A}{V} - k_{d2} * Cln1/2 \quad (2)$$

$$\frac{dB^R}{dt} = k_{growth} * \left(\frac{k_R}{k_R + k_{Am} + k_{Ad}}\right) * mB^R * B^R * \frac{A}{V} \quad (3)$$

$$\frac{dB^{Am}}{dt} = k_{growth} * \left(\frac{k_{Am}}{k_R + k_{Am} + k_{Ad}}\right) * mB^A * B^R * \frac{A}{V} \quad (4)$$

$$\frac{dB^{Ad}}{dt} = k_{growth} * \left(\frac{k_{Ad}}{k_R + k_{Am} + k_{Ad}}\right) * mB^R * B^R * \frac{A}{V} \quad (5)$$

$$mB^R = \text{constant} \quad (6)$$

$$mB^A = \text{constant} \quad (7)$$

$$f(mCLN1/2,\ t) = \begin{cases} mCLN1/2(t_i) + \text{randint}(0,1) & \text{if } t = t_i \\ mCLN1/2(t) & \text{otherwise} \end{cases} \quad (8)$$

$$\Delta A^m = k_x * \Delta B^{Am} \quad (9)$$

$$\Delta A^d = k_x * \Delta B^{Ad} \quad (10)$$

$$A(t) = A^m(t) + A^d(t) \quad (11)$$

$$V^x(t) \propto [A^x(t)]^{3/2} \text{ for } x \in \{m, d\} \quad (12)$$

$$V(t) = V^m(t) + V^d(t) \quad (13)$$

Conclusions kinetic multi-scale models

- Course-grained models help analyse the dynamics of the interactions between multiple functions of the cell
  - Minimal assumptions on molecular mechanisms at work
  - Models easier to calibrate and to explore
  - Focused on understanding qualitative trends rather than quantitative precision
- However, models are usually valid only within limited range of conditions!

  Implicit assumptions on environment

Conclusions

- Adaptation of bacteria to their environment involves reorganisation of cellular physiology
- Increasingly powerful methods have become available to experimentally quantify cellular adaptation
  - Transcriptomics, proteomics, fluxomics, metabolomics, …
- Adaptation process achieved by large and complex regulatory networks
  - Nonlinear dynamical systems with feedback across different time-scales
- Fundamental questions on network functioning remain unanswered and require integrated models of the cell
  - Multiple functions, multiple regulatory levels, interactions with environment and ecosystem, …

Conclusions

- Several approaches have been tried to develop and exploit integrated models of the cell
  - Flux balance models
  - Kinetic models of cellular functions: towards whole-cell models
  - Resource allocation models
- Issues for development of such models:
  - Scope
  - Granularity
  - Mathematical methods
  - …

Conclusions

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- Issues for development of such models
  - Scope
  - Granularity
  - Mathematical methods
  - …
- Most importantly, models are tools for a purpose: **a different model for a different question**

Most fundamental questions are still open

- How does the multi-level **feedback structure** of the network give rise to **dynamical properties** of adaptive response?

  Can we formulate **general laws** that explain a variety of phenomena on the molecular level?

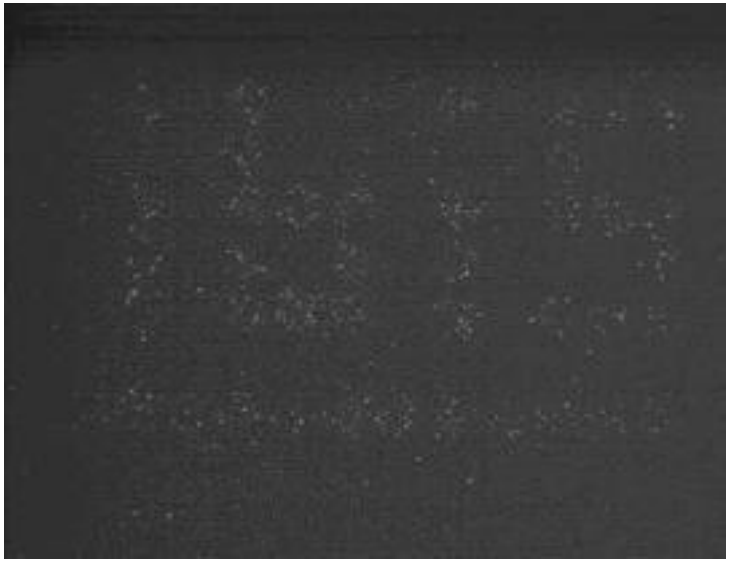
- How does repertoire of dynamical properties of the cell respond to **challenges from ecosystem**?

  Why have these properties been **evolutionary conserved** in environment?

  How do bacterial cells cooperate and evolve in **consortia of microorganisms**?

Internships in IBIS

- Challenging problems for biologists, physicists, computer scientists, mathematicians, …
- … in a multidisciplinary working environment
- Contact: Hidde.deJong@inria.fr and ibis.inrialpes.fr



Courtesy Guillaume Baptist (2008)

Merci !

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