Introduction

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INRIA Grenoble - Rhône-Alpes and IBIS

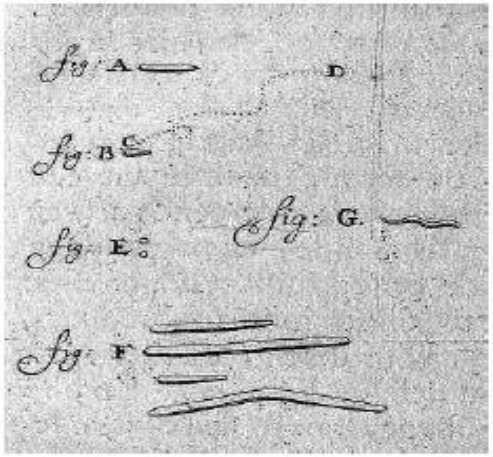
- IBIS: systems biology group at INRIA/Université Grenoble-Alpes
  - Analysis of bacterial regulatory networks by means of models and experiments
  - Biologists, computer scientists, mathematicians, physicists, …

http://team.inria.fr/ibis

Bacteria

- Bacteria were first observed by Antonie van Leeuwenhoek, using a single-lens microscope of his own design

http://commons.wikimedia.org/



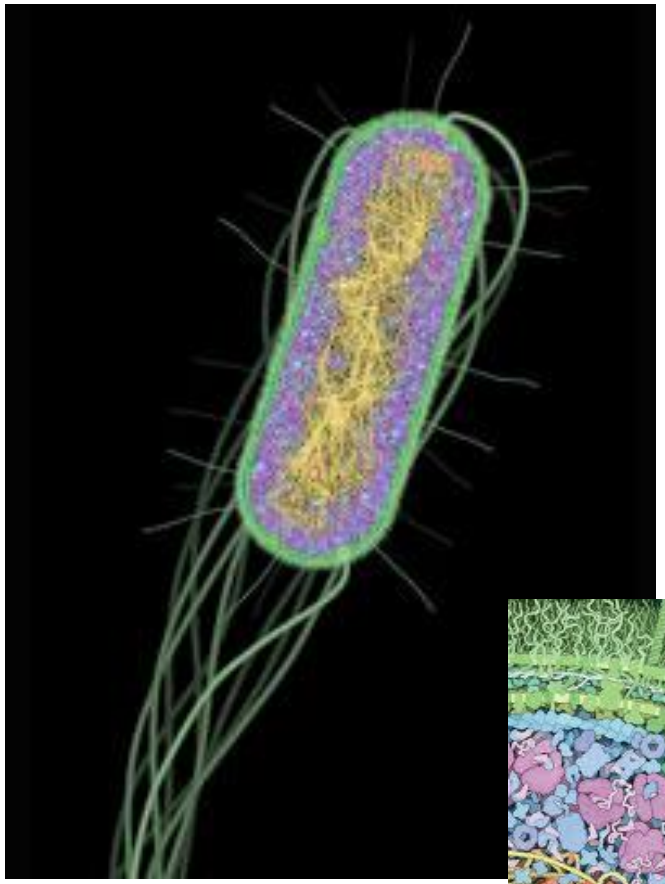
www.euronet.nl/users/warnar/leeuwenhoek.html.

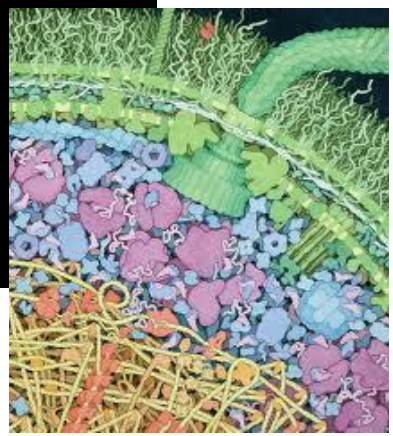
van Leeuwenhoek A (1684), *Philosophical Transactions (1683–1775)* 14: 568–574

"In the morning I used to rub my teeth with salt and rinse my mouth with water and after eating to clean my molars with a toothpick.... I then most always saw, with great wonder, that in the said matter there were many very ***little living animalcules****, very prettily a-moving. The biggest sort had a very strong and swift motion, and shot through the water like a pike does through the water; mostly these were of small numbers."*

Bacteria are complex living systems

- Bacterial cells are complex biochemical and biophysical machines
  - Wide range of shapes, typically 0.5-5 µm in length
  - 10^6 bacterial cells in 1 ml of fresh water
  - About as much bacterial cells as human cells in human body

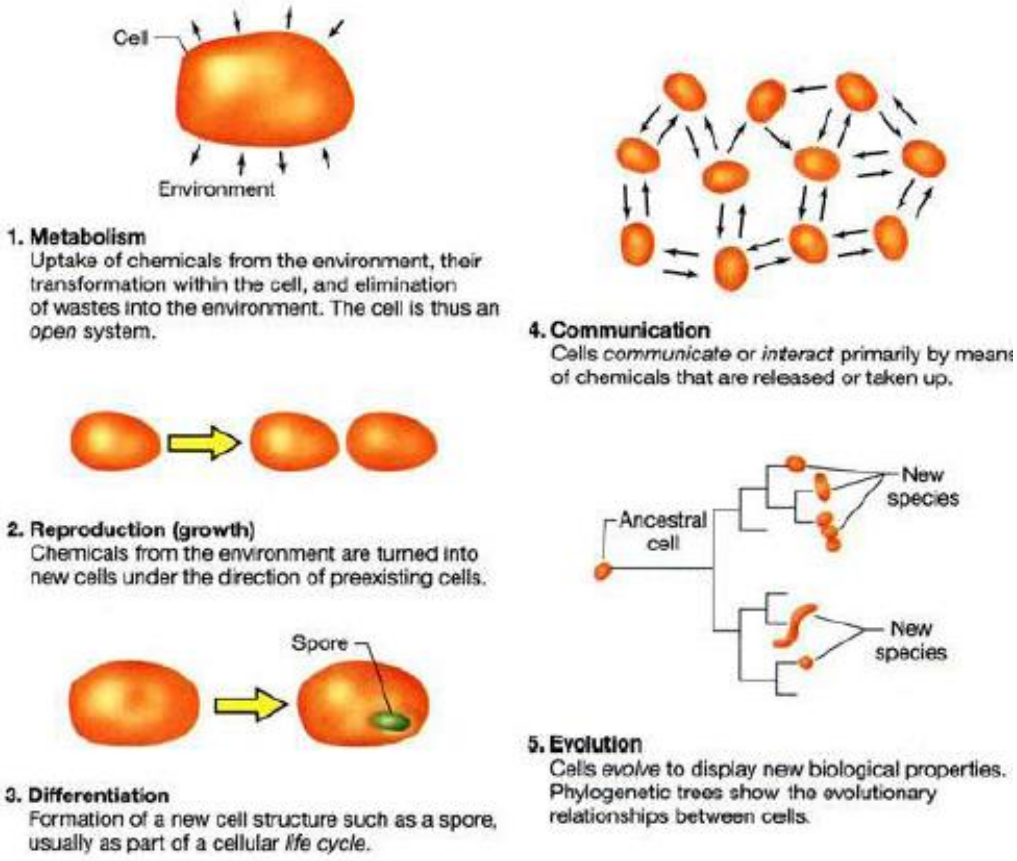




Goodsell (2010), *The Machinery of Life, Springer,* 2nd ed.

Bacteria are complex living systems

- Bacterial cells are complex biochemical and biophysical machines
- Bacteria possess characteristics shared by most living systems:
  - Metabolism
  - Growth and reproduction
  - Differentiation
  - Communication
  - Evolution

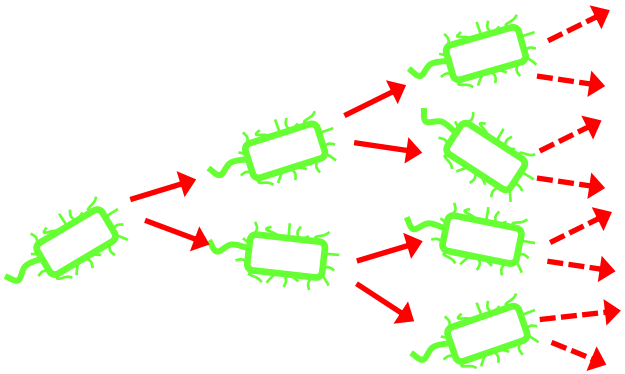


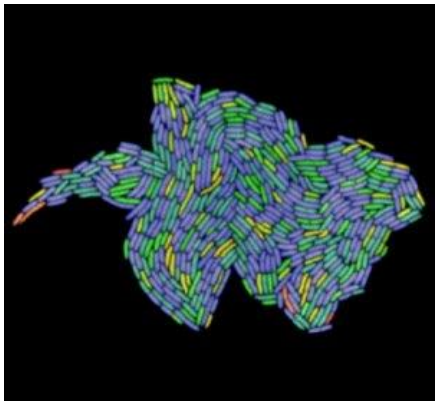
Madigan *et al.* (2003), *Brock Biology of Microorganisms*, Prentice Hall, 10th ed.

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

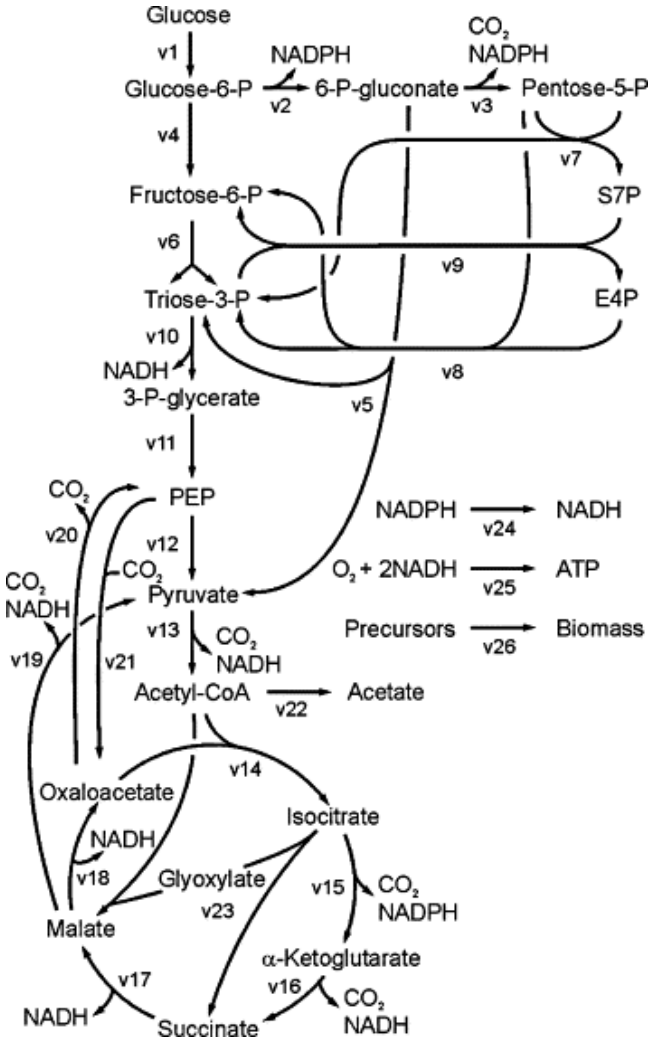
- **Metabolism** fuels growth by production of energy and building blocks for macromolecules, using nutriments from environment

  ATP, amino acids, nucleotides, …

Bacterial growth and metabolism

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

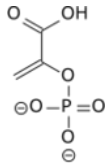
  Glucose, acetate, lactose, …

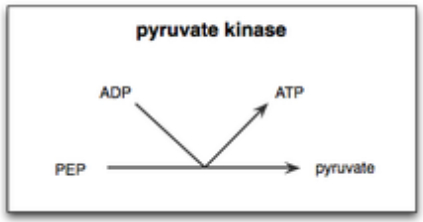


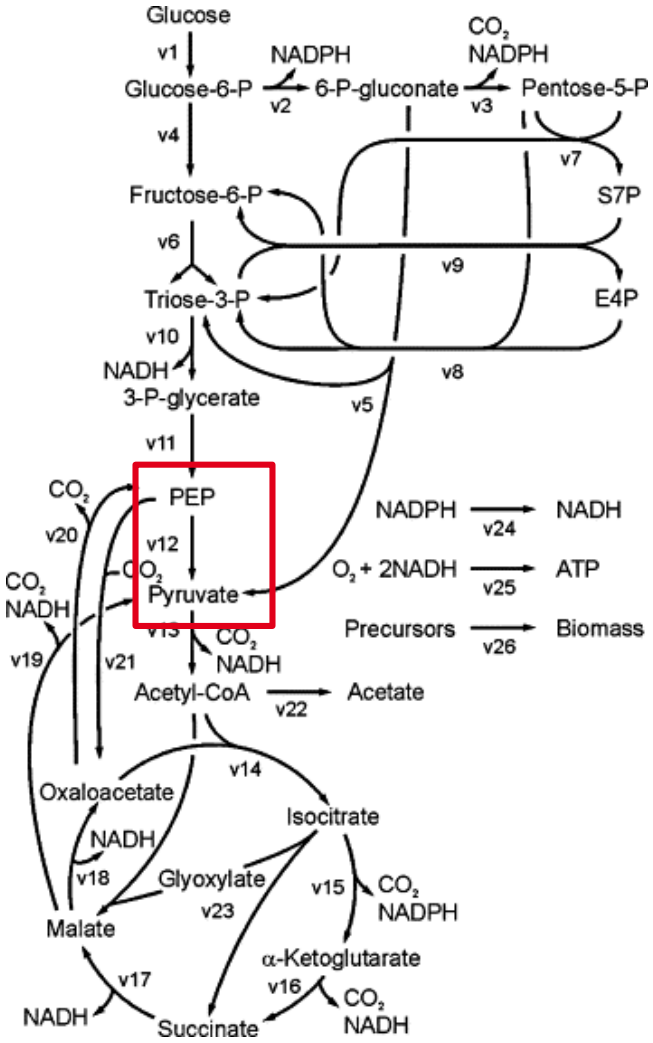
Fischer *et al.* (2004), *Anal. Biochem.*, 325(2):308–16

Bacterial growth and metabolism

- Central **carbon metabolism** breaks down carbon sources for energy production and macromolecular synthesis
  - Glucose, acetate, lactose, …
- **Enzymes** catalyse individual steps in metabolic network
  - Pyruvate kinase transforms phosphoenolpyruvate (PEP) into pyruvate





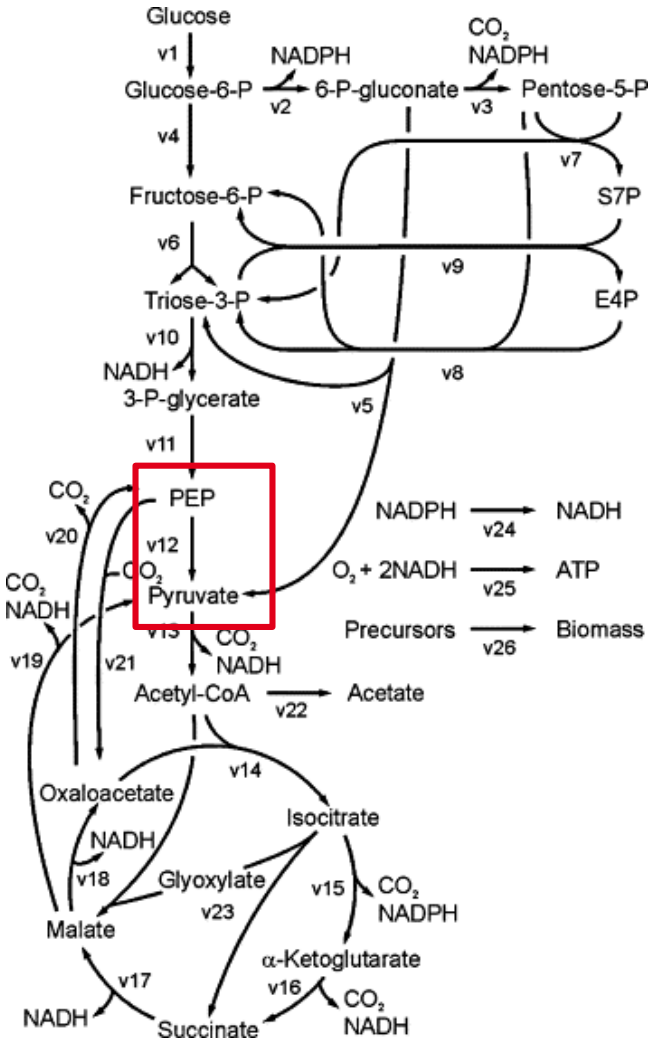


Bacterial growth and metabolism

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

  Glucose, acetate, lactose, …

- Enzymes produced from information encoded in **genes**
  - *pykF* is gene encoding pyruvate kinase

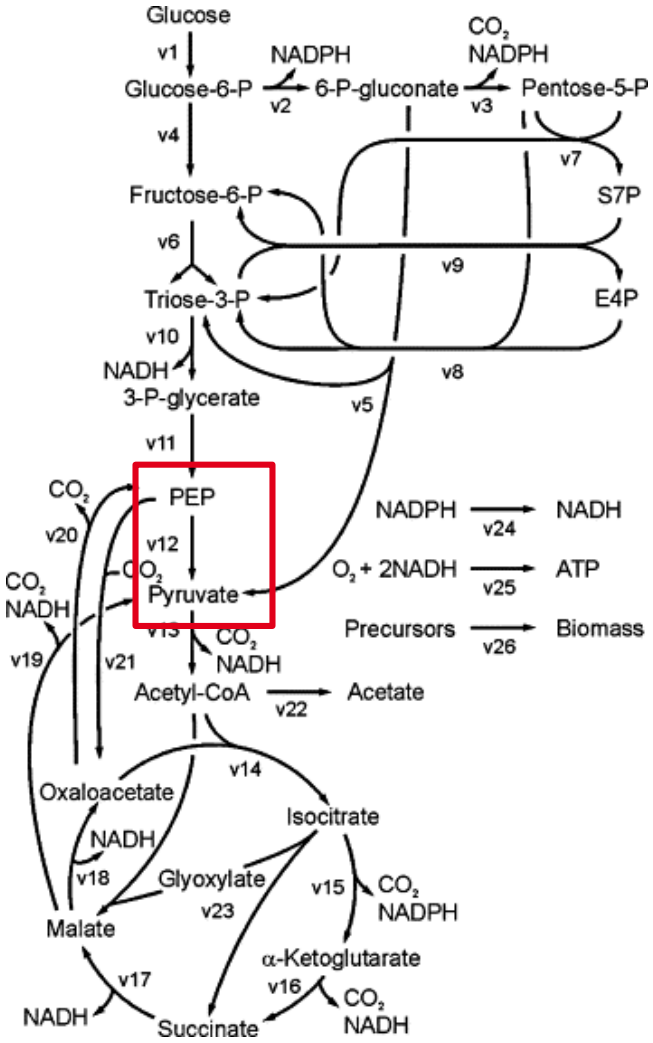


Bacterial growth and metabolism

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

  Glucose, acetate, lactose, …

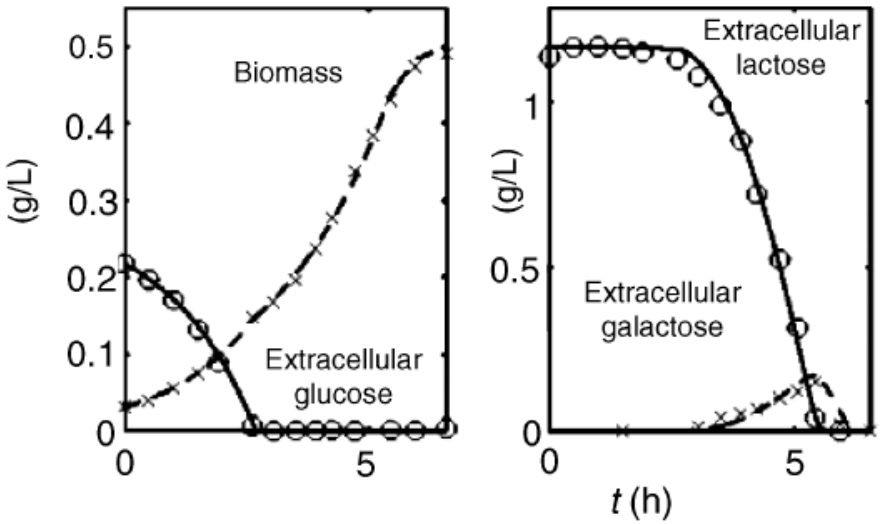
- Enzymes produced from information encoded in **genes**
  - *pykF* is gene encoding pyruvate kinase
  - Expression of *pykF* regulated by transcription factor Cra



Bacterial growth and metabolism

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

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



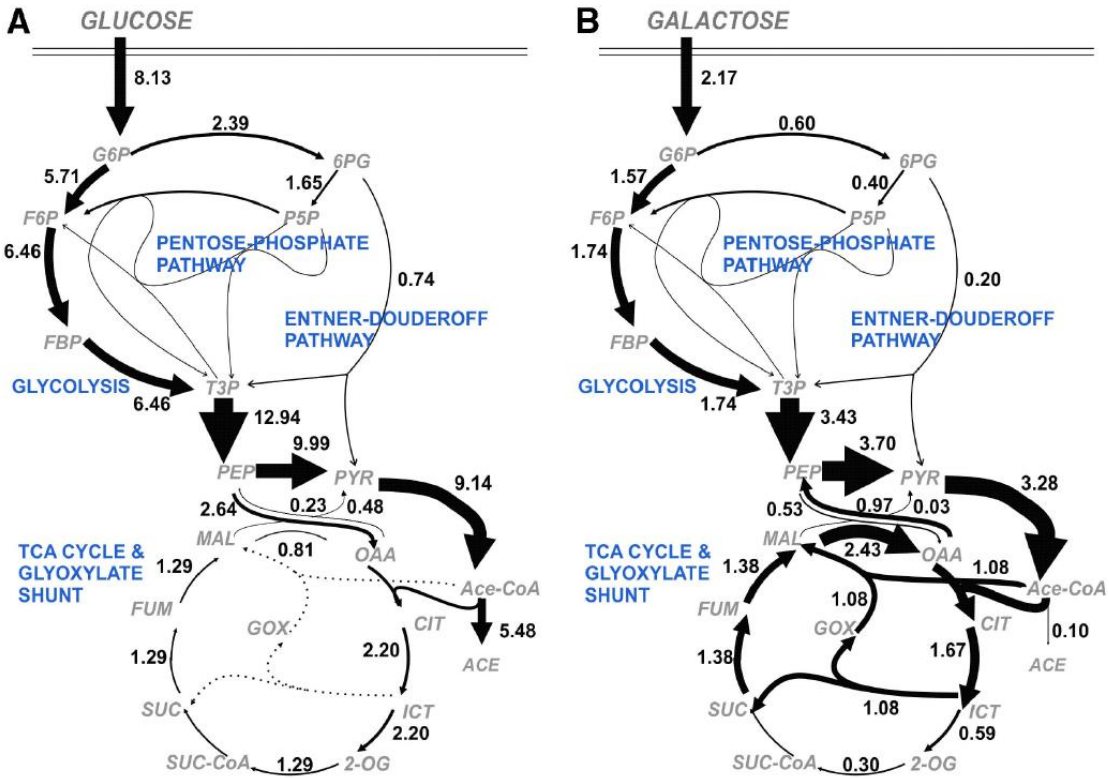
Bettenbrock *et al.* (2006), *J. Biol. Chem.*, 281(5):2578-84

- Adaptation of bacterial physiology to different carbon sources

Growth transition and metabolism

- Adaptation to different carbon source involves changes in **metabolic fluxes**

  Different flux distribution in central metabolism of *E. coli* during growth on glucose and galactose



Haverkorn van Rijsewijk *et al.* (2011), *Mol. Syst. Biol.*, 7:477

Growth transition and metabolism

- Adaptation to different carbon source involves adjustment of **metabolite concentrations**
  - Different metabolite concentrations in *E. coli* cells growing on glucose and acetate

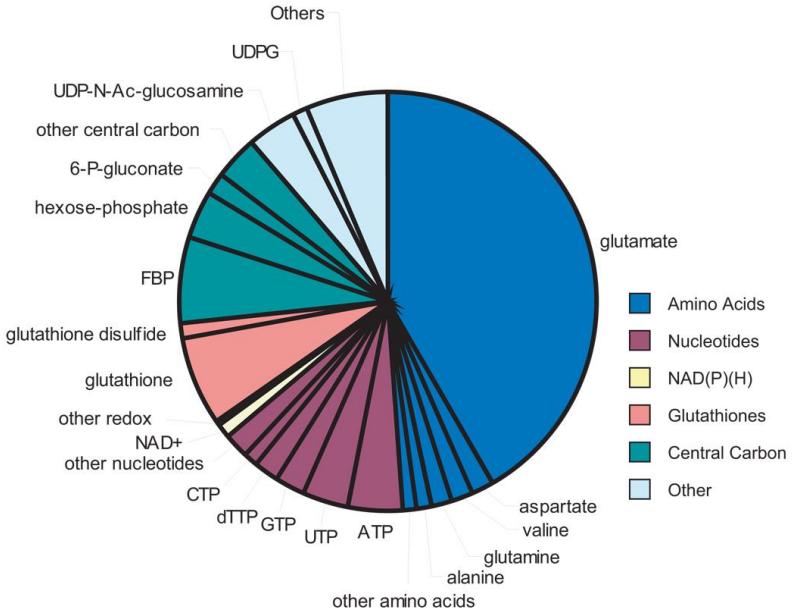


Table 1 Intracellular metabolite concentrations in glucose-fed, exponentially growing *E. coli*

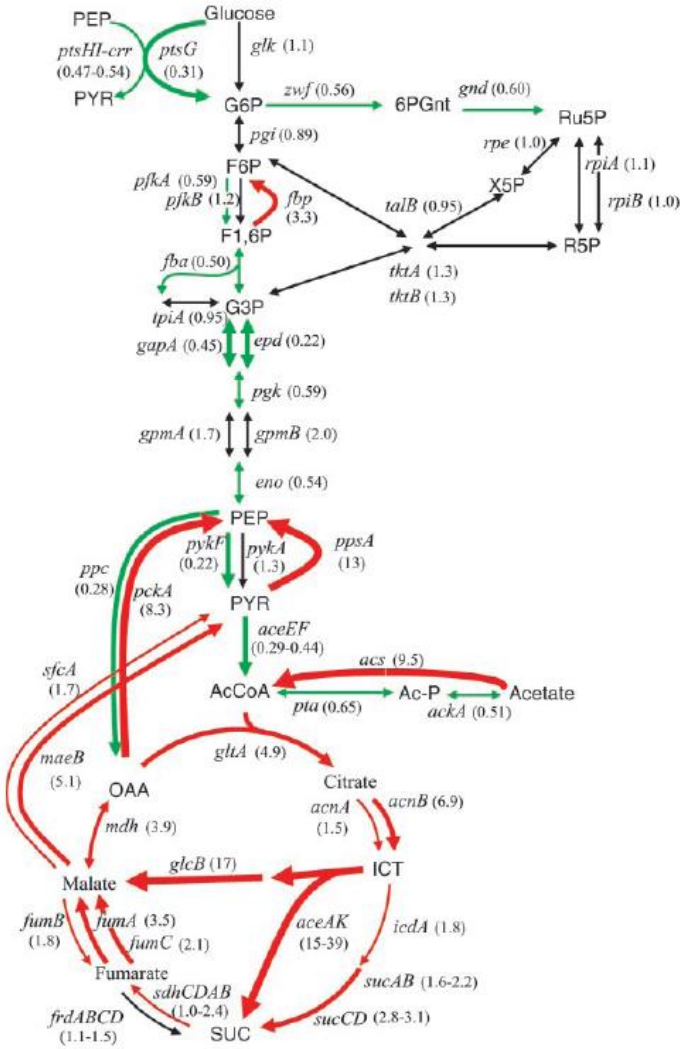
Metabolite	mol l^{-1}	Metabolite	mol l^{-1}
Glutamate	9.6×10^{-2}	UDP-glucuronate (51)	5.7×10^{-4}
Glutathione	1.7×10^{-2}	ADP	5.6×10^{-4}
Fructose-1,6-bisphosphate	1.5×10^{-2}	Asparagine (52)	5.1×10^{-4}
ATP	9.6×10^{-3}	α-Ketoglutarate	4.4×10^{-4}
UDP-*N*-acetylglucosamine (29)	9.2×10^{-3}	Lysine (53)	4.1×10^{-4}
Hexose-P[a]	8.8×10^{-3}	Proline (54)	3.9×10^{-4}
UTP (30)	8.3×10^{-3}	dTDP (55)	3.8×10^{-4}
GTP (31)	4.9×10^{-3}	Dihydroxyacetone phosphate	3.7×10^{-4}
dTTP	4.6×10^{-3}	Homocysteine (56)	3.7×10^{-4}
Aspartate	4.2×10^{-3}	CMP (57)	3.6×10^{-4}
Valine (32)	4.0×10^{-3}	Deoxyribose-5-P (58)	3.0×10^{-4}
Glutamine	3.8×10^{-3}	Isoleucine (59) + leucine (60)	3.0×10^{-4}
6-Phosphogluconate	3.8×10^{-3}	AMP	2.8×10^{-4}

Bennett *et al.* (2009), *Nat. Chem. Biol.*, 5(8):593-9

Growth transition and gene expression

- Adaptation to different carbon source involves adjustment of **expression of enzymatic genes**

  Difference in expression levels of genes encoding enzymes in central metabolism of *E. coli* during growth on glucose and acetate

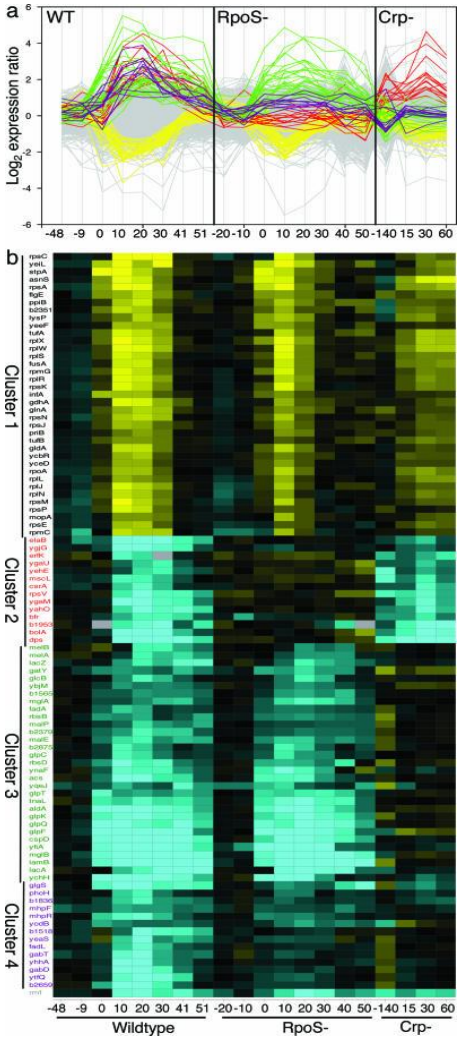


Oh *et al.* (2002), *J. Biol. Chem.*, 277(15):13175–83

Growth transition and gene expression

- Adaptation to different carbon source involves **genome-wide reorganisation of gene expression**

  Gene expression during glucose-lactose shift in *E. coli*



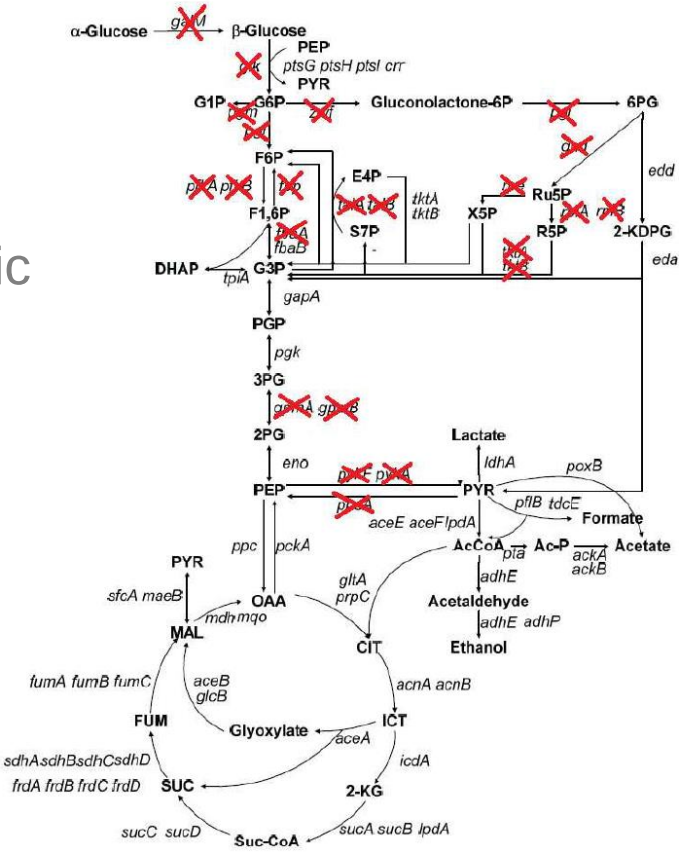
Traxler *et al.* (2006), *Proc. Natl. Acad. Sci. USA*, 103(7):2374–9

Adaptation on multiple levels

- Adaptation to different carbon source involves **adjustments on multiple levels** at the same time!

  Parallel measurement of enzyme and metabolite concentrations, and metabolic fluxes in a variety of experimental conditions

Ishii *et al.* (2007), *Science*, 316(5284):593-7



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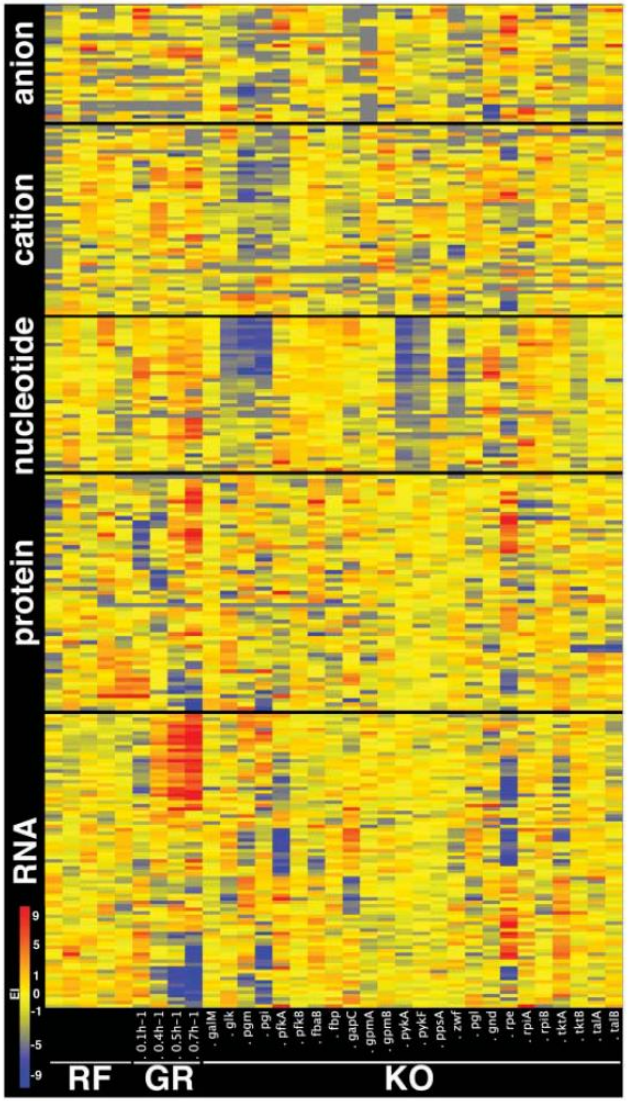
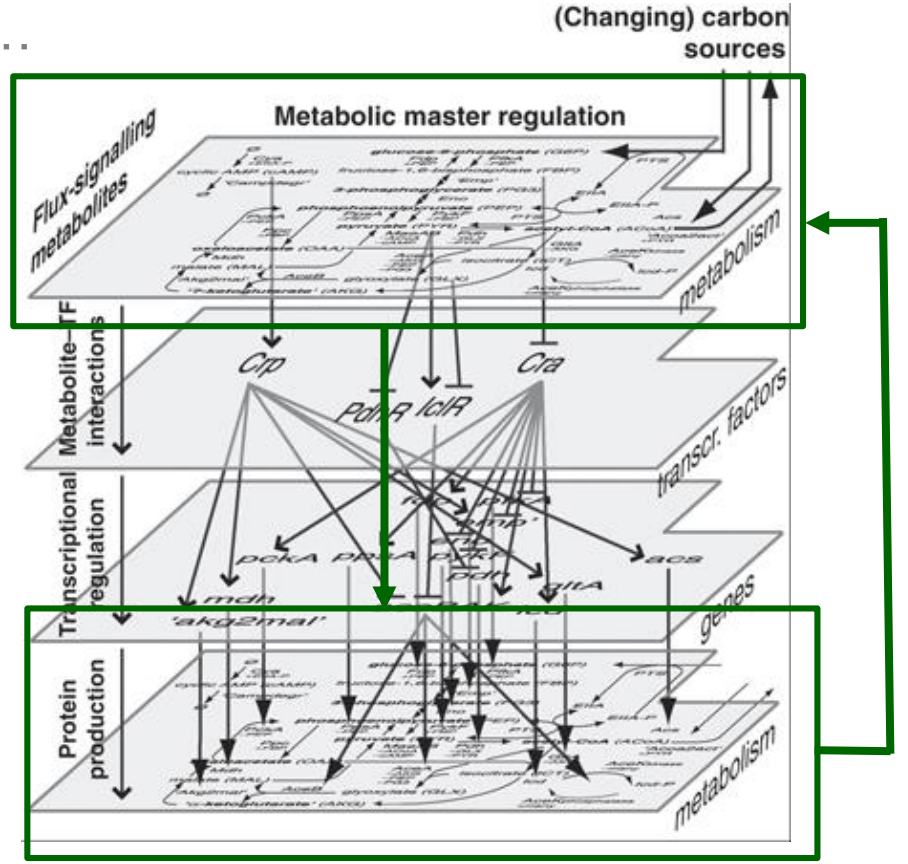


General question on cellular adaptation

- Cells are capable of responding to a variety of changes in their environment by adapting their physiology
  - Change in carbon source, starvation, population density, …
- On the molecular level, these responses involve adjustment of metabolism and gene expression
  - Cellular concentrations of metabolites, enzymes, transcription factors, …
- **Question**: how does cell coordinate these adaptive responses?

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

No global view on network functioning

- Coordination of adaptative responses of bacterial cell achieved by large and complex regulatory networks
- Abundant knowledge on biochemical mechanisms underlying interactions between network components
- Accumulation of data on multi-level response of network to external perturbations

  Metabolic fluxes and cellular concentrations of metabolites, enzymes, transcription factors, signalling molecules, …
- However, **global view on functioning of entire network** is difficult to achieve and largely absent today

Mathematical models and systems biology

- Regulatory networks are **complex nonlinear dynamical systems**, evolving on different time-scales
- **Challenge:** can mathematical models and computer tools help us understand how these systems function?
  - Integration of interaction structure and heterogenous data sources into mathematical models
  - Use of models to analyse and predict dynamical behaviour of system
  - Emergence of new discipline: **systems biology**…

Alon (2007), *An Introduction to Systems Biology*, Chapman & Hall/CRC Press

Historical note

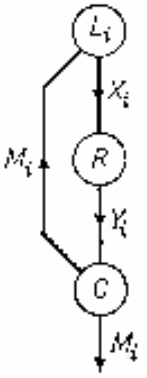
- Systems biology, and more particularly the mathematical modeling and computer simulation of biochemical reaction networks, have a long history

Westerhoff and Palsson (2004), *Nat. Biotechnol.*,22(10):1249-52

- Simulation of metabolic pathways (glycolysis)

Garfinkel *et al.* (1970), *Ann. Rev. Biochem.*, 39:473-98

- Modeling of gene regulatory networks



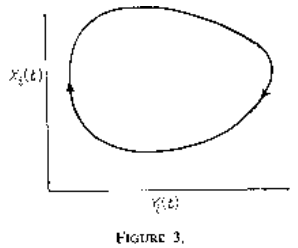
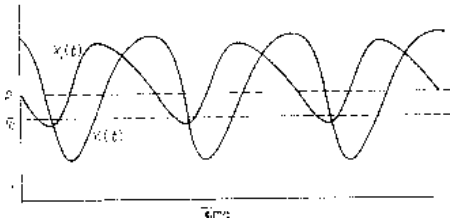


FIGURE 3.



Goodwin (1963), *Temporal Organization in Cells*, London

Mathematical modeling of biochemical reaction networks

- Well-established framework for modeling of biochemical reaction networks using **ordinary differential equation (ODE)** models
- General form of ODE models of biochemical reaction networks

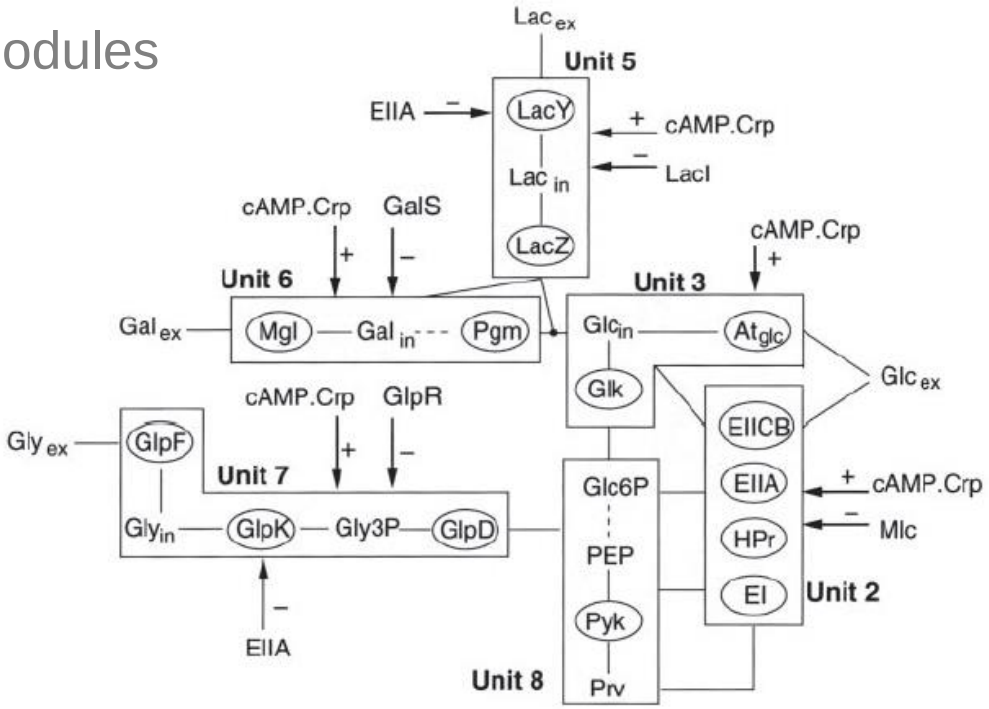
$$\dot{x} = N\, v(x)$$

  - Concentration variables $x \in \mathbb{R}^n_+$
  - Reaction rates $v : \mathbb{R}^n_+ \rightarrow \mathbb{R}^q$
  - Stoichiometry matrix $N \in \mathbb{Z}^{n \times q}$
- Various forms of kinetic rate laws: mass-action, Michaelis-Menten, Hill, Monod-Wyman-Changeux, …

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

Example of network modeling

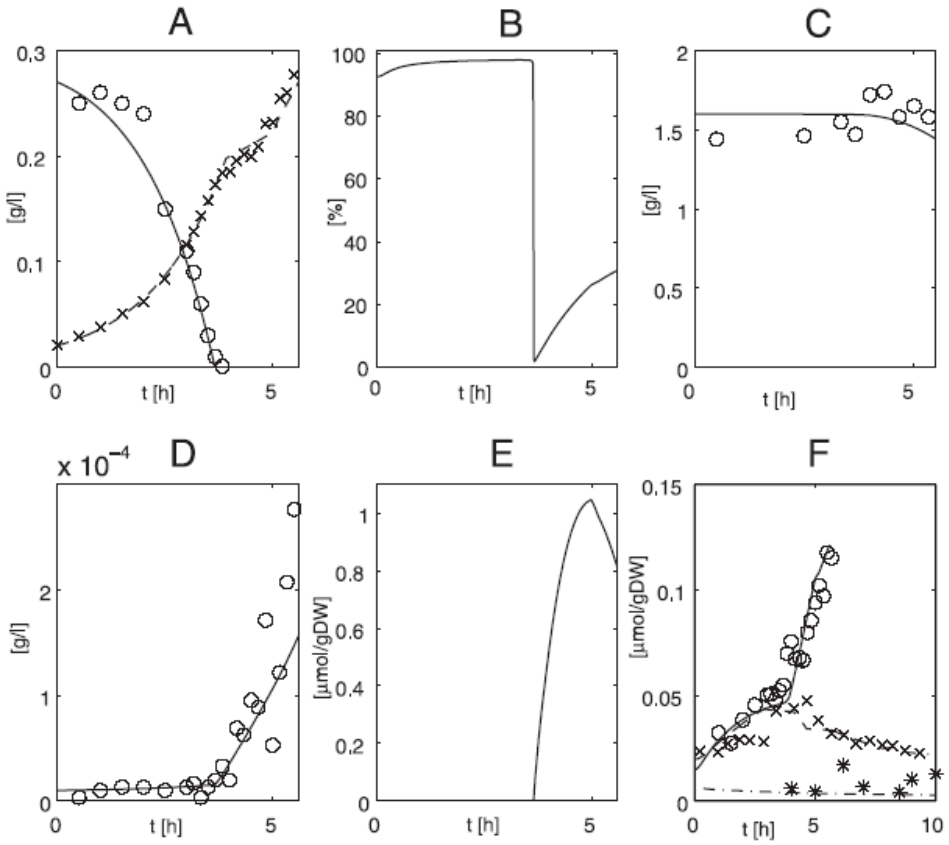
- Model of uptake of carbon sources (glucose, lactose, glycerol, …) by *E. coli*
  - Several dozens of equations and more than a hundred parameters, many of them unknown or unreliable
  - Mostly metabolic modules



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

Example of network modeling

- Estimation of parameter values from time-series measurements of metabolite concentrations on wild-type and mutant strains



- Model has good predictive capability

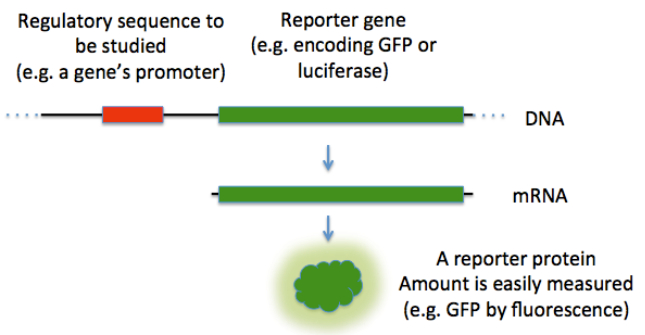
Bettenbrock *et al.* (2005), *J. Biol. Chem.*, 281(5): 2578-2584

Issues in mathematical modeling

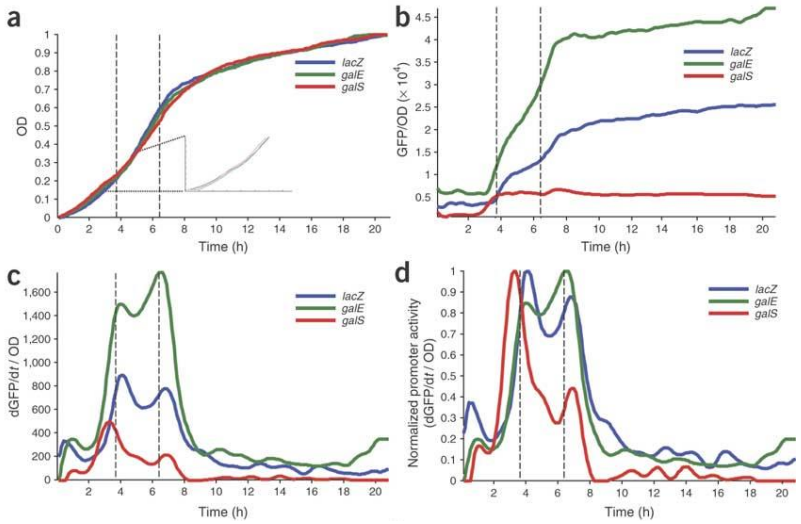
- Mathematical models are used for explanation, prediction, and control
- Modeler confronted with several **practical problems**
  - Models of actual networks are large systems of nonlinear ODEs
  - Parameter values are generally unknown and difficult to measure directly
  - Reaction mechanisms are often unknown
  - Experimental measurements of variables are scarce, noisy, and indirect
- This raises issues in model reduction and approximation, parameter estimation, network inference, data analysis, ...
- But also: issues in experimental **data acquisition**

Fluorescent reporter genes

- Use of **fluorescent reporter genes** allows expression from host promoter to be monitored *in vivo* and in real time
  - Different colors (emission peaks): GFP, YFP, RFP, …
  - Reporter genes on plasmids and on chromosome
  - Transcriptional and translational reporters

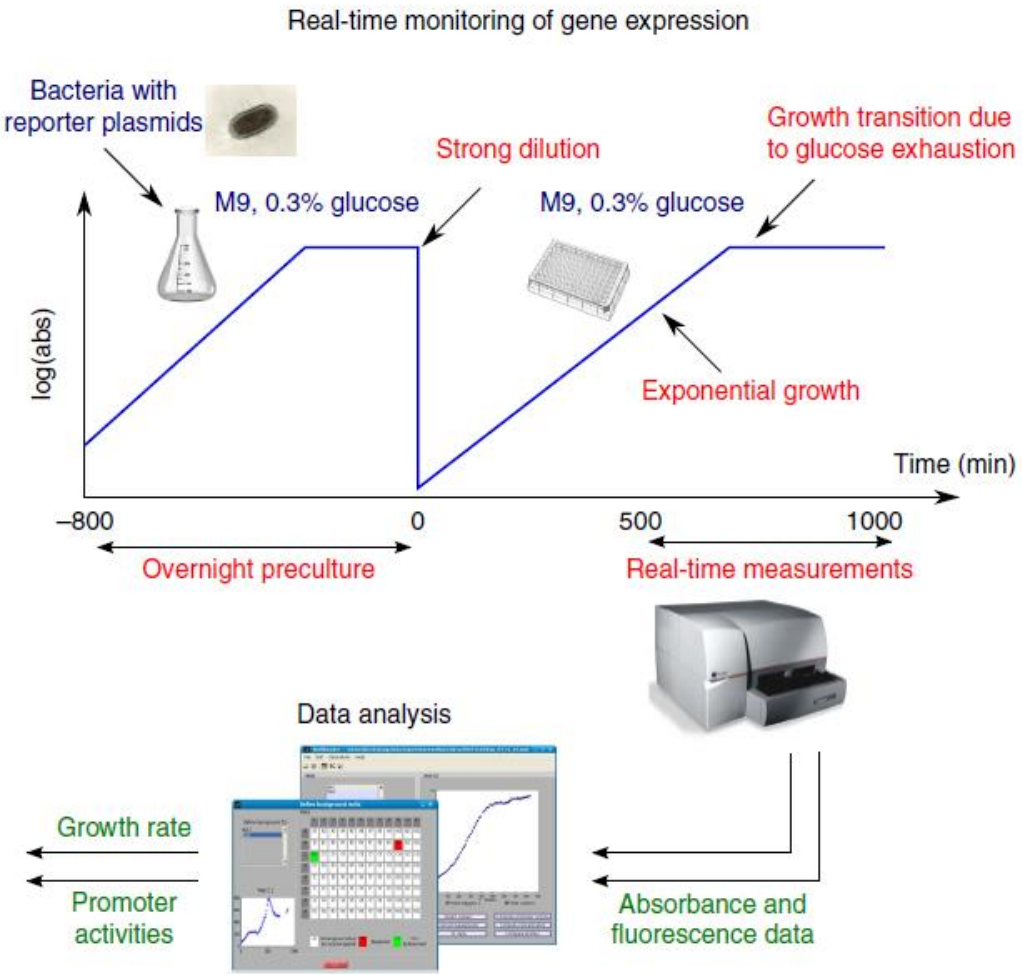


- Library of fluorescent transcriptional reporter genes in *E. coli*



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

Microplate readers



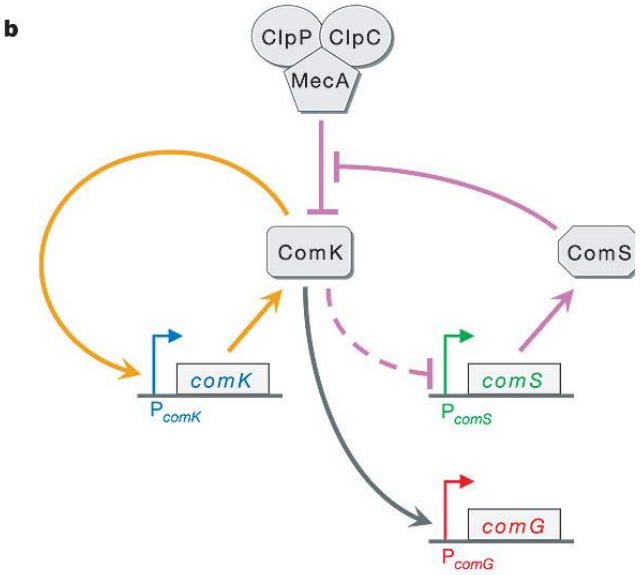
- Monitoring of gene expression on population level using fluorescent reporters and **automated microplate readers**

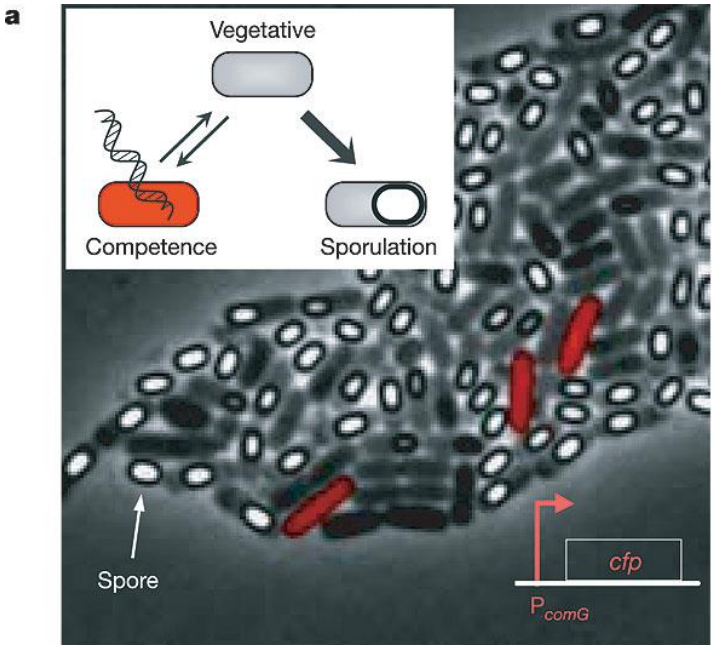
Berthoumieux *et al.* (2013), *Mol. Syst. Biol.*, 9:634

Single-cell microscopy

- Monitoring of gene expression in single cells using fluorescent reporters, **automated time-lapse microscopy**, and **image analysis**
- Monitoring onset of competence in *B. subtilis*

Süel *et al.* (2006), *Nature*, 440:545-50





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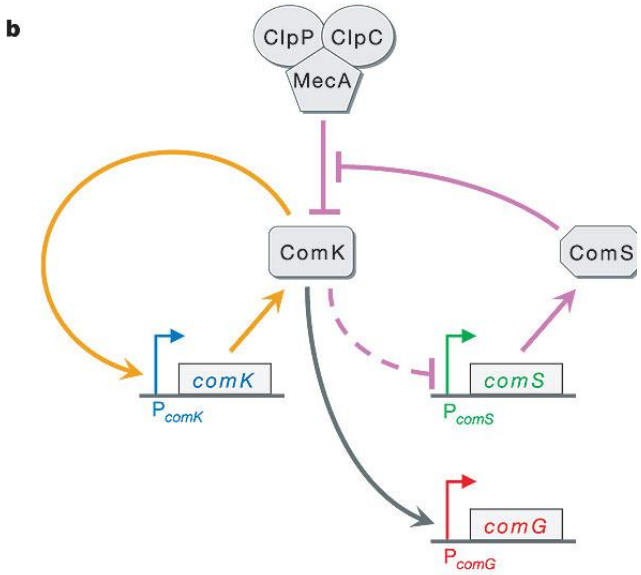
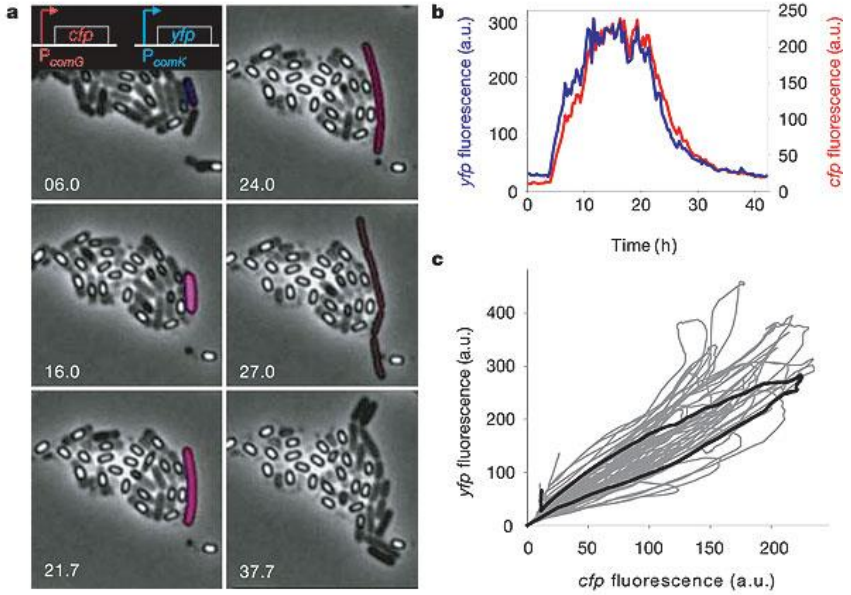
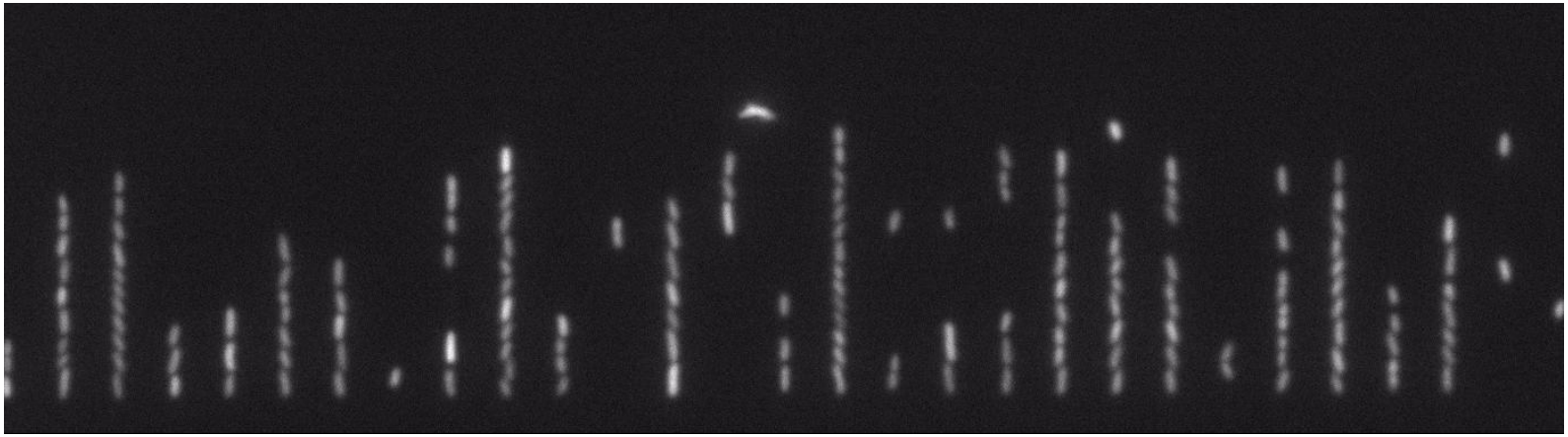




Single-cell microscopy and microfluidics

- **Microfluidic** trapping devices for long-term acquisition of single-cell data

Bennett and Hasty (2009), *Nat. Rev. Genet.*, 10(9):628-38



- Microfluidic devices allow tight control of environmental perturbations

Izard, Gomez Balderas *et al.* (2015), *Mol. Syst. Biol.*, 11:840

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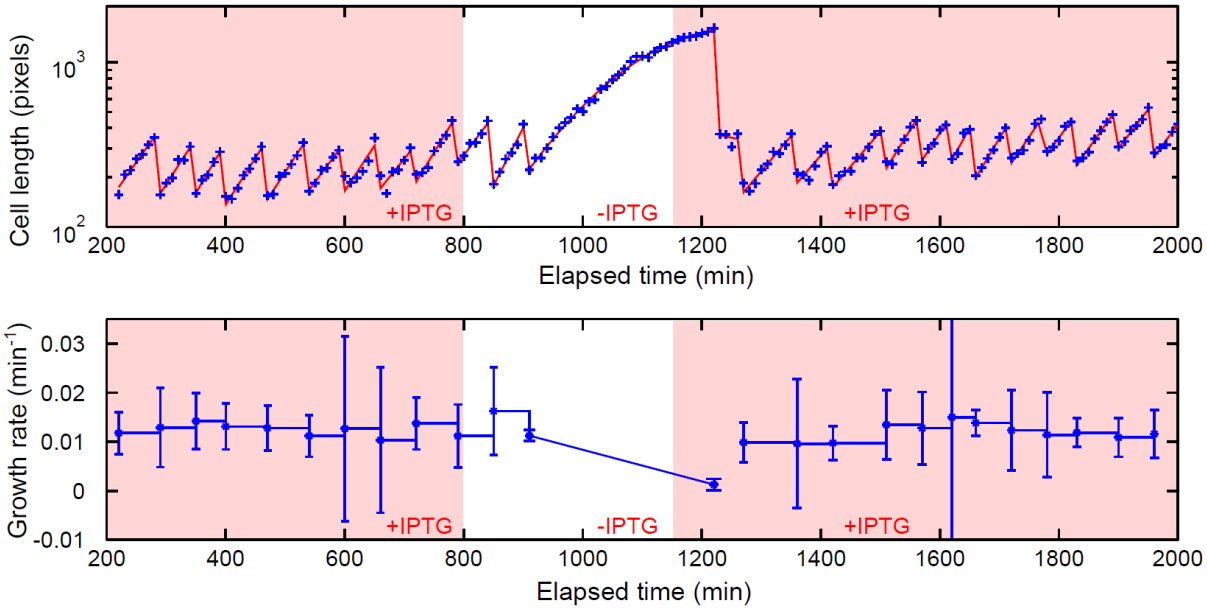

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Izard, Gomez Balderas *et al.* (2015), *Mol. Syst. Biol.*, 11:840

Objective of course "Modeling of biological networks"

- **Course objective** is to learn the modelling of cellular networks, in particular **metabolic networks** and **gene regulatory networks**
  - Both the theoretical foundations and concrete applications to diverse systems of biological regulation
  - Applications will rely on the practical use of computer tools for the modelling, analysis and simulation of biological networks

Course program

- Part 1. Systems biology and kinetic modeling (courses 4 h)
  - Introduction
  - Kinetic modeling of biochemical reaction networks
- Part 2. Metabolic network modeling (courses and practical 10 h)
  - Kinetic modeling of metabolism
  - Metabolic control analysis (MCA)
  - Flux balance analysis (FBA)
  - Practical on flux balance analysis (COBRA)

Course program

- Part 3. Gene regulatory network modeling (courses and practical 12 h)
  - Quantitative modeling of gene regulatory networks
  - Qualitative modeling of gene regulatory networks
  - Stochastic modeling of gene regulatory networks
  - Practical on integrated models of bacterial growth (Matlab)

Course organisation

- Schedule: courses 2h-3h on Wednesdays
- Credits: 2 units or 50 h:
  - Courses: 25 h
  - Mini-project: 25 h
- Articles to read, associated with courses
- Contact: Hidde de Jong (Hidde.de-Jong@inria.fr)
- Slides and articles will be made available on course web site: https://team.inria.fr/ibis, go to *Teaching*
- Mailing list 5BIM and Master students?

Mini-projects

- Evaluation based on **individual mini-projects**
- Mini-projects develop specific aspects of course
- Mini-projects proposed by students or selected from list of suggestions
- Mini-projects may be literature study, implementation of algorithm, construction of model, …
- Results of mini-projects described in report (~10 p)
  - Introduction (context, problem/question, approach)
  - Methods
  - Results
  - Discussion and conclusions
- Reports discussed with teacher (feedback)

Mini-projects

- Suggestions for **mini-projects**:
  - Whole-cell modeling
  - Biotechnological applications of flux balance analysis (FBA)
  - Coarse-grained resource allocation models
  - Resource balance analysis (RBA) and other resource allocation variants of FBA
  - Feedback control of synthetic networks
  - Model checking of biological networks
  - Evolution of regulatory networks
  - Machine learning approaches for the modeling and inference of biological networks
  - Acceleration of stochastic simulation using parallel computing
  - Scaling up the stochastic analysis of regulatory networks using Finite State Projection (FSP)

Mini-projects

- Possible topics for **mini-projects** (cont'd):
  - Automated design of synthetic networks
  - Simulation of cellular processes on the single-molecule level
  - Modelling communities of microorganisms
  - Large-scale modeling of signaling networks using Boolean logic
  - Experimental design for predicting the most informative experiments
  - Tracking individual cells using image analysis and machine learning
  - …

Examples of mini-projects

- Examples of mini-projects last year:
  - « Boolean model of immunology network activated by Covid-19 »
  - « Simulation of *E. coli* cell division process on the single molecular level »
  - « Accélération des simulations stochastiques en utilisant les capacités de parallélisation des processeurs graphiques »
  - « Modélisation de communautés bactériennes impliquées dans la méthanisation »
  - …

Merci !

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