



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>

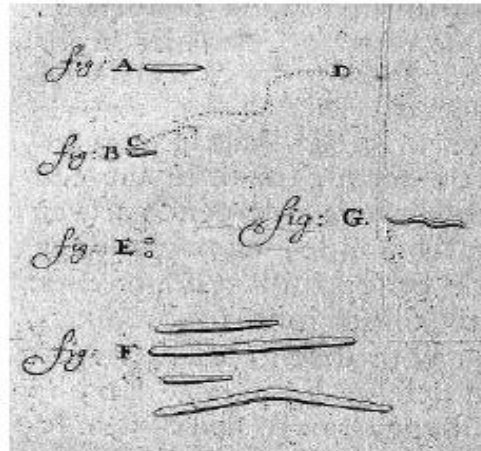


Bacteria

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



<http://commons.wikimedia.org/>



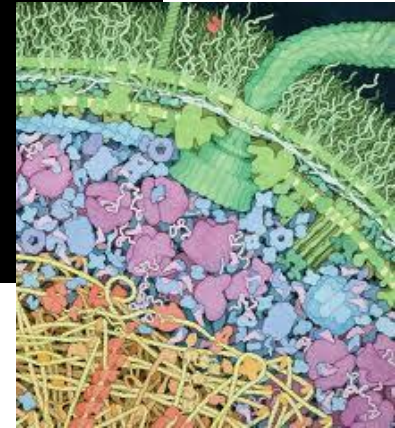
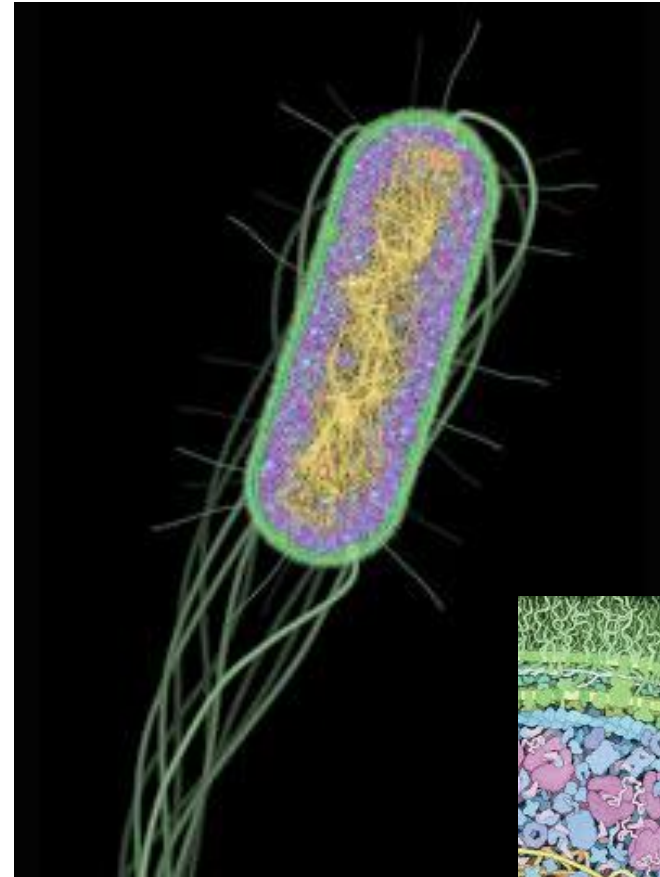
www.euronet.nl/users/wamar/leeuwenhoek.html

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

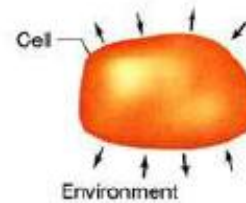
- Bacterial cells are complex biochemical and biophysical machines
 - Wide range of shapes, typically 0.5-5 μm in length
 - 10^6 bacterial cells in 1 ml of fresh water
 - 10 times as much bacterial cells as human cells in human body



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



1. Metabolism

Uptake of chemicals from the environment, their transformation within the cell, and elimination of wastes into the environment. The cell is thus an open system.



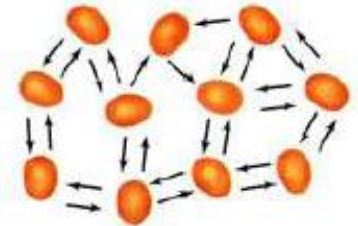
2. Reproduction (growth)

Chemicals from the environment are turned into new cells under the direction of preexisting cells.



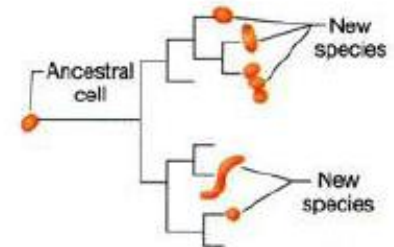
3. Differentiation

Formation of a new cell structure such as a spore, usually as part of a cellular life cycle.



4. Communication

Cells communicate or interact primarily by means of chemicals that are released or taken up.



5. Evolution

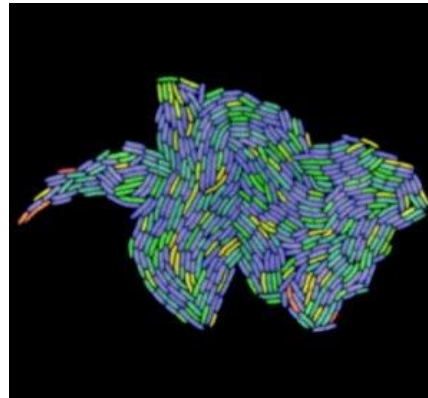
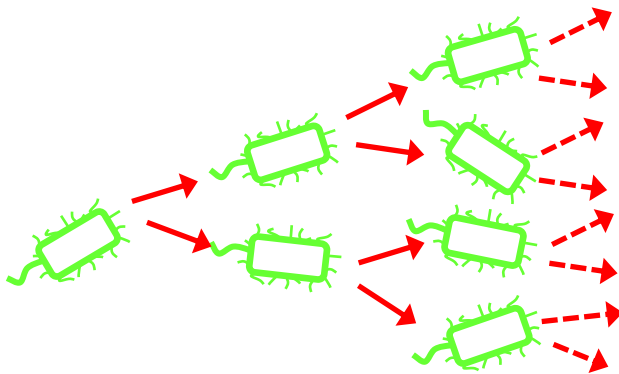
Cells evolve to display new biological properties. Phylogenetic trees show the evolutionary relationships between cells.

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

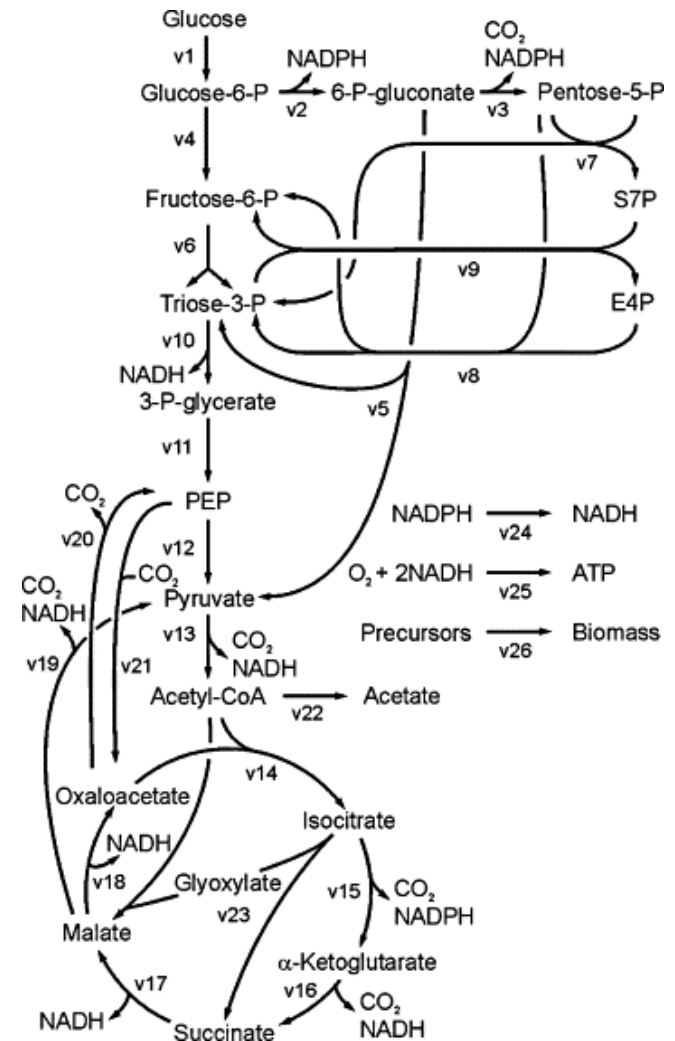
- **Metabolism** fuels growth by production of energy and building blocks for macromolecules, using nutriment from environment

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

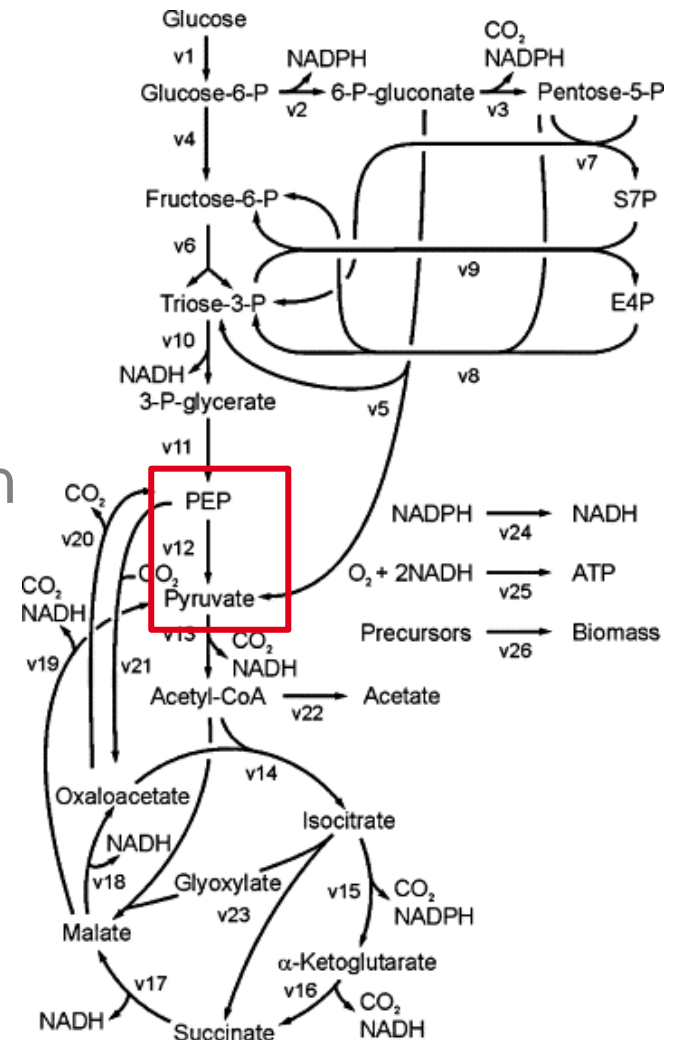
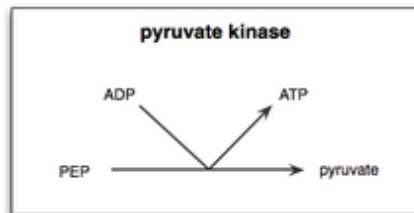
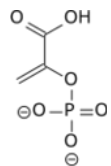
Bacterial growth and metabolism

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Glucose, acetate, lactose, ...

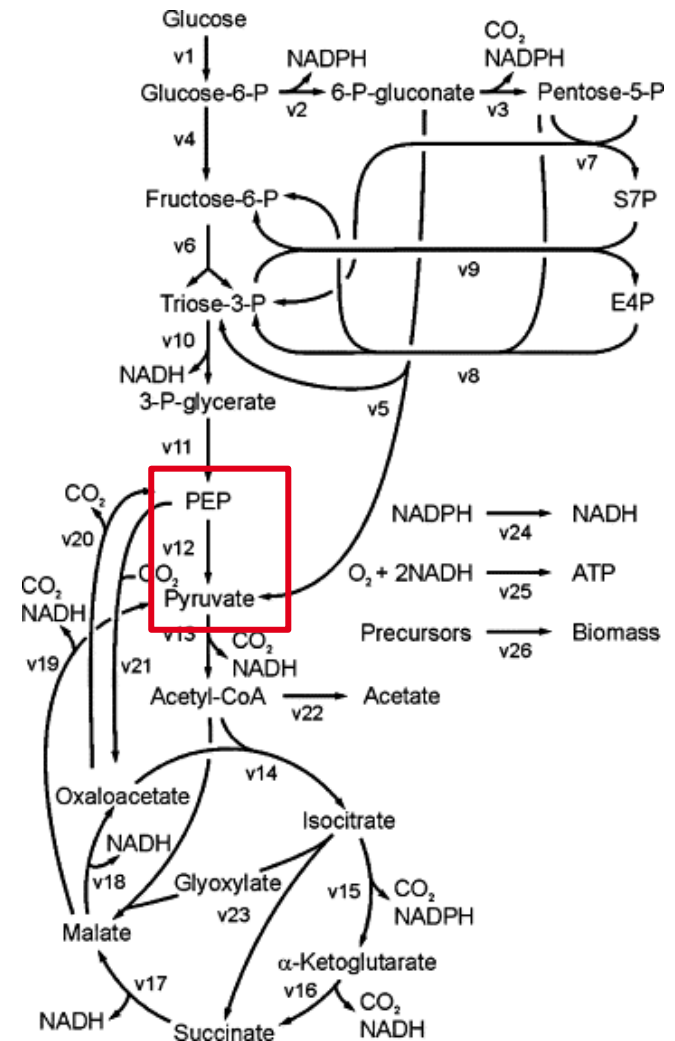
- Enzymes** catalyse individual steps in metabolic network

Pyruvate kinase transforms phosphoenolpyruvate (PEP) into pyruvate



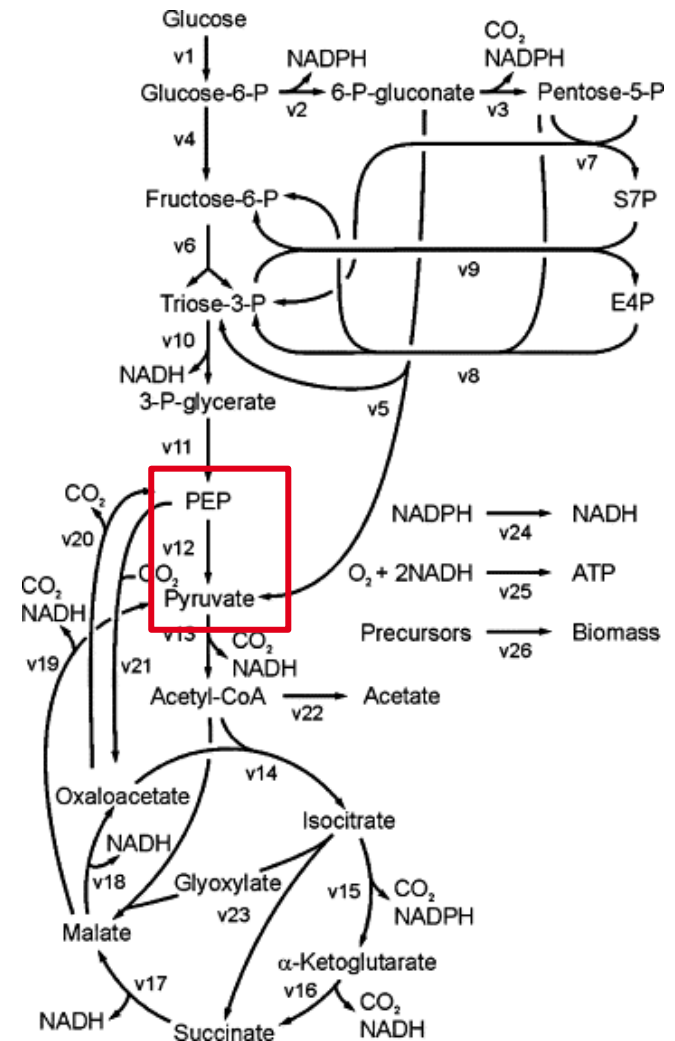
Bacterial growth and metabolism

- 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

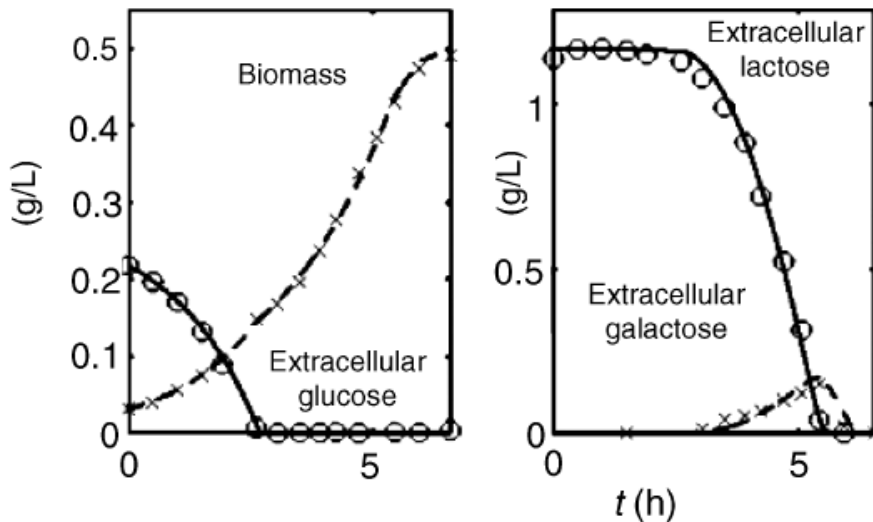
- Central **carbon metabolism** breaks down carbon sources for energy production and macromolecular synthesis
 - Glucose, acetate, lactose, ...
- Enzymes produced from information encoded in **genes**
 - *pykF* is gene encoding pyruvate kinase
 - Expression of *pykF* regulated by transcription factor 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



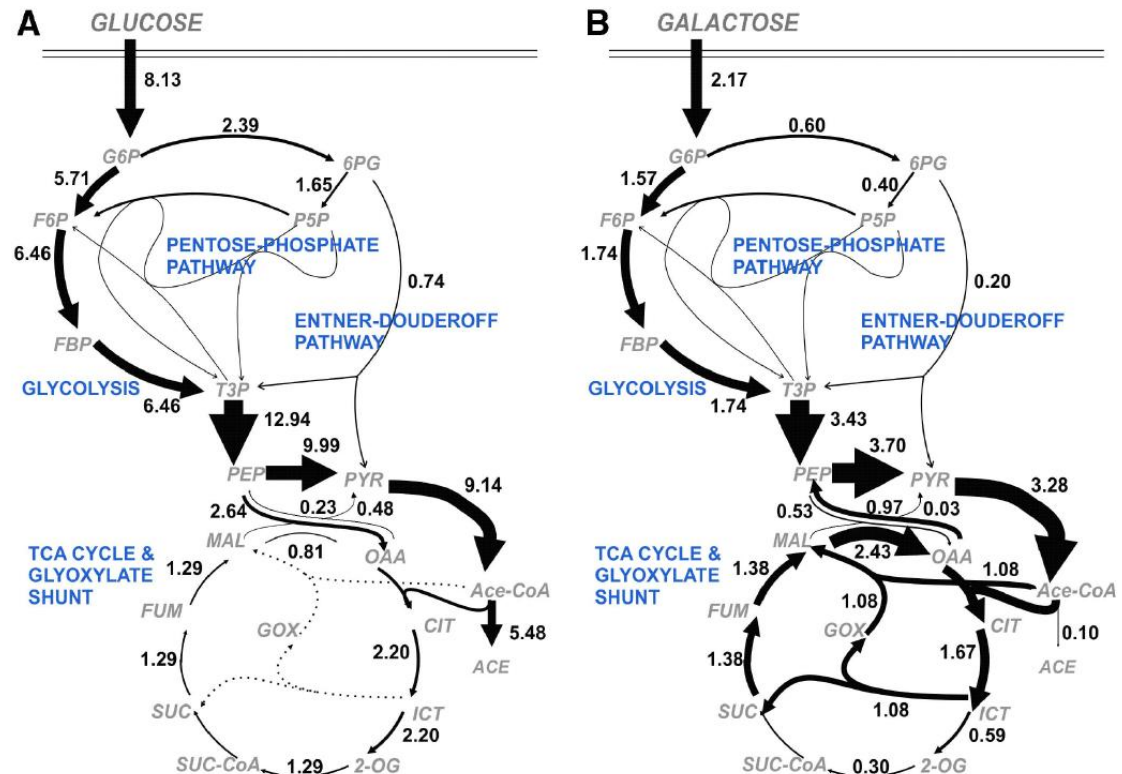
Bettenbrock *et al.* (2006), *J. Biol. Chem.*, 281(5):2578-84

- 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

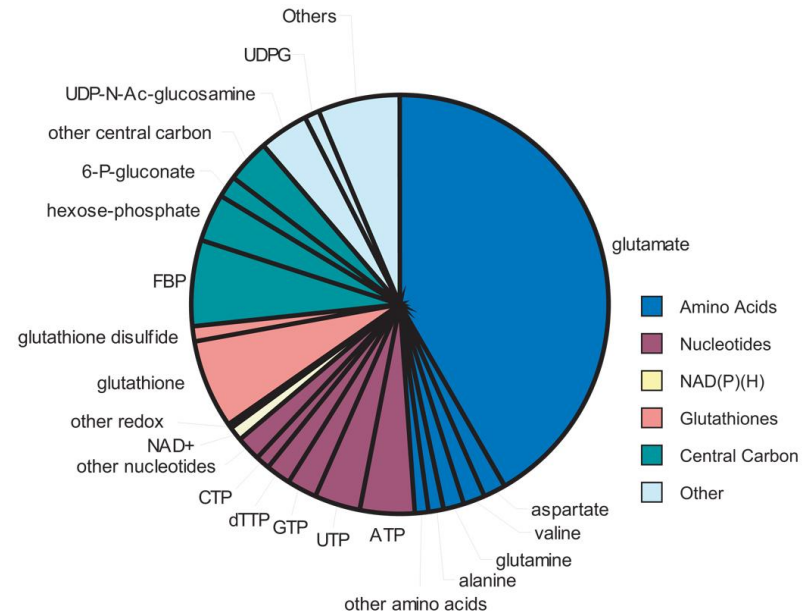


Table 1 Intracellular metabolite concentrations in glucose-fed, exponentially growing *E. coli*

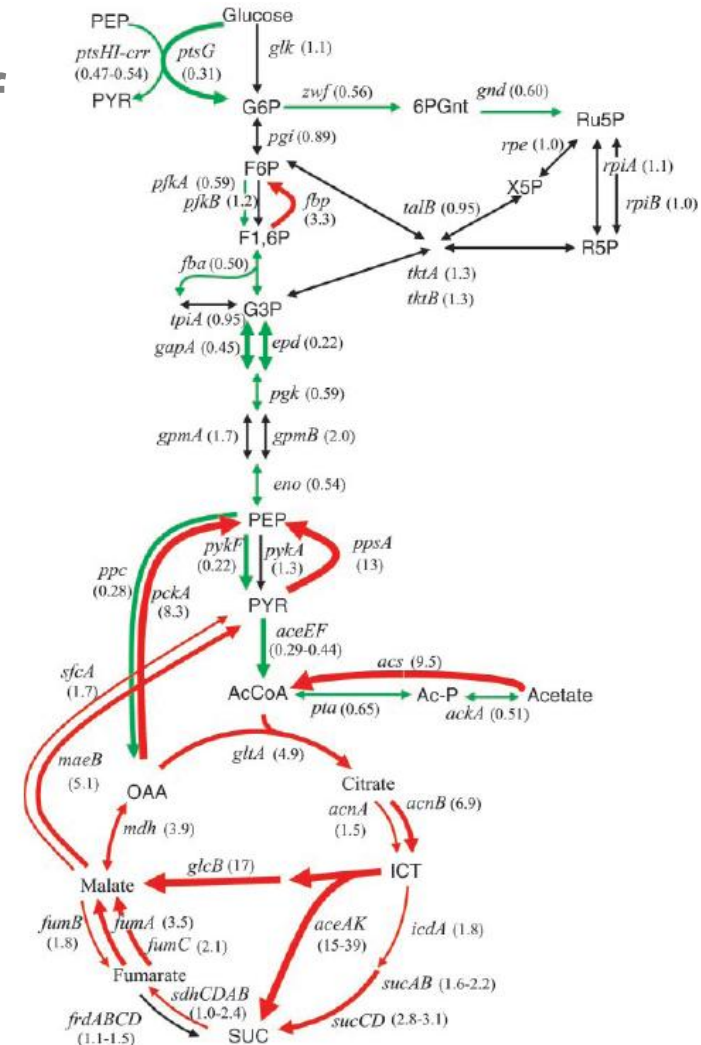
Metabolite	mol l ⁻¹	Metabolite	mol l ⁻¹
Glutamate	9.6×10^{-2}	UDP-glucuronate (51)	5.7×10^{-4}
Glutathione	1.7×10^{-2}	ADP	5.6×10^{-4}
Fructose-1,6-bisphosphate	1.5×10^{-2}	Asparagine (52)	5.1×10^{-4}
ATP	9.6×10^{-3}	α -Ketoglutarate	4.4×10^{-4}
UDP-N-acetylglucosamine (29)	9.2×10^{-3}	Lysine (53)	4.1×10^{-4}
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

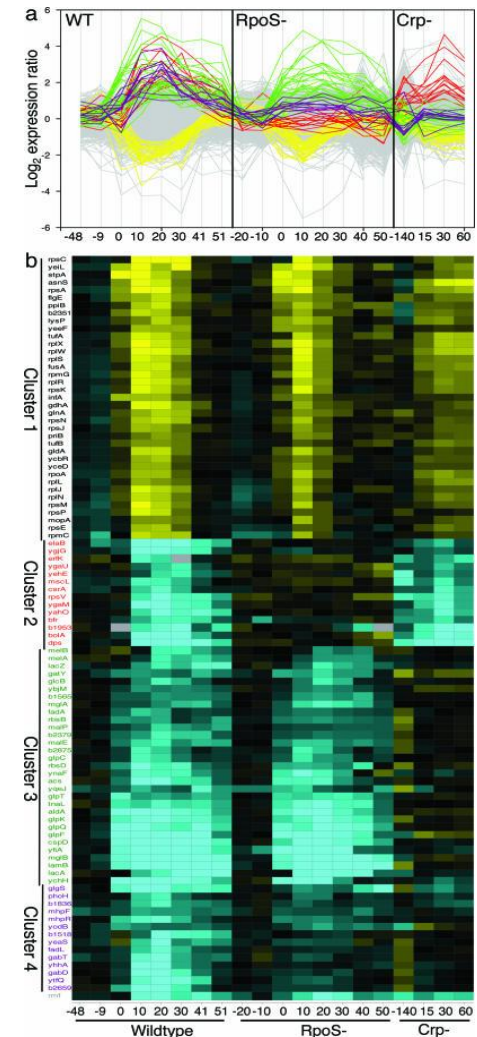


Oh et al. (2002), *J. Biol. Chem.*, 277(15):13175–83

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*



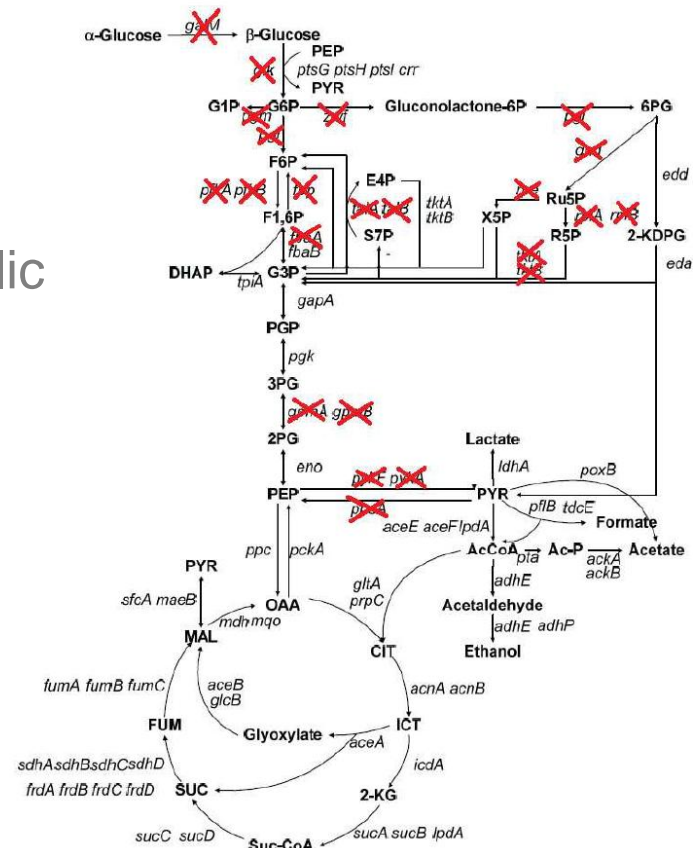
Traxler *et al.* (2006), *Proc. Natl. Acad. Sci. USA*, 103(7):2374–9

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

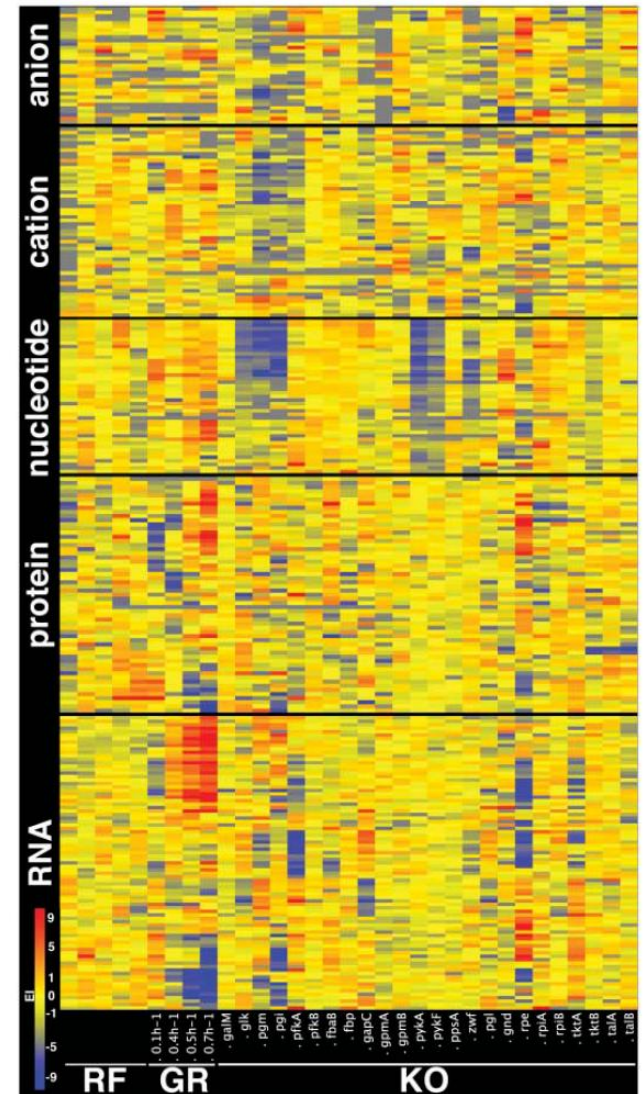


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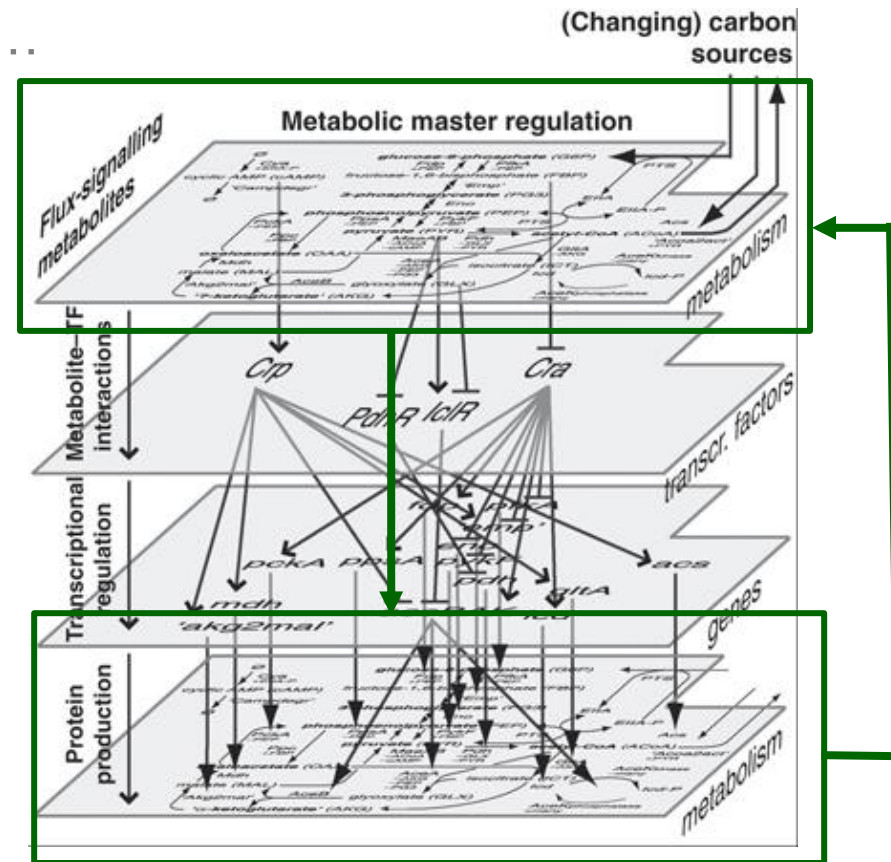


General question on cellular adaptation

- 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 heterogeneous 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

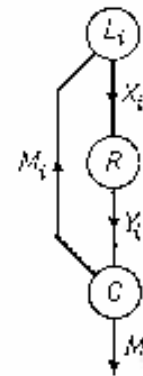
- Systems biology, and more particularly the mathematical modeling and computer simulation of biochemical reaction networks, have a long history

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



Goodwin (1963), *Temporal Organization in Cells*

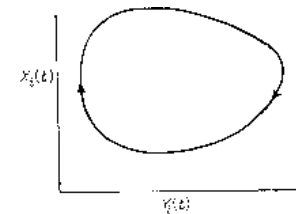
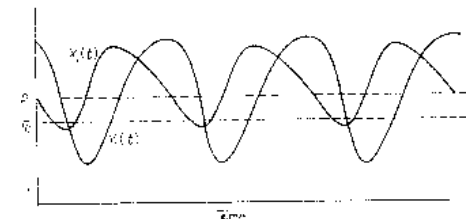


FIGURE 3.



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

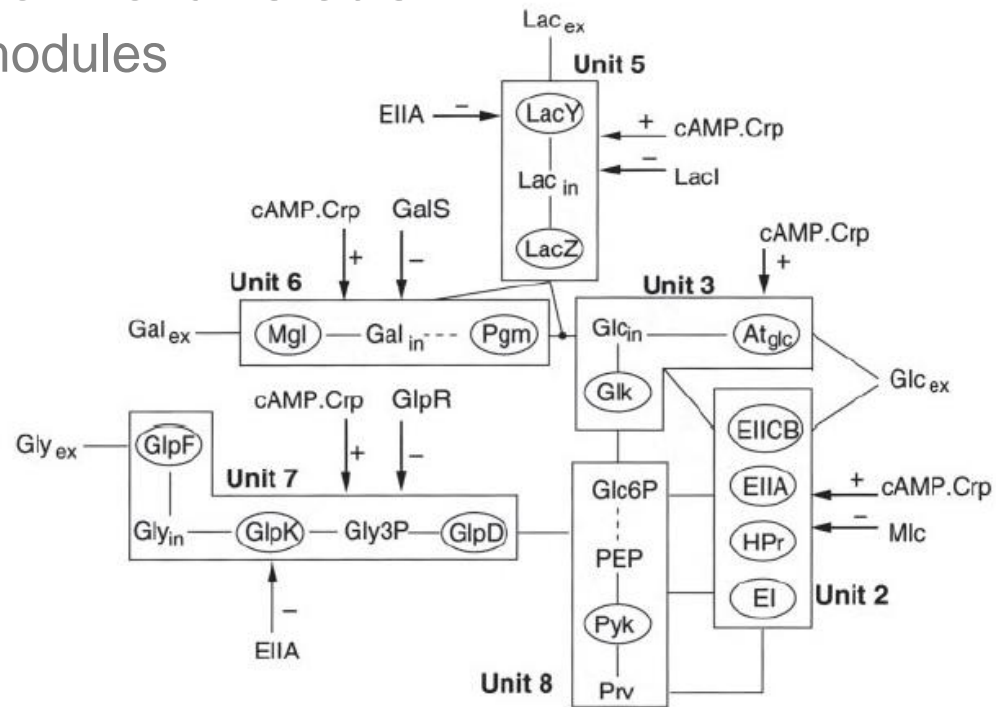
$$\dot{x} = N v(x)$$

- 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

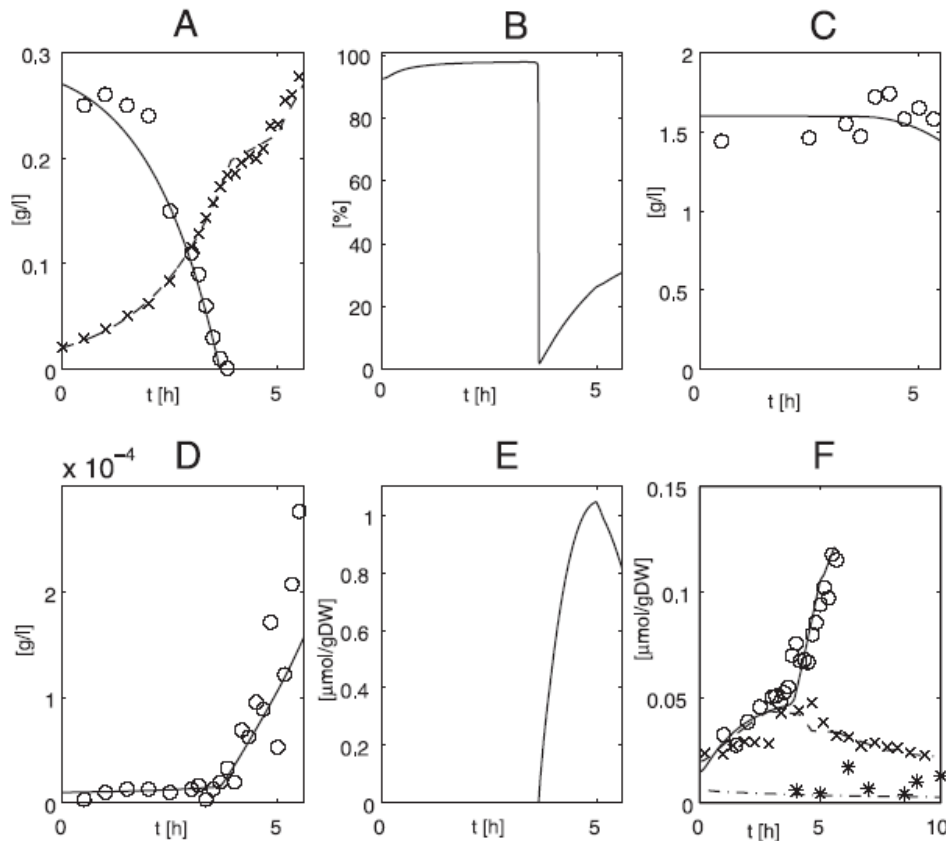
- Model of uptake of carbon sources (glucose, lactose, glycerol, ...) by *E. coli*
 - Several dozens of equations and more than a hundred parameters, many of them unknown or unreliable
 - Mostly metabolic modules



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 4 h)
 - Introduction to regulatory systems (Hans Geiselmann)
 - Reminders on kinetic modeling and enzymology (Daniel Kahn)
- Part 2. Metabolic network modeling (courses and practical 13 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