Introduction

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INRIA Grenoble - Rhône-Alpes and IBIS

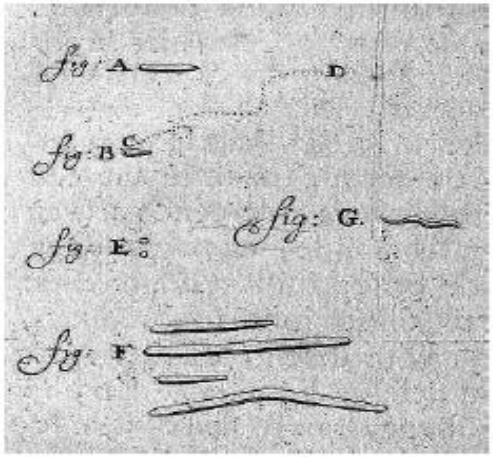
- IBIS: systems biology group at INRIA/Université Joseph Fourier/CNRS
  - Analysis of bacterial regulatory networks by means of models and experiments
  - Biologists, computer scientists, mathematicians, physicists, …

http://ibis.inrialpes.fr

Bacteria

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

http://commons.wikimedia.org/



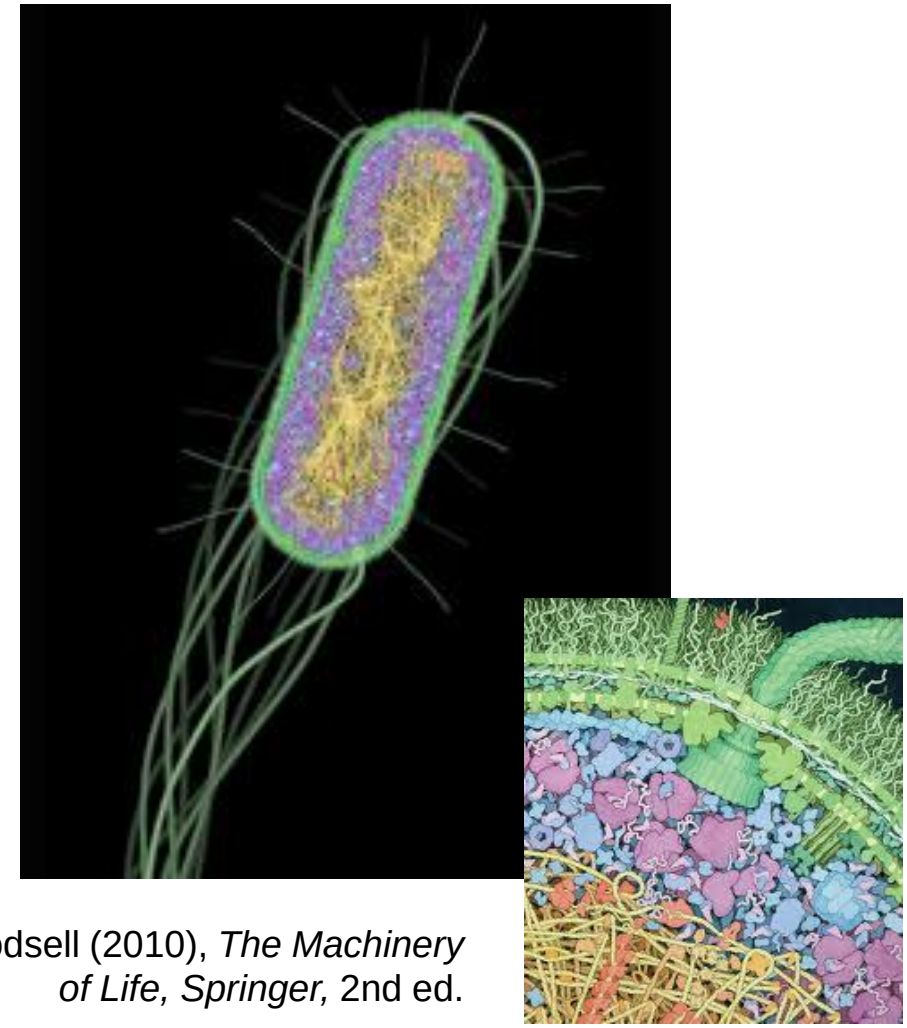
www.euronet.nl/users/warnar/leeuwenhoek.html.

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

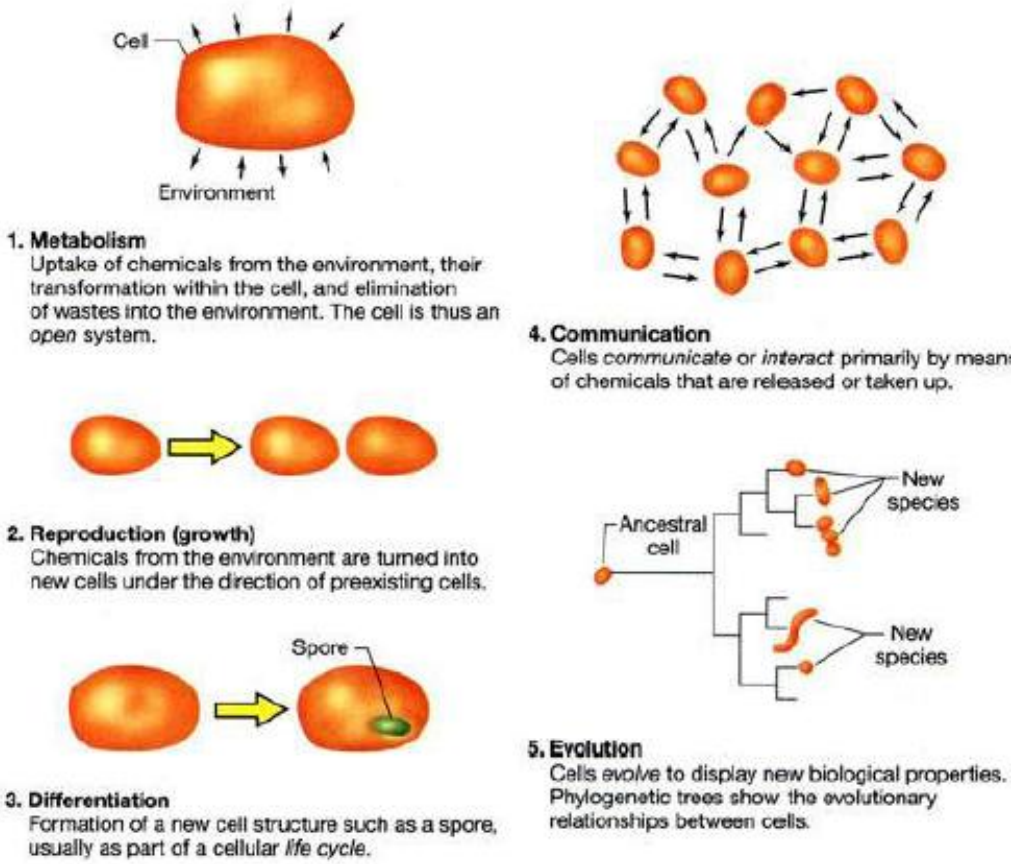
- Bacterial cells are complex biochemical and biophysical machines
  - Wide range of shapes, typically 0.5-5 µm in length
  - 10^6 bacterial cells in 1 ml of fresh water
  - 10 times as much bacterial cells as human cells in human body



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

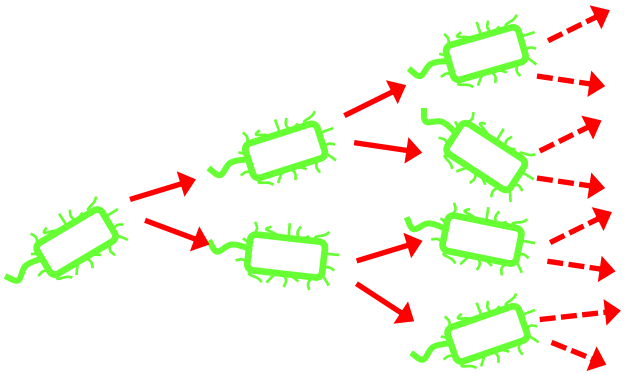


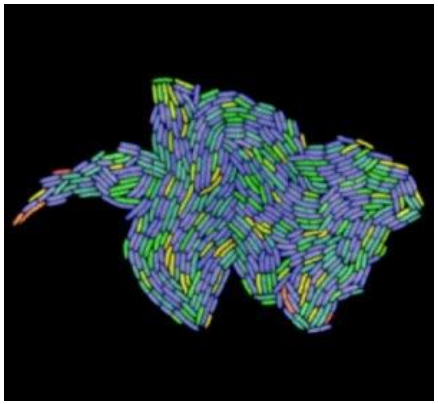
Madigan *et al.* (2003), *Brock Biology of Microorganisms*, Prentice Hall, 10th ed.

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

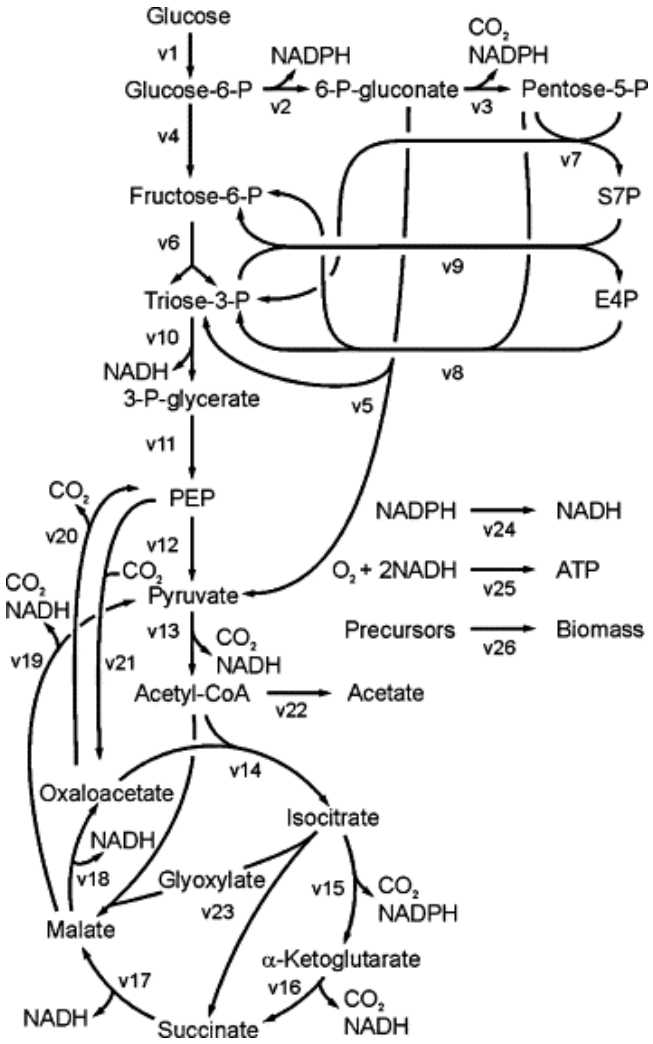
- **Metabolism** fuels growth by production of energy and building blocks for macromolecules, using nutriments from environment

  ATP, amino acids, nucleotides, …

Bacterial growth and metabolism

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

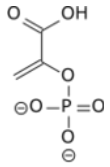
  Glucose, acetate, lactose, …

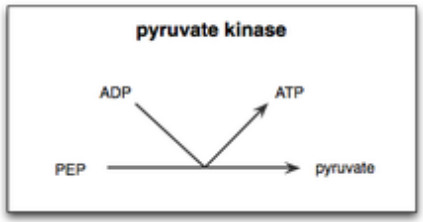


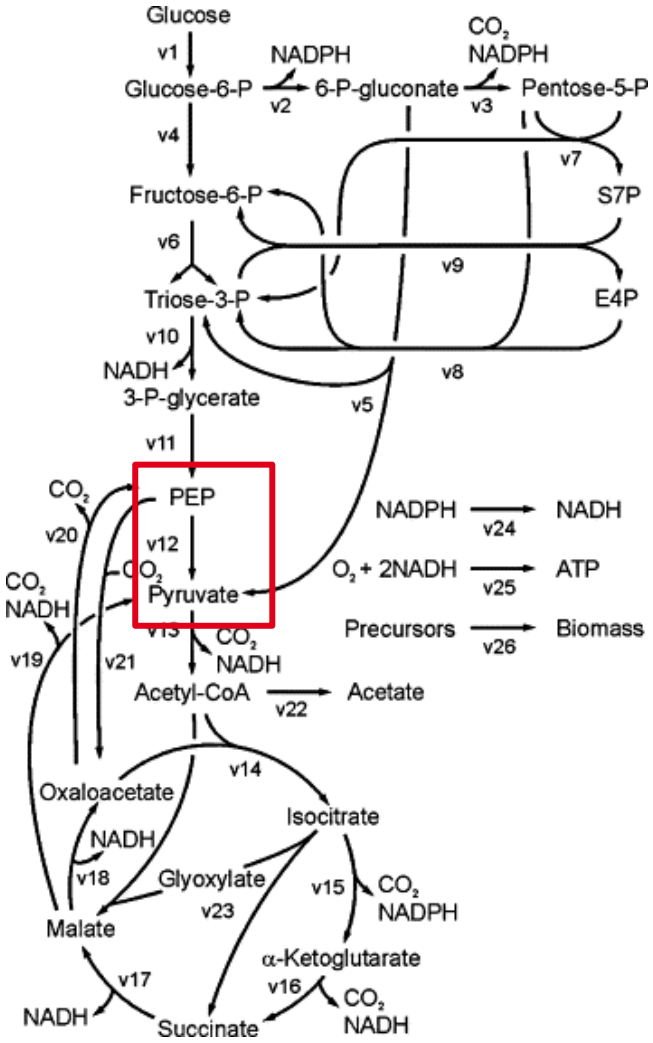
Fischer *et al.* (2004), *Anal. Biochem.*, 325(2):308–16

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





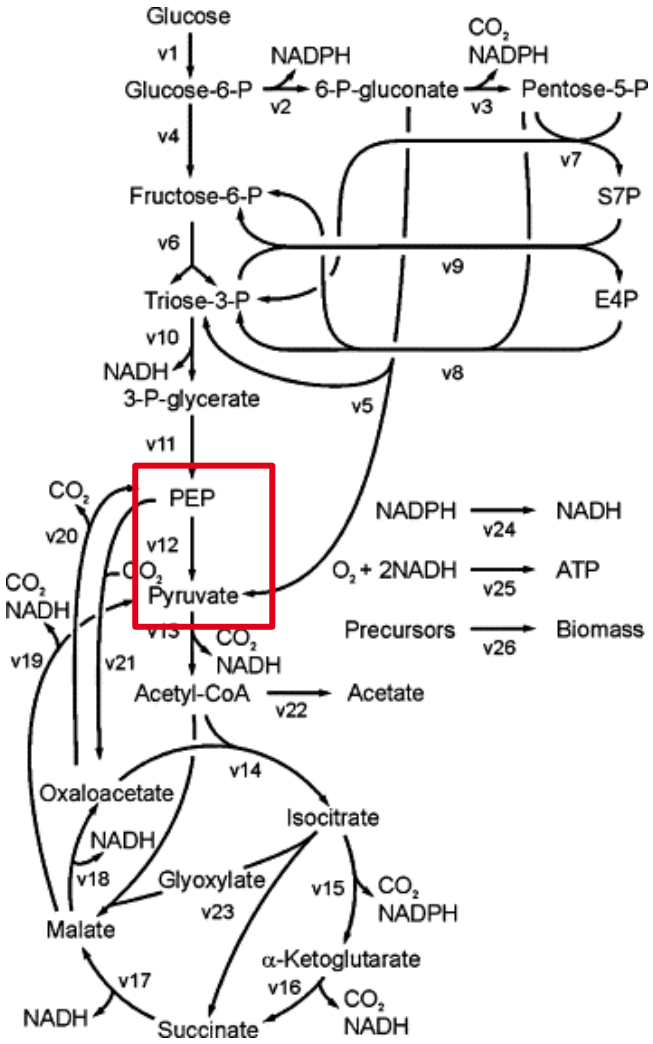


Bacterial growth and metabolism

- 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

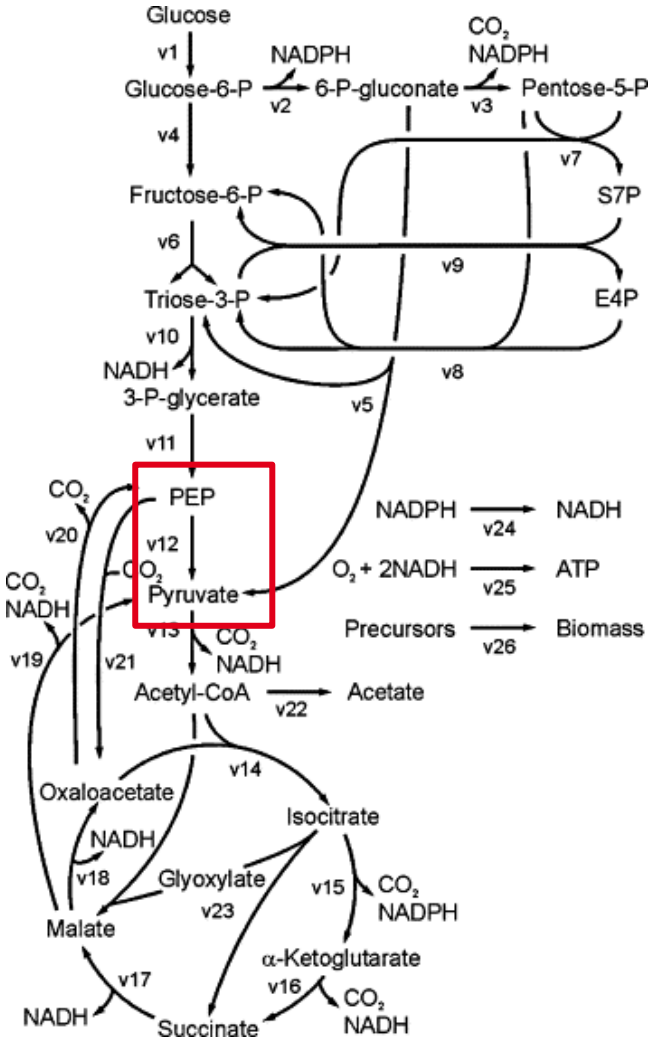


Bacterial growth and metabolism

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

  Glucose, acetate, lactose, …

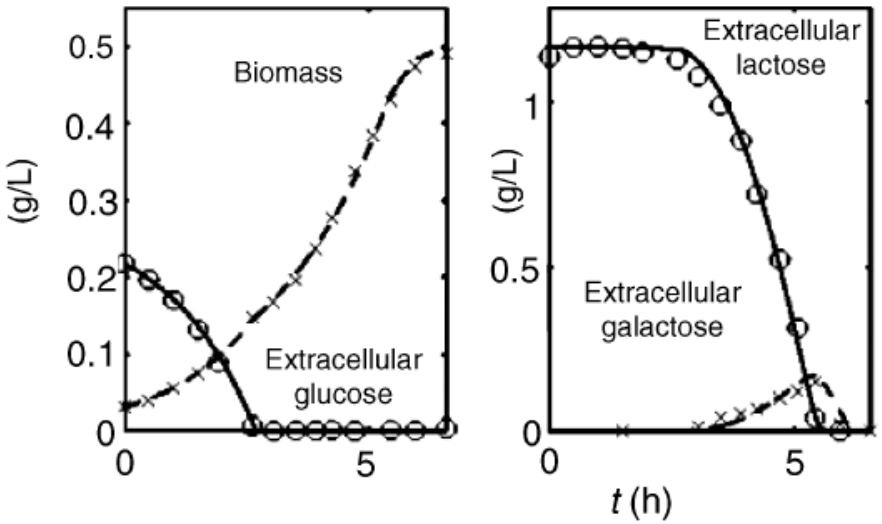
- Enzymes produced from information encoded in **genes**
  - *pykF* is gene encoding pyruvate kinase
  - Expression of *pykF* regulated by transcription factor Cra



Bacterial growth and metabolism

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

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



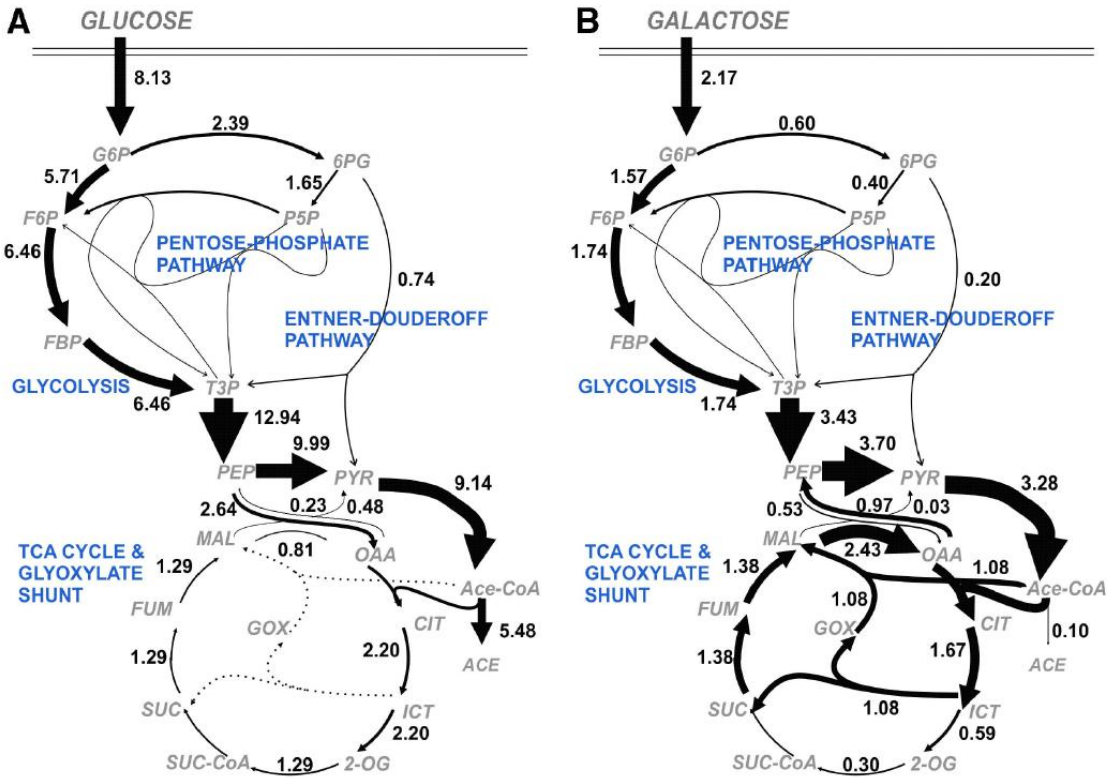
Bettenbrock *et al.* (2006), *J. Biol. Chem.*, 281(5):2578-84

- 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

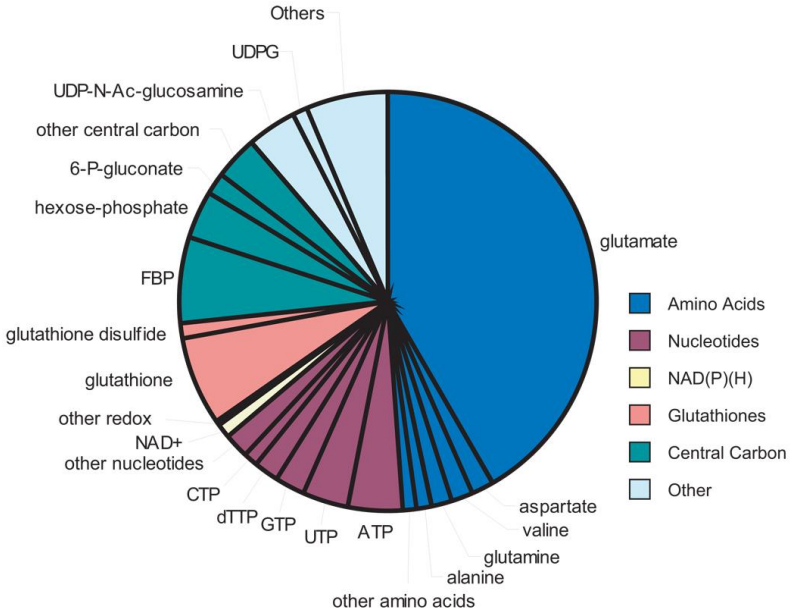


Table 1 Intracellular metabolite concentrations in glucose-fed, exponentially growing *E. coli*

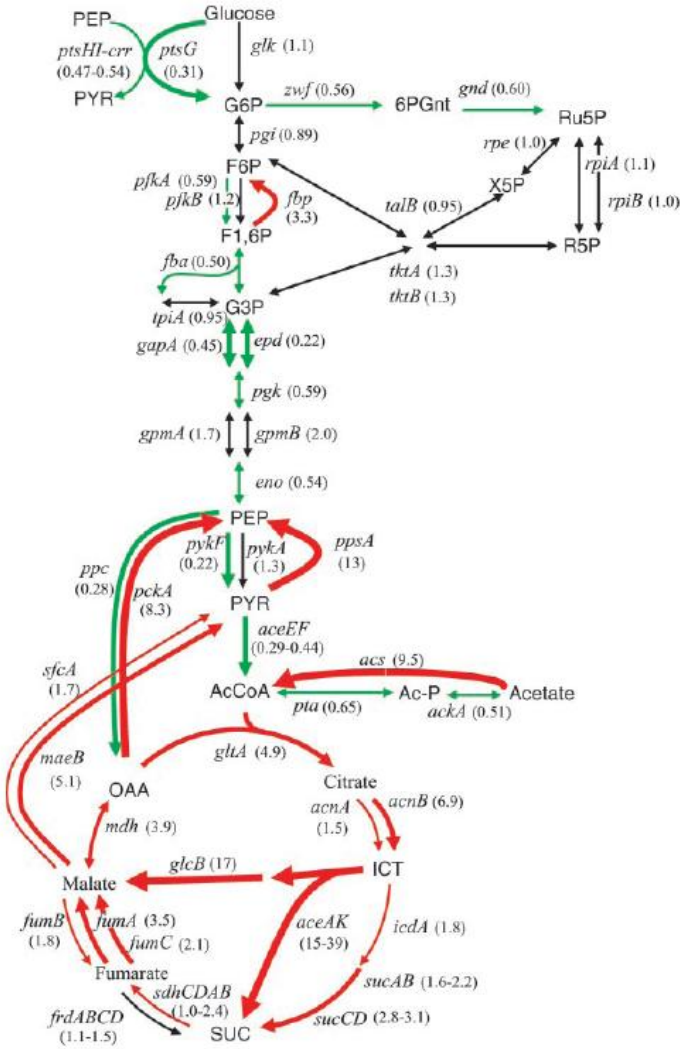
Metabolite	mol l^{-1}	Metabolite	mol l^{-1}
Glutamate	9.6×10^{-2}	UDP-glucuronate (51)	5.7×10^{-4}
Glutathione	1.7×10^{-2}	ADP	5.6×10^{-4}
Fructose-1,6-bisphosphate	1.5×10^{-2}	Asparagine (52)	5.1×10^{-4}
ATP	9.6×10^{-3}	α-Ketoglutarate	4.4×10^{-4}
UDP-*N*-acetylglucosamine (29)	9.2×10^{-3}	Lysine (53)	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

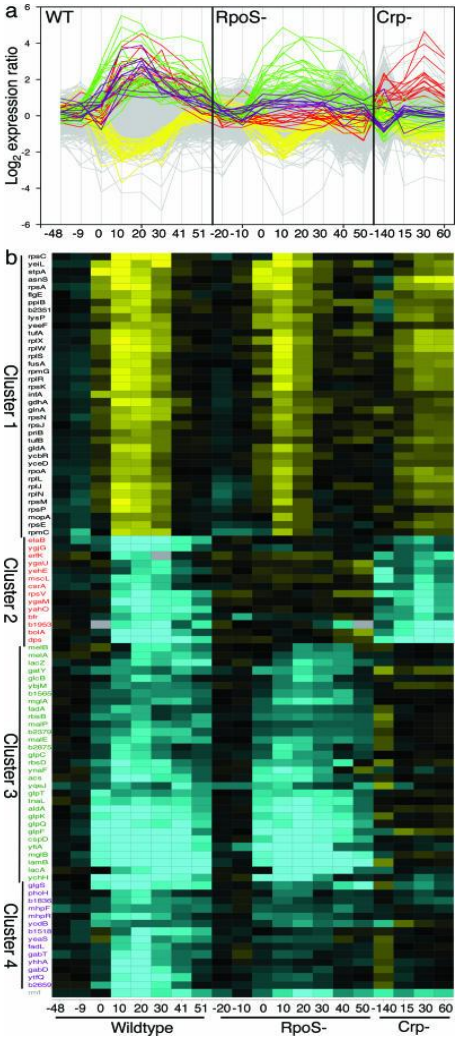


Oh *et al.* (2002), *J. Biol. Chem.*, 277(15):13175–83

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*



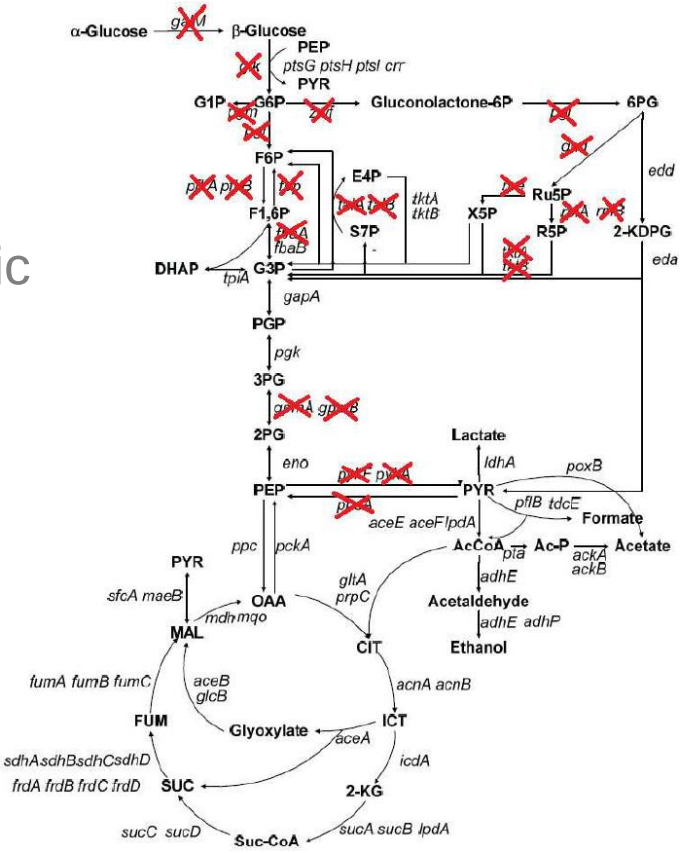
Traxler *et al.* (2006), *Proc. Natl. Acad. Sci. USA*, 103(7):2374–9

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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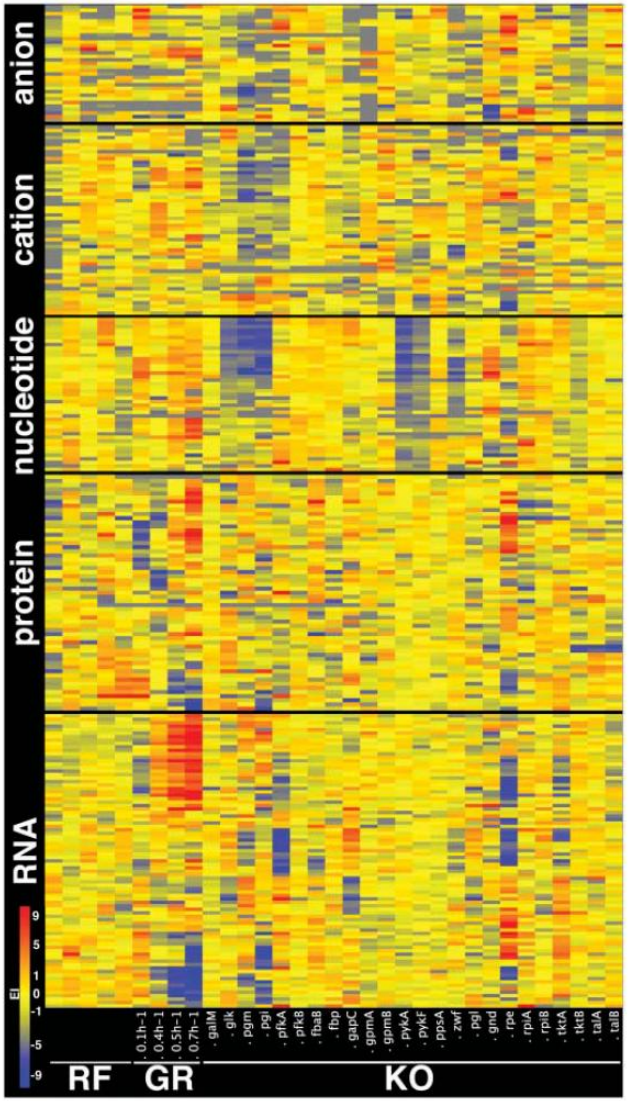
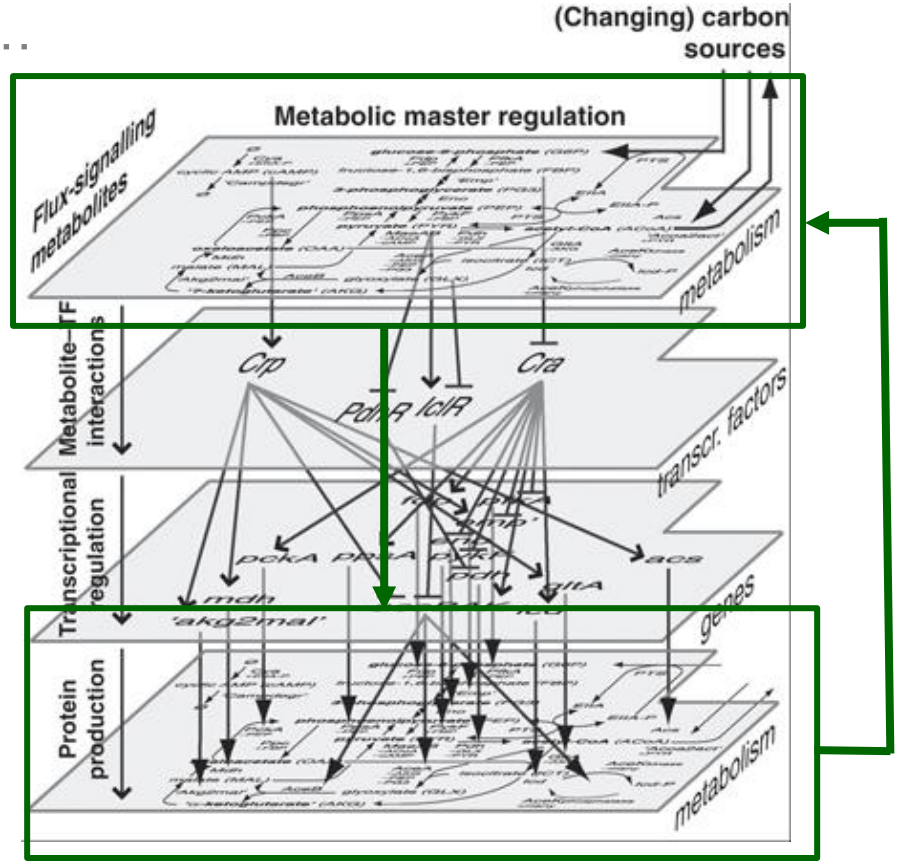


General question on cellular adaptation

- 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 heteregenous 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

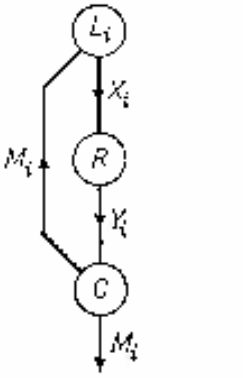
- Systems biology, and more particularly the mathematical modeling and computer simulation of biochemical reaction networks, have a long history

Westerhoff and Palsson, *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



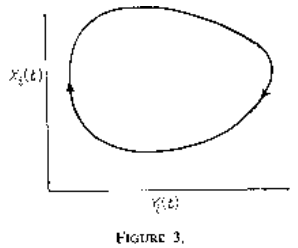
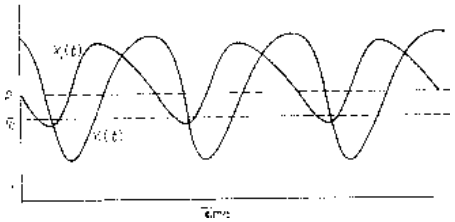


FIGURE 3.



Goodwin (1963), *Temporal Organization in Cells*

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

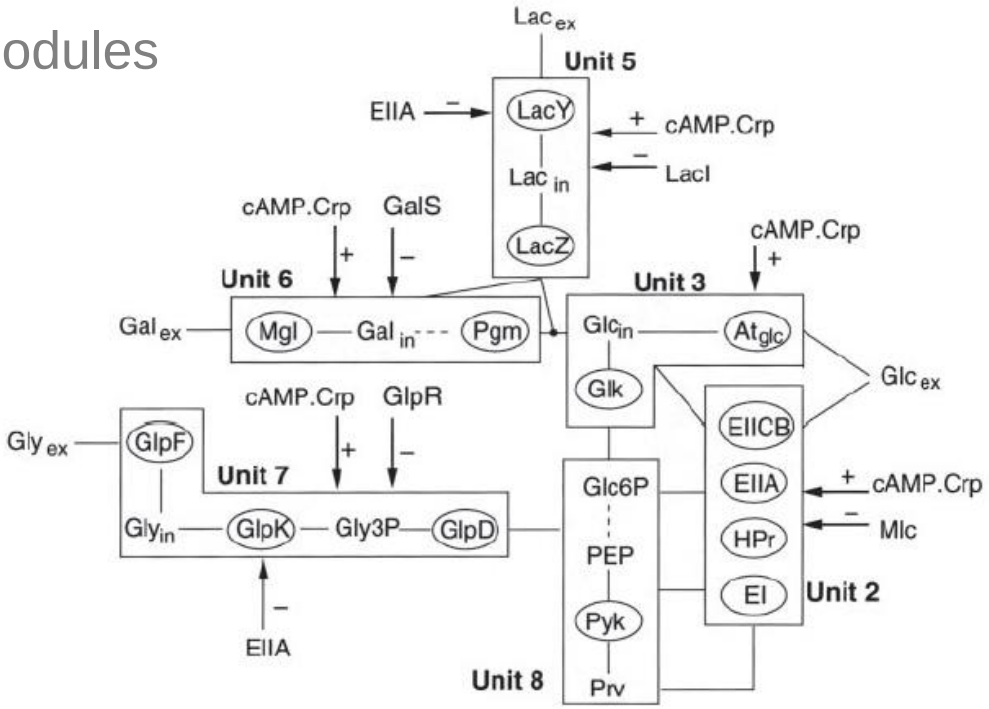
$$\dot{x} = N\, v(x)$$

  - 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

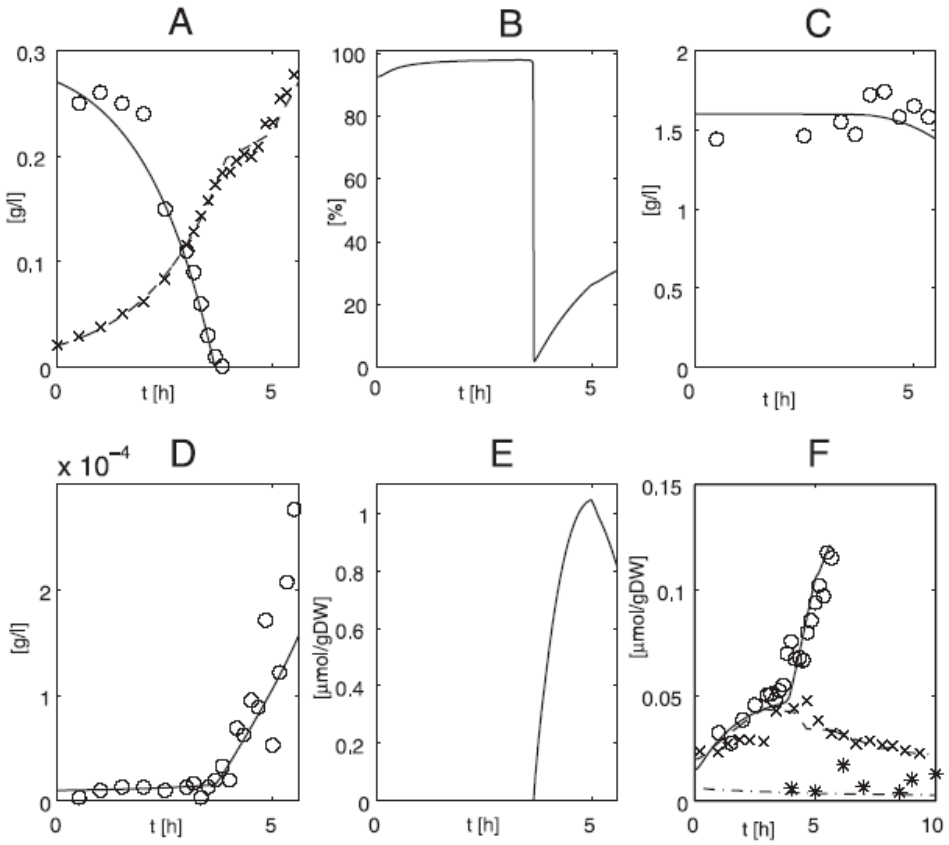
- Model of uptake of carbon sources (glucose, lactose, glycerol, …) by *E. coli*
  - Several dozens of equations and more than a hundred parameters, many of them unknown or unreliable
  - Mostly metabolic modules



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

Objective of course "Modeling of biological networks"

- **Course objective** is to master kinetic modelling as applied to metabolic 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

Program and teachers

- Part 1. Systems biology and kinetic modeling (courses 8 h)
  - Reminders on dynamical systems (Hidde de Jong)
  - Introduction to regulatory systems (Hans Geiselmann)
  - Reminders on kinetic modeling and enzymology (Daniel Kahn)
- Part 2. Metabolic network modeling (courses and practical 18 h)
  - Introduction to metabolic networks (Daniel Kahn)
  - Metabolic Control Theory (Daniel Kahn)
  - Practical on the modeling of a metabolic system using COPASI (Daniel Kahn)

Program and teachers

- Part 3. Gene regulatory network modeling (courses 14 h, and practicals 6 h)
  - Introduction to recent techniques for measuring gene expression (Hidde de Jong)
  - Kinetic models of gene expression and dynamics of gene regulatory networks (Hidde de Jong)
  - Identification and inference of gene network models (Eugenio Cinquemani)
  - Practical on the qualitative modeling of bacterial regulatory networks, using GNA (Hidde de Jong)
- Part 4. Concluding course in cooperation with SeMoVi (presentation 2 h)

Evaluation

- Metabolic network modeling:
  - Exercises handed out during course
- Gene regulatory network modeling:
  - Questions on articles handed out during course
  - Or: literature review on specific topic of interest
- Grade is average of grades for two subparts of course
- Articles will be made available via course web site

Merci

www.inrialpes.fr/ibis