

Modeling and Simulation of Gene Regulatory Networks

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INRIA Grenoble - Rhône-Alpes and IBIS



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

Overview

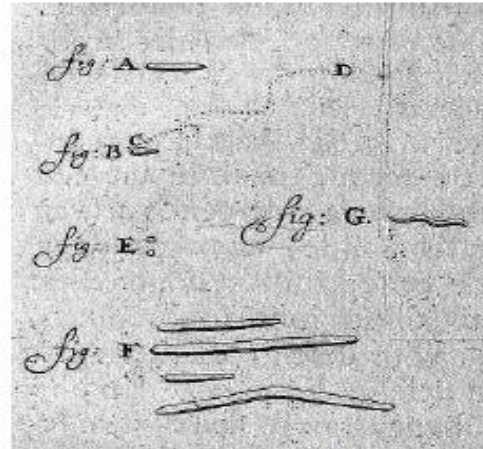
- 1. Gene regulatory networks in bacteria**
- 2. Novel methods for measuring gene expression**
- 3. Quantitative modeling of gene regulatory networks**
 - Ordinary differential equations
 - Stochastic master equations
- 4. Qualitative modeling of gene regulatory networks**
 - Piecewise-linear differential equations
- 5. Conclusions and perspectives**

Bacteria

- ❖ Bacteria were first observed by Antonie van Leeuwenhoek in 1676, using a single-lens microscope of his own design



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



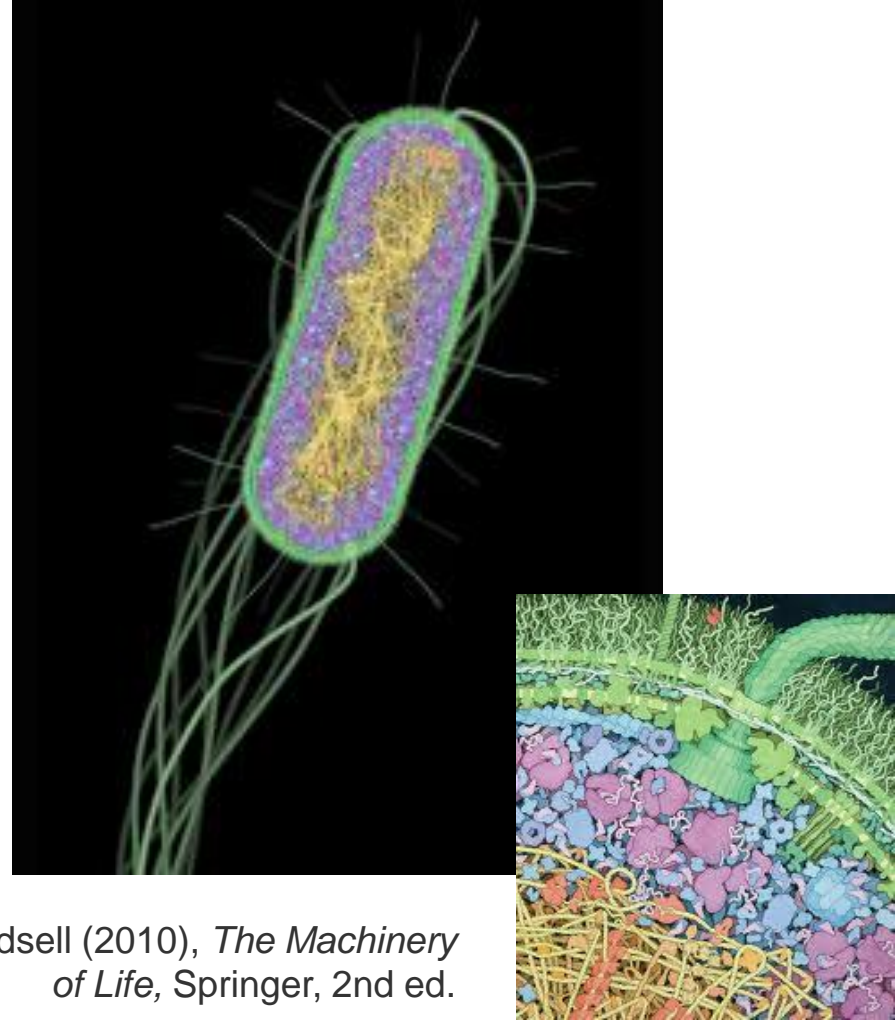
www.euronet.nl/users/wamar/leeuwenhoek.html

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

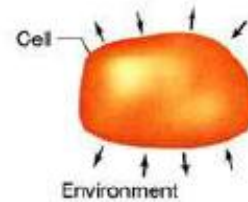
- ❖ Bacterial cells are complex biochemical and biophysical machines
 - Wide range of shapes, typically 0.5-5 μm in length
 - 10^6 bacterial cells in 1 ml of fresh water
 - 10 times as much bacterial cells as human cells in human body



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.



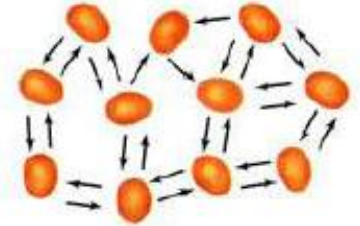
2. Reproduction (growth)

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



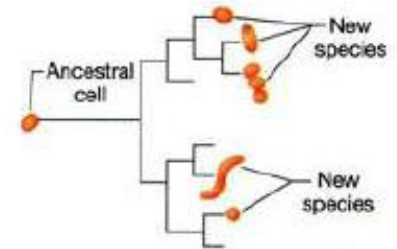
3. Differentiation

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

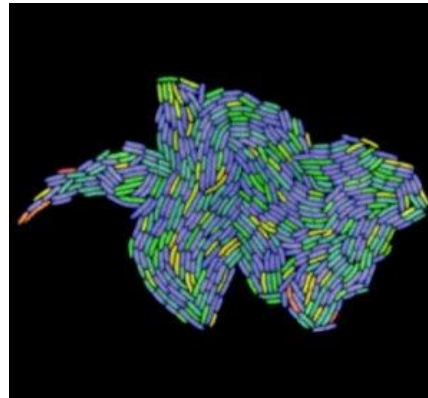
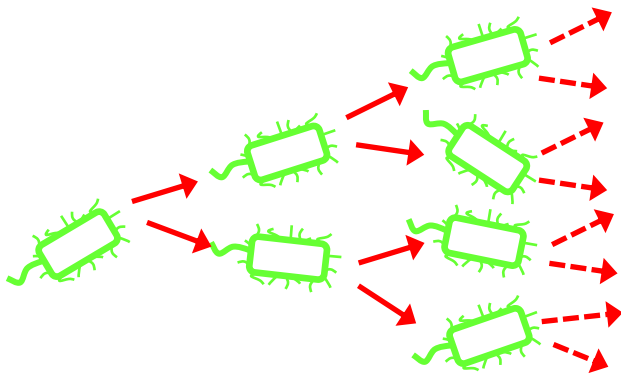
Cells evolve to display new biological properties. Phylogenetic trees show the evolutionary relationships between cells.

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

Bacterial growth and metabolism

- ❖ Bacteria are 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, ...

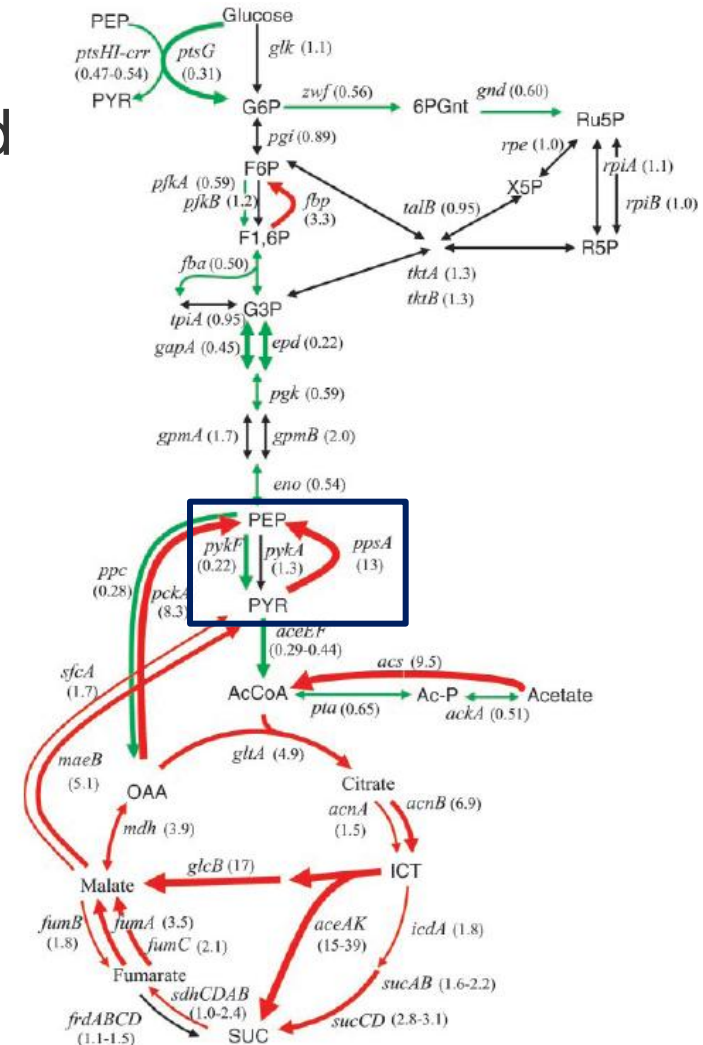
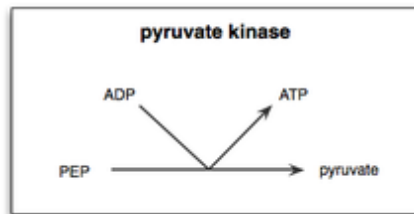
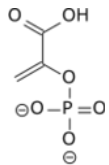
Bacterial growth and metabolism

- ❖ Central carbon metabolism breaks down sugars for energy production and macromolecular synthesis

Glucose, acetate, lactose, ...

- ❖ Enzymes catalyze individual steps in metabolic network

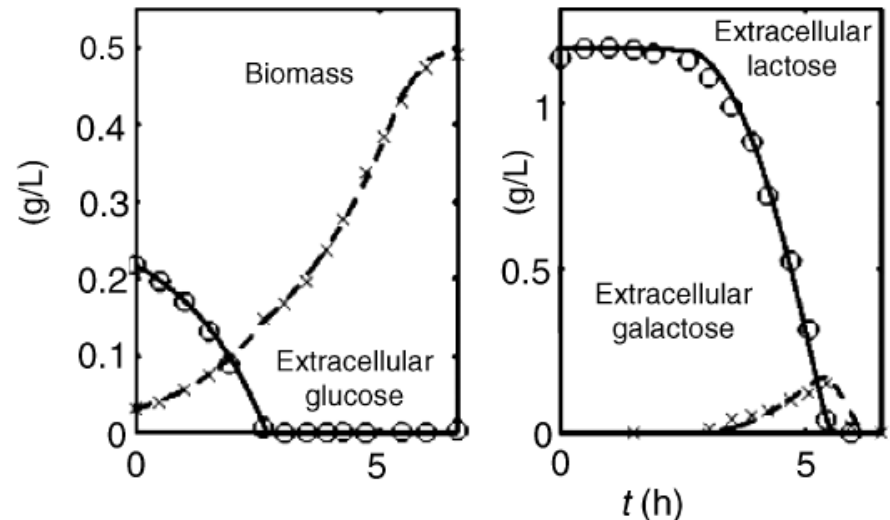
Pyruvate kinase transforms phosphoenolpyruvate (PEP) into pyruvate



Bacterial growth and metabolism

- ❖ Bacteria can sequentially use different sugars, in preferential order

Diauxic growth on glucose and lactose



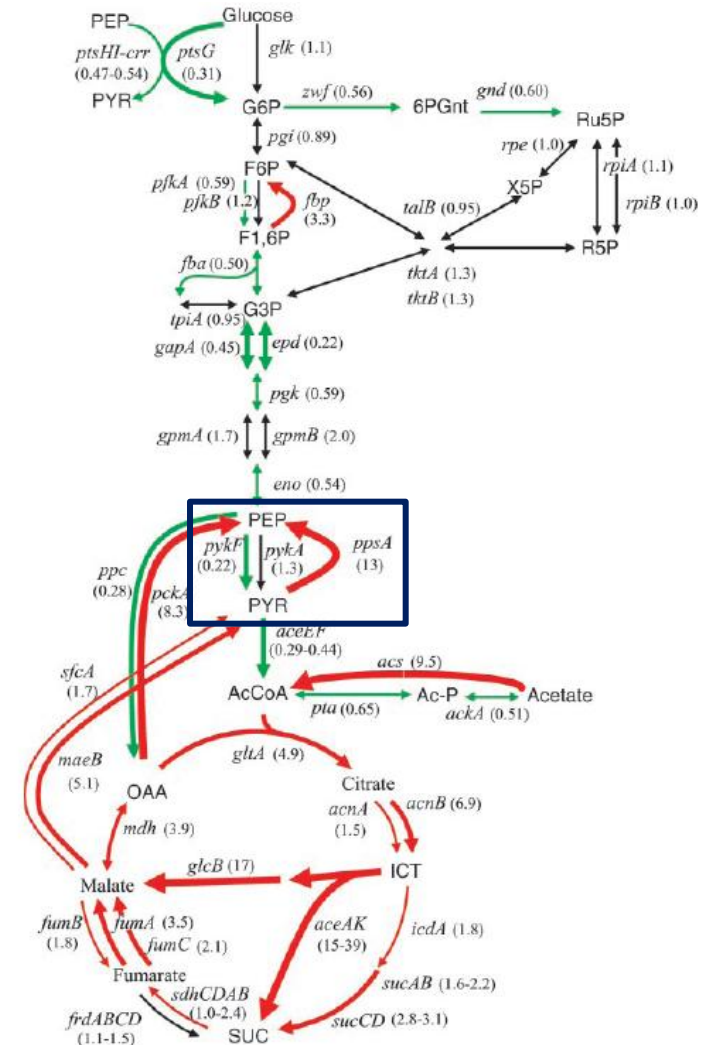
Bettenbrock *et al.* (2006), *J. Biol. Chem.*,
281(5):2578-84

Bacterial growth and metabolism

- ❖ Bacteria can sequentially use different sugars, in preferential order
- ❖ Adaptation of bacteria to growth on different sugar involves adjustment of **enzyme levels**

Differences in expression of enzymes in central metabolism of *E. coli* during growth on glucose or acetate

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

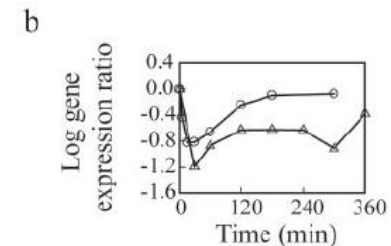
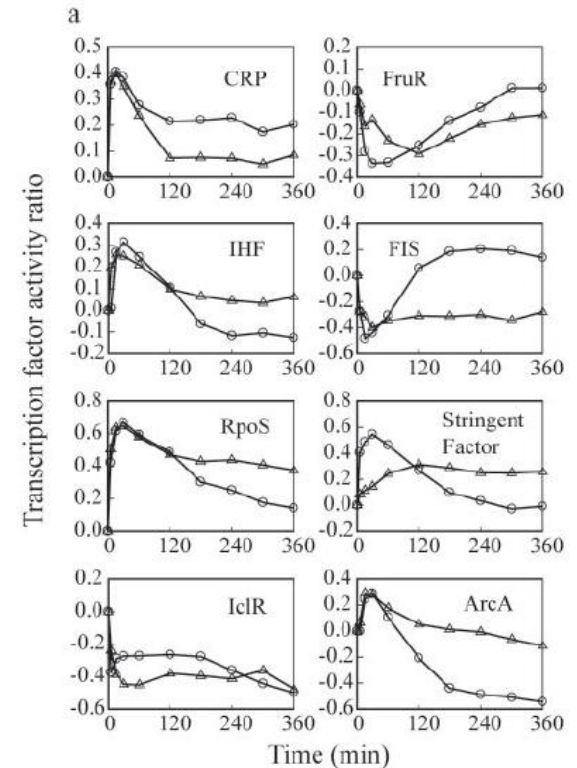


Bacterial growth and metabolism

- ❖ Bacteria can sequentially use different sugars, in preferential order
- ❖ Adaptation of bacteria to growth on different sugar involves adjustment of **transcription factor levels**

Changes in activity and concentration of transcription factors during glucose-acetate diauxie in *E. coli*

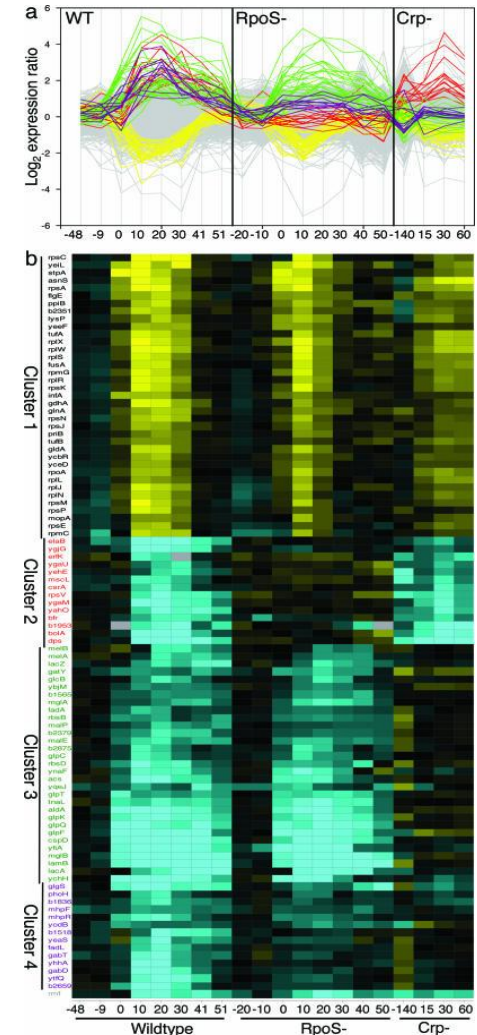
Kao *et al.* (2005), *J. Biol. Chem.*, 280(43):36079–87



Growth adaptation and gene expression

❖ Genome-wide reorganization of gene expression following growth transitions in bacteria

Gene expression during glucose-lactose diauxie in *E. coli*, in wild-type and transcription factor mutants



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

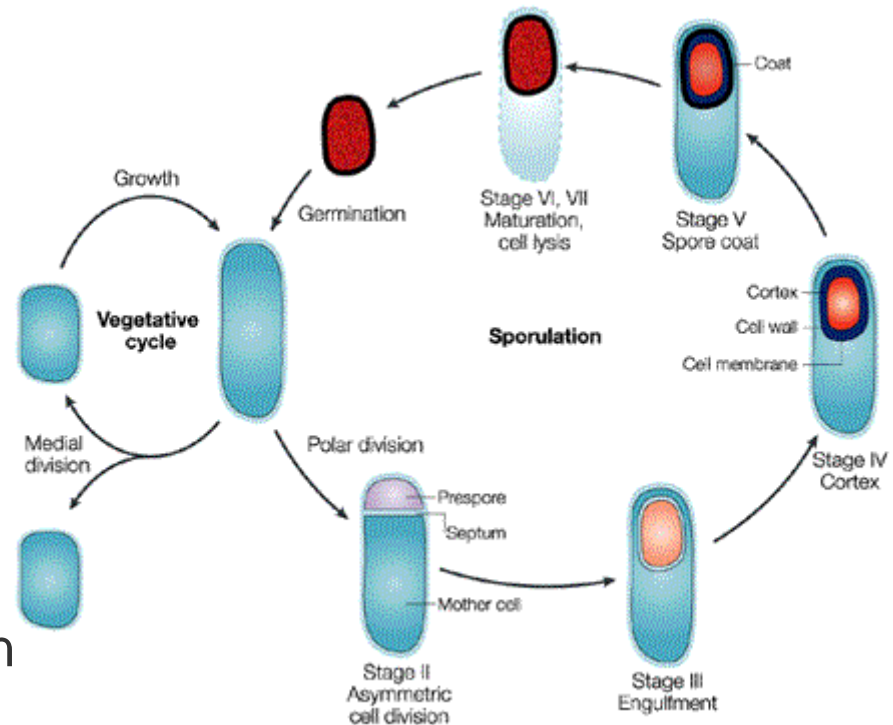
Sporulation and cell differentiation

- ❖ *Bacillus subtilis* cells can form resistant **spores** when environmental conditions become unfavorable (starvation)

Ultimate response in repertoire of stress responses (motility, toxin release, competence, ...)

- ❖ Asymmetric cell division produces smaller forespore cell and larger mother cell

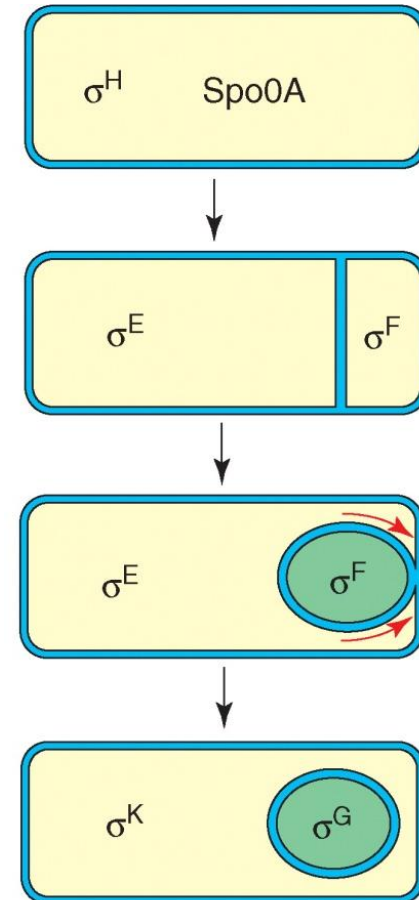
Prototype of cell differentiation and intercellular signalling



Errington (2005), *Nat. Rev. Microbiol.*, 1(2):117-126

Sporulation and cell differentiation

- ❖ Precise temporal ordering of events on molecular level
 - ❖ Specific proteins control differentiation processes in different stages of sporulation
- Transcription factors, **sigma factors**

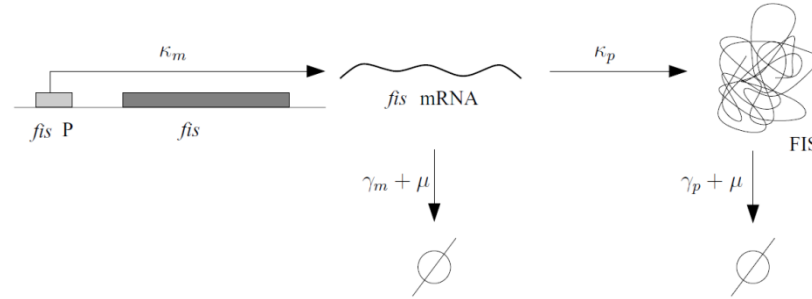


Piggot, Hilbert (2004), *Curr. Opin. Microbiol.*, 7(6):579-86

General questions 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 protein concentrations in the cell
 - Enzymes, sigma factors, transcription factors, ...
- ❖ **Question:** how can protein concentrations change in response to specific environmental changes?
- ❖ **Question:** how does cell coordinate changes in concentration of a variety of proteins?

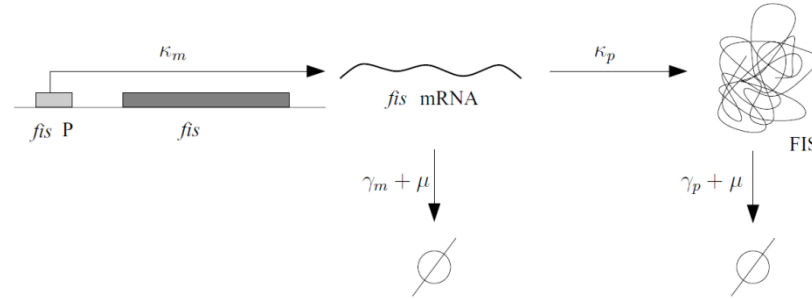
Gene expression



❖ Typically, and simplifying quite a bit, **gene expression** in bacteria involves:

- Transcription by RNAP (mRNA)
- Translation by ribosomes (proteins)
- Degradation of mRNA and protein

Regulation of gene expression

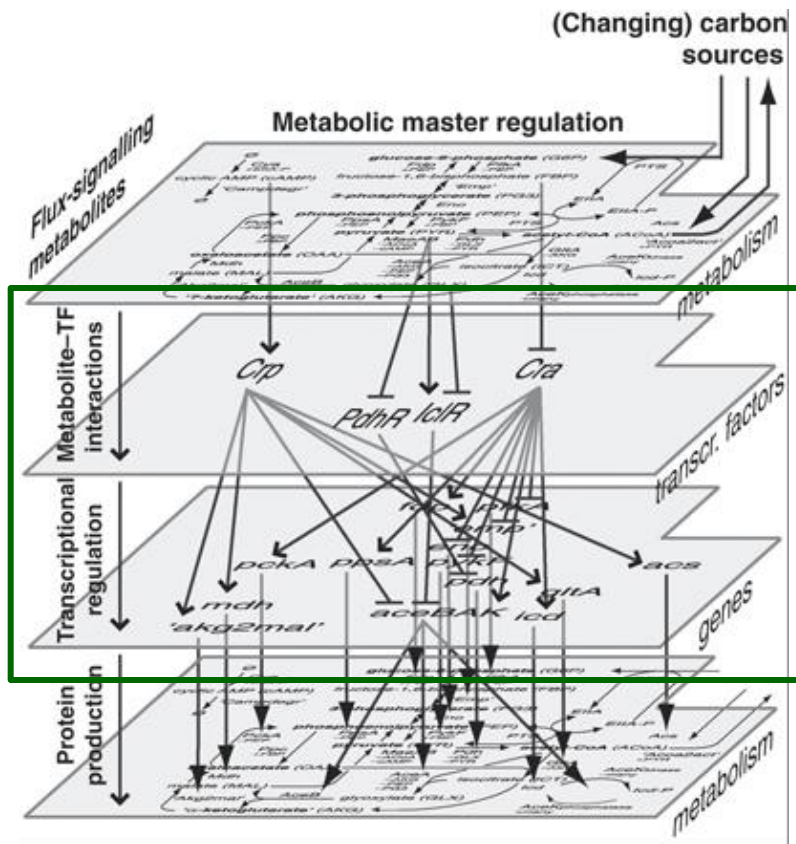


❖ Typically, and simplifying quite a bit, **regulation of gene expression** in bacteria involves:

- Transcription regulation by transcription factors
- Translational regulation by small RNAs
- Regulation of degradation by proteases

Gene regulatory networks

- ❖ Gene regulatory networks control changes in gene expression levels in response to environmental perturbations



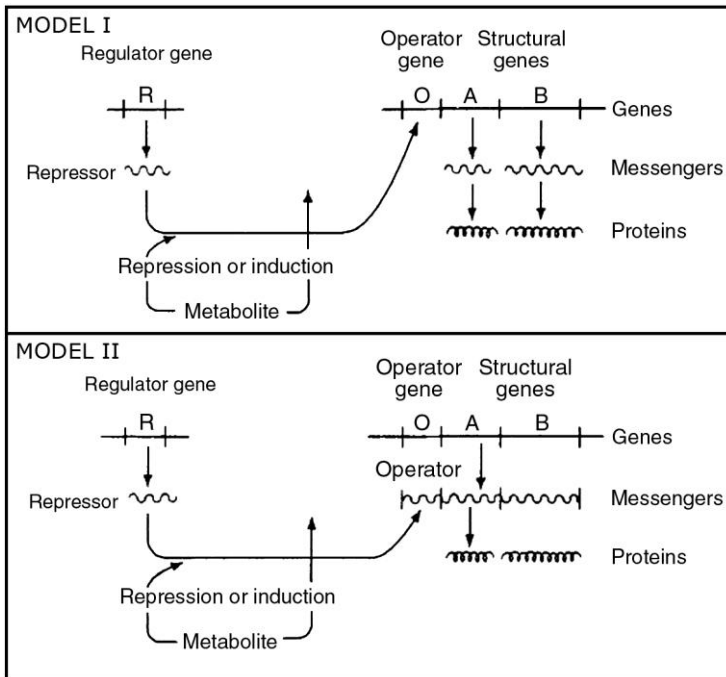
- ❖ Gene regulatory networks consist of genes, gene products, signalling metabolites, and their mutual regulatory interactions

Global regulators of transcription involved in glucose-acetate diauxie in *E. coli*

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

Gene regulatory networks

- ❖ **Gene regulatory networks** control changes in expression levels in response to environmental perturbations



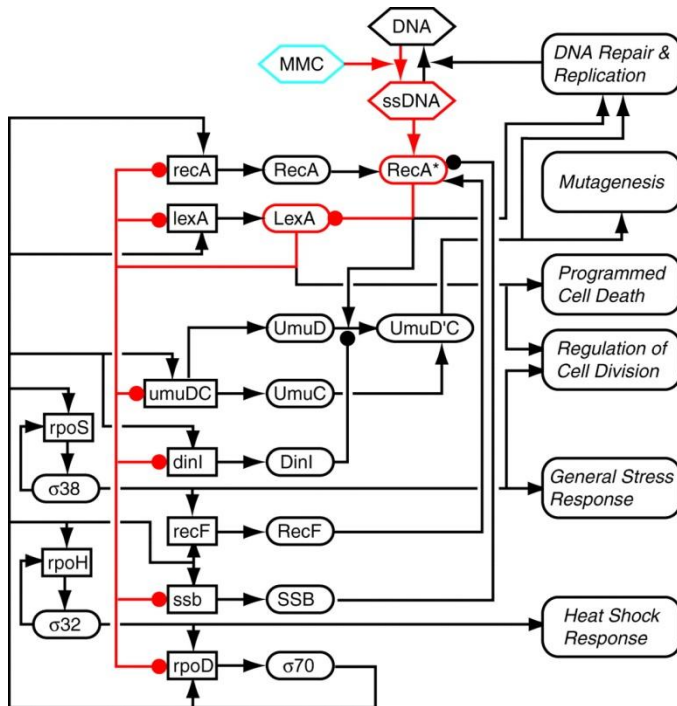
- ❖ Gene regulatory networks consist of genes, gene products, signalling metabolites, and their mutual regulatory interactions

Original *lac* operon model

Jacob and Monod (1961), *J. Mol. Biol.*, 3(3):318-56

Gene regulatory networks

- ❖ **Gene regulatory networks** control changes in expression levels in response to environmental perturbations



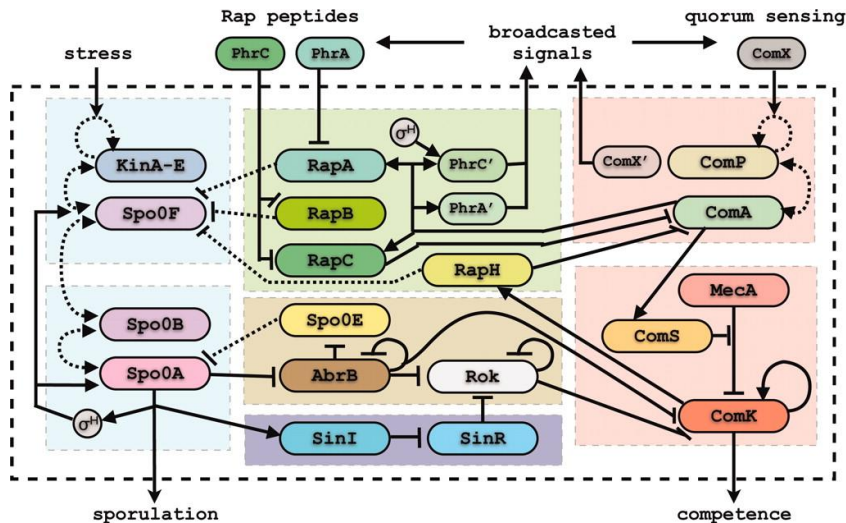
- ❖ Gene regulatory networks consist of genes, gene products, signalling metabolites, and their mutual regulatory interactions

SOS response network in *E. coli*

Gardner *et al.* (2011), *Science*, 301(5629):102-5

Gene regulatory networks

- ❖ **Gene regulatory networks** control changes in expression levels in response to environmental perturbations



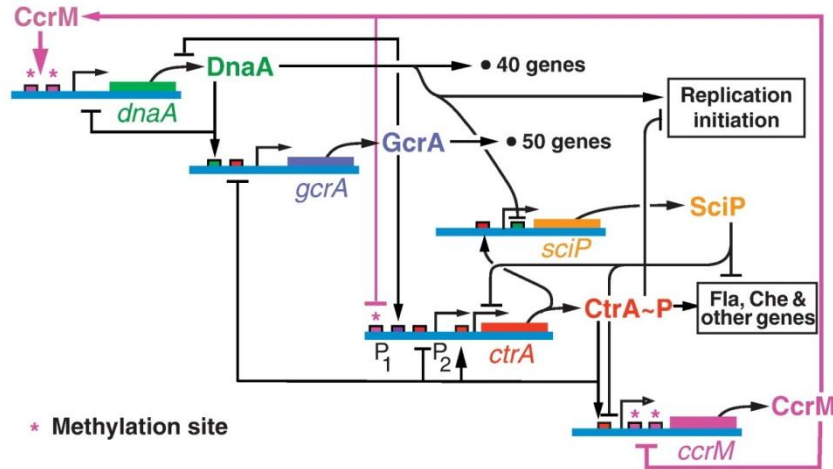
- ❖ Gene regulatory networks consist of genes, gene products, signalling metabolites, and their mutual regulatory interactions

Sporulation and competence network in *B. subtilis*

Schultz *et al.* (1961), *Proc. Natl. Acad. Sci.*, 106(50):21027-34

Gene regulatory networks

- ❖ **Gene regulatory networks** control changes in expression levels in response to environmental perturbations



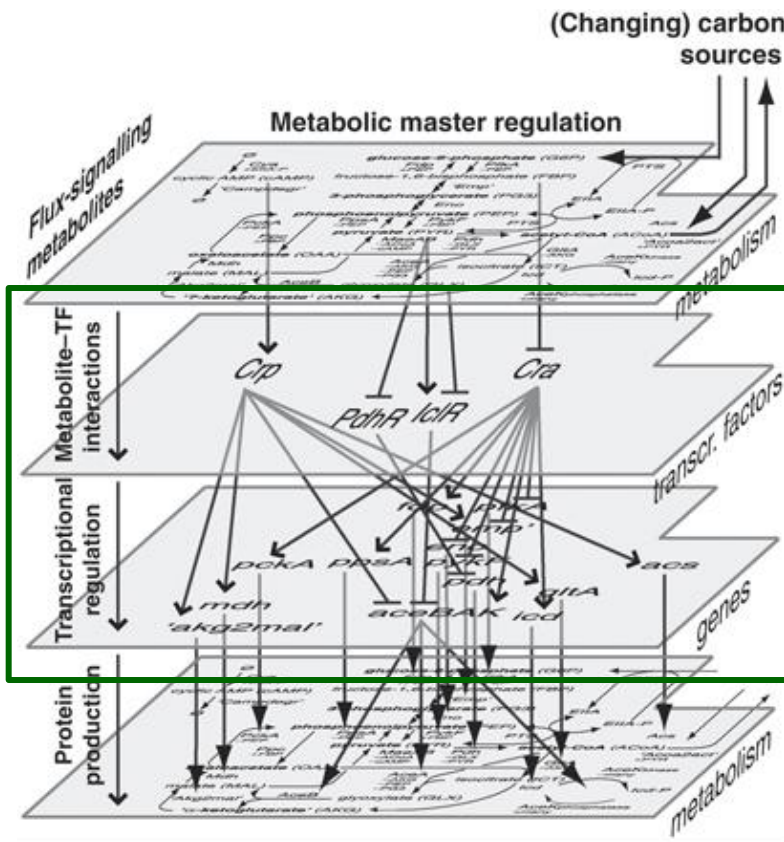
- ❖ Gene regulatory networks consist of genes, gene products, signalling metabolites, and their mutual regulatory interactions

Caulobacter cell cycle network

McAdams and Shapiro (2011), *J. Mol. Biol.*, 409(1):28-35

Broader view on gene regulatory networks

- ❖ Gene regulatory networks control changes in expression levels in response to environmental perturbations

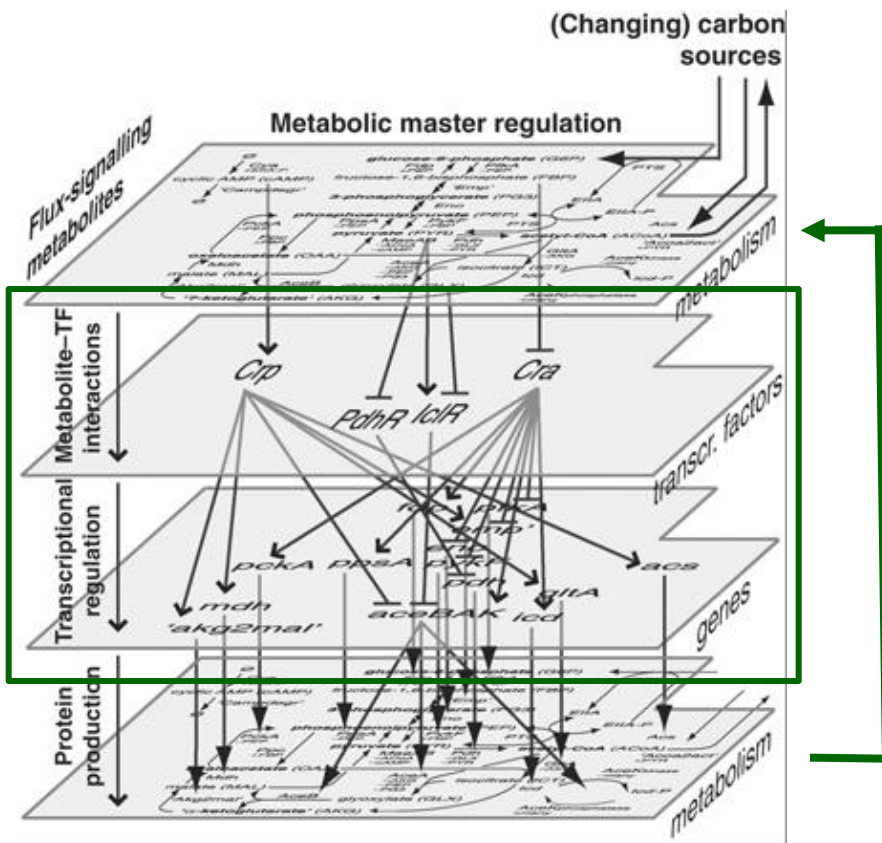


- ❖ **But:** adaptation of gene expression leads to changes in metabolism which feed back into regulatory network
- ❖ Gene regulatory networks are intertwined with metabolic and signaling networks

Complex, heterogeneous systems evolving on different time-scales

Broader view on gene regulatory networks

- ❖ Gene regulatory networks control changes in expression levels in response to environmental perturbations



- ❖ Feedback through metabolism leads to indirect regulatory interactions: **metabolic coupling**

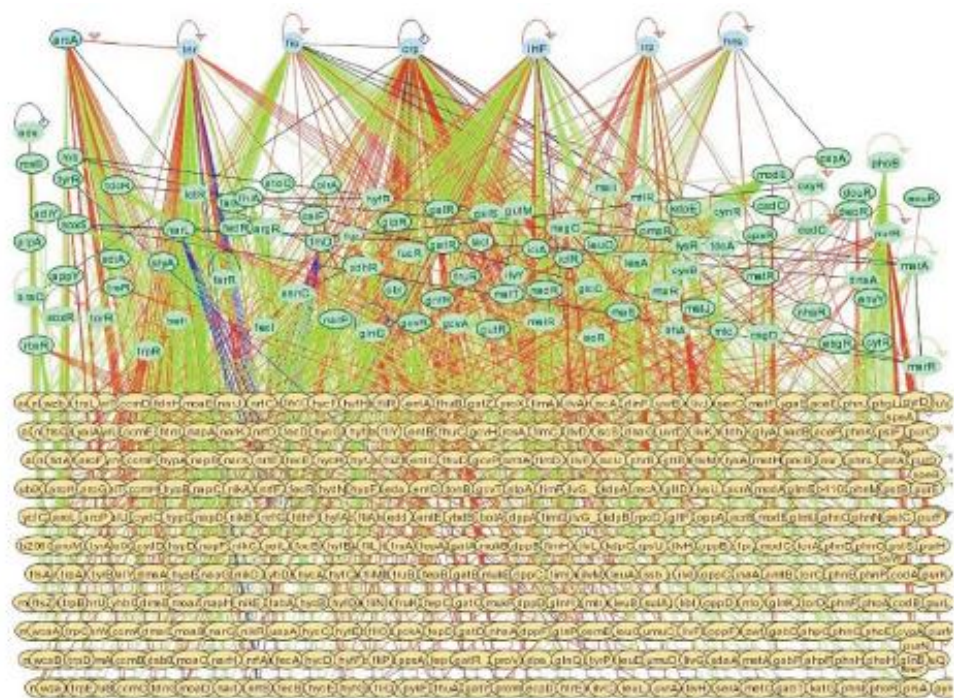
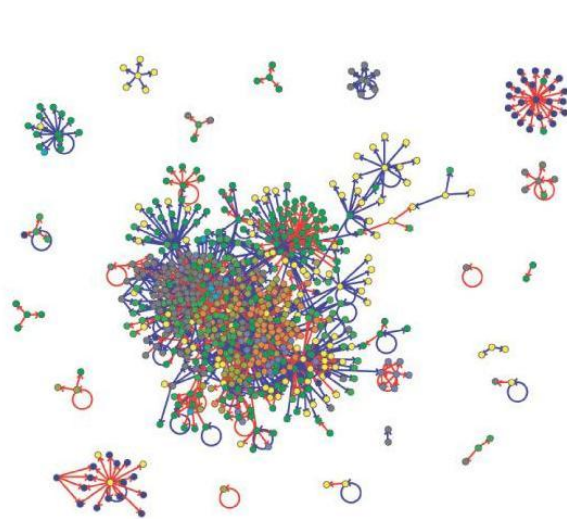
Regulatory effects of enzymes on gene expression

Baldazzi *et al.* (2010), *PLoS Comput. Biol.*, 6(6):e1000812

Complexity of gene regulatory networks

- Most gene regulatory networks of biological interest are large and complex

E. coli has 4200 genes coding for several hundreds of transcription factors



Cases and de Lorenzo (2005), *Nat. Rev. Microbiol.*, 3(2):105-18

Martinez-Antonio et al. (2003), *Curr. Opin. Microbiol.*, 6(5):482-9

Systems biology

❖ Most gene regulatory networks of biological interest are large and complex

❖ **No global view of functioning** of network available, despite abundant knowledge on network components

Understanding of dynamics requires **experimental tools** for monitoring gene expression over time

Understanding of dynamics requires **mathematical modeling** and **computer analysis and simulation**

Discipline now often referred to as **systems biology**

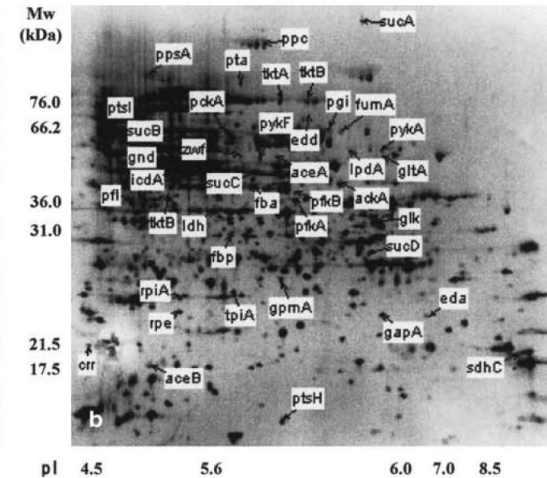
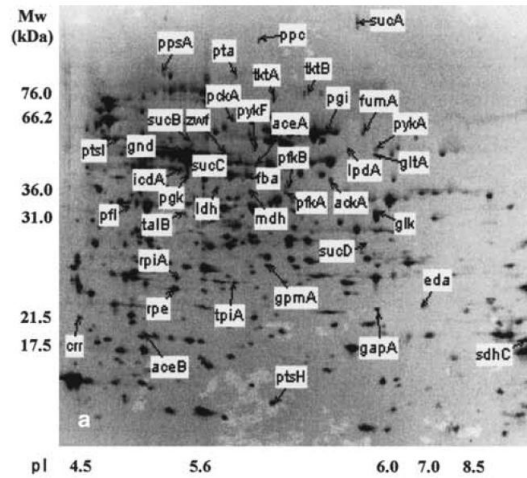
Alon (2007), *An Introduction to Systems Biology*, Chapman & Hall/CRC Press

Experimental tools

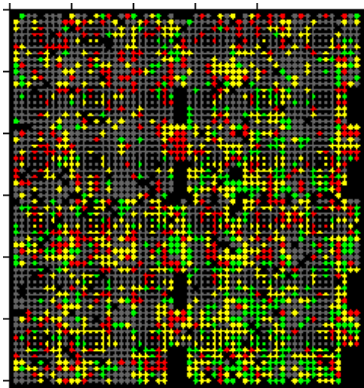
- ❖ A variety of experimental tools allow gene expression to be measured, by quantifying mRNA and protein abundancies

Peng and Shimizu (2003),
App. Microbiol. Biotechnol., 61:163-78

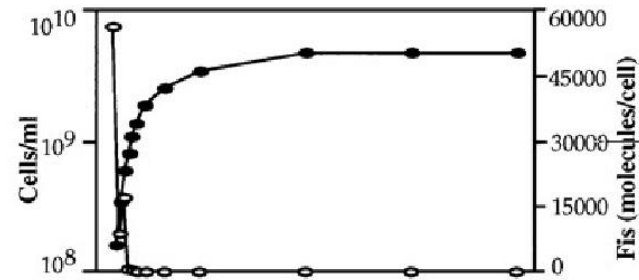
2D gels



DNA
microarrays



Western blots

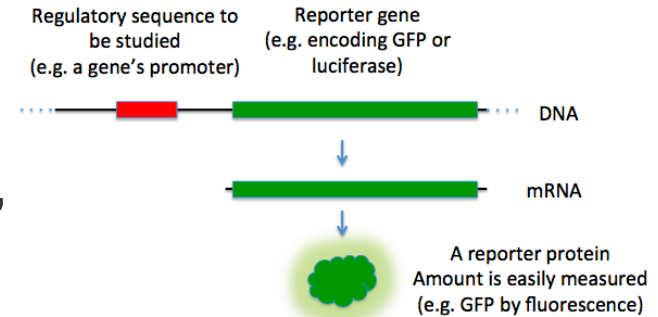


Ali Azam et al. (1999), *J. Bacteriol.*, 181(20):6361-70

Fluorescent reporter genes

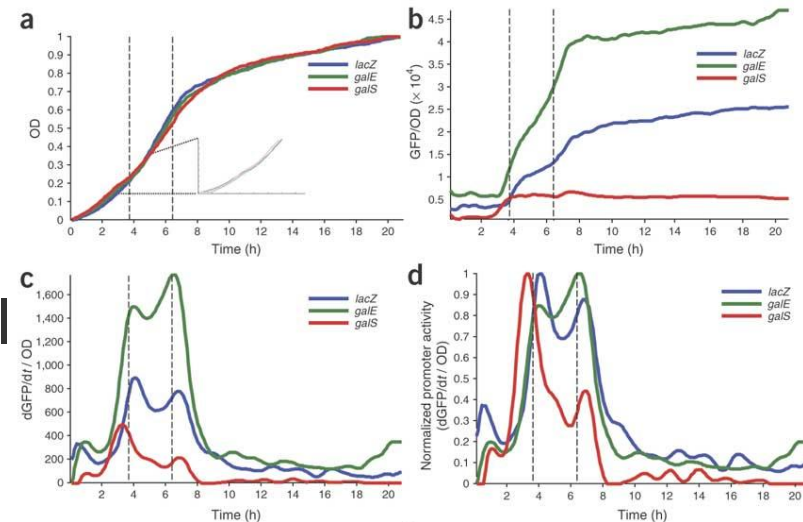
❖ Use of fluorescent reporter genes allows expression from host promoter to be monitored *in vivo* and in real time

- 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*

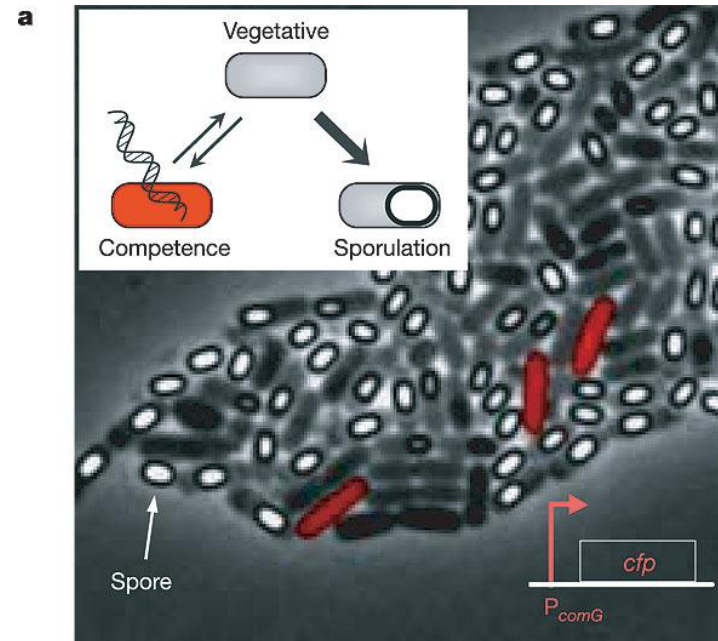
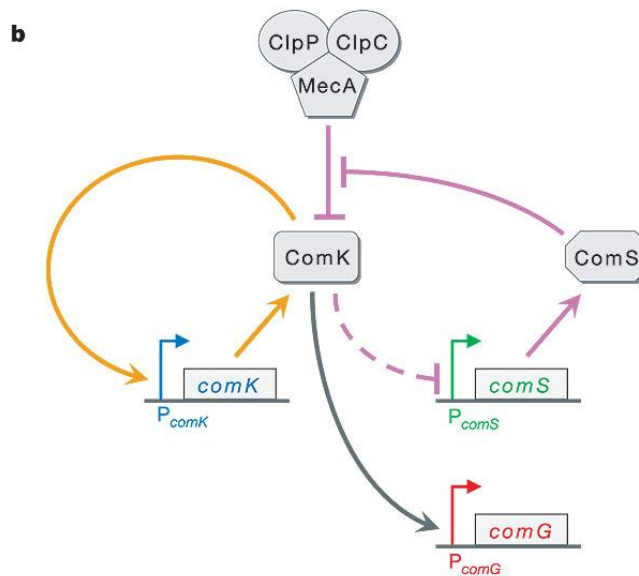
Zaslaver *et al.* (2006), *Nat. Methods*, 3(8):623-8



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*

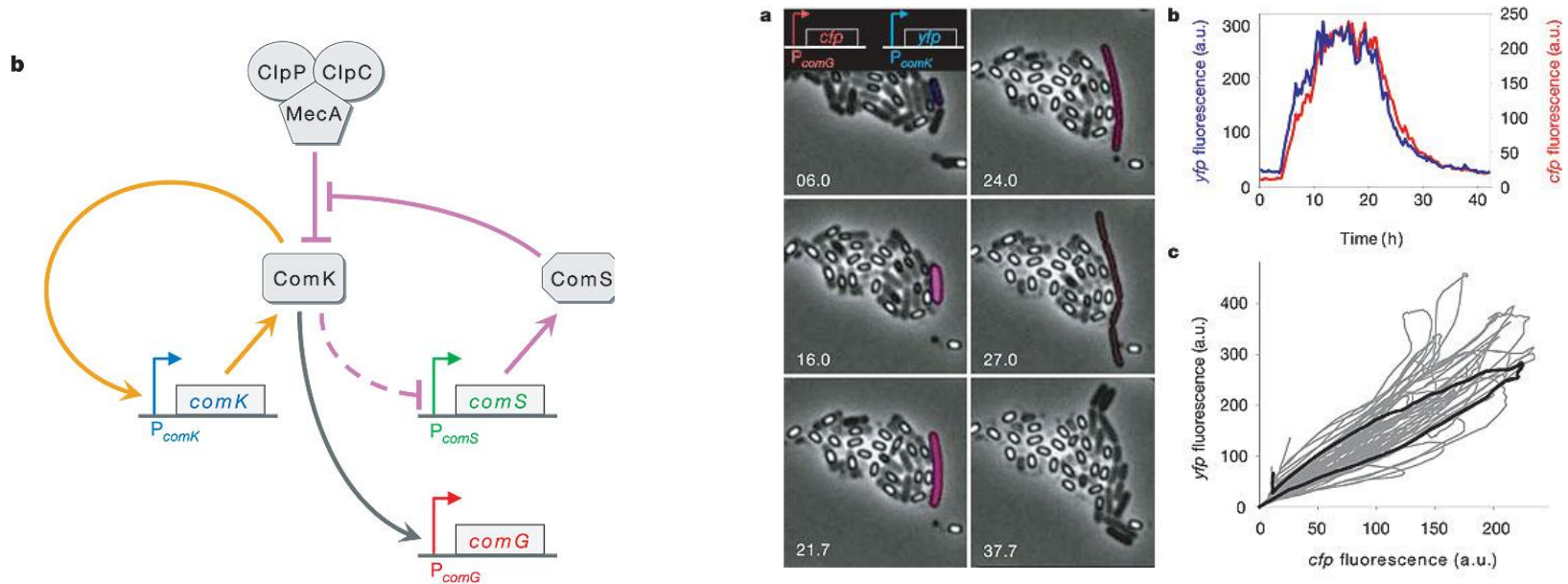
Süel *et al.* (2006), *Nature*, 440:545-50



Single-cell microscopy

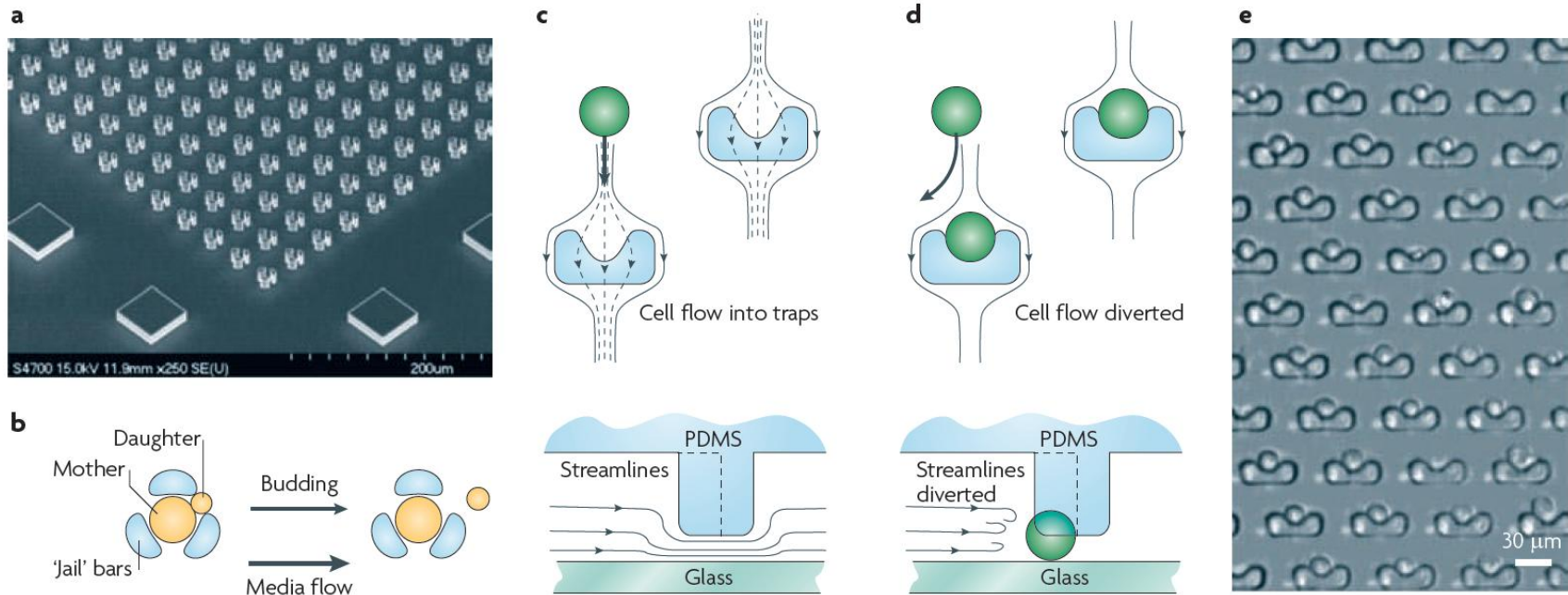
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- ❖ Monitoring onset of competence in *B. subtilis*

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



Single-cell microscopy and microfluidics

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



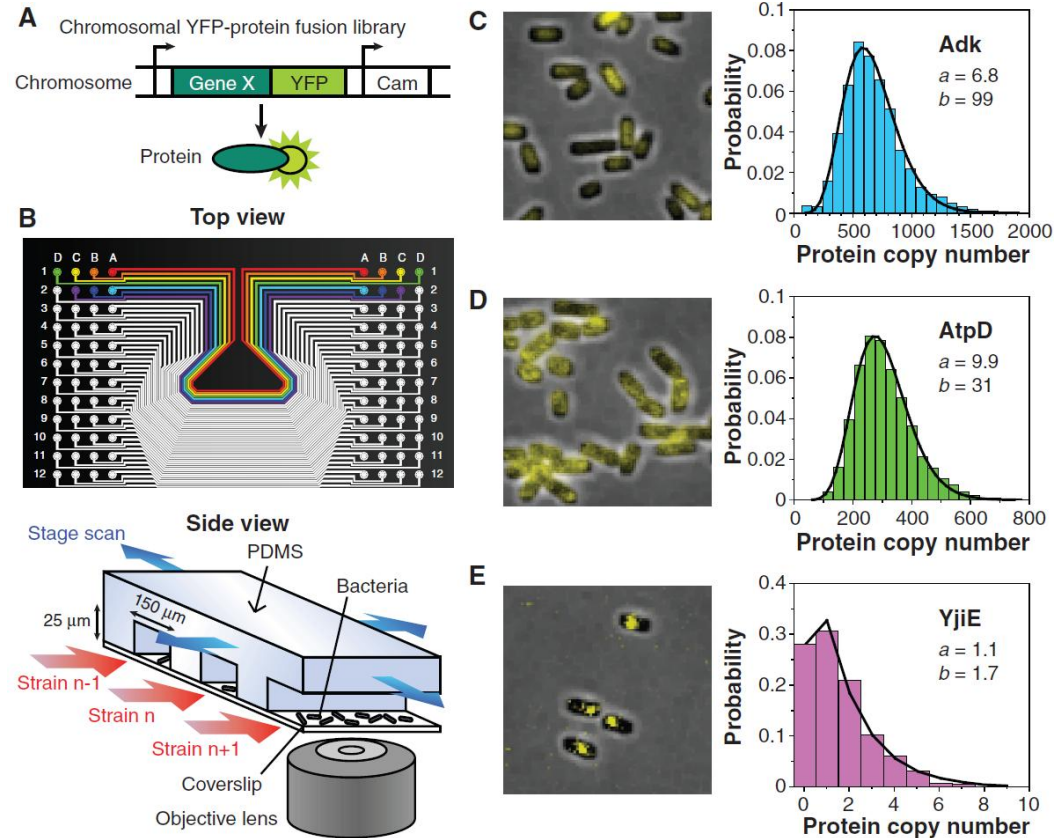
- ❖ **Microfluidic** devices allow tight control of environmental perturbations

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

Single-molecule quantification

❖ Measurement of gene expression at **single-molecule level** using fluorescence reporter genes, microfluidic device, fluorescence microscopy, and calibration

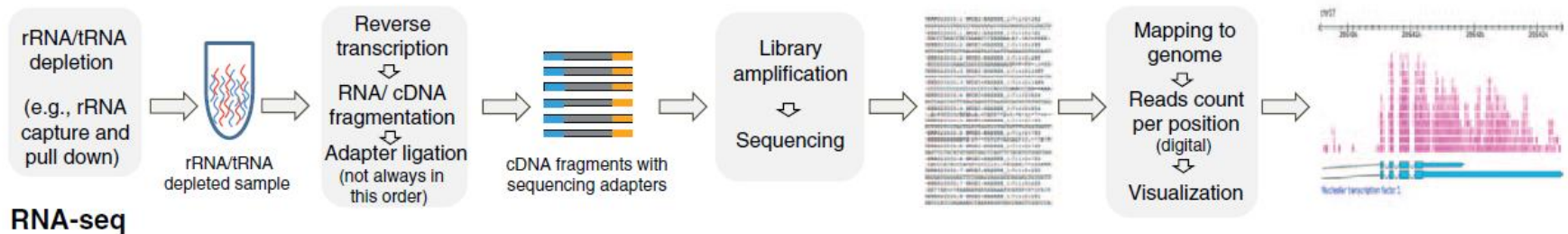
❖ Measurement of expression of thousand *E. coli* genes using YFP-tagged chromosomal reporters



Taniguchi *et al.* (2010), *Science*, 329(5991):533-9

RNA sequencing

- ❖ **RNA sequencing (RNA-seq)** exploits new generation of sequencing technologies for quantifying RNA levels

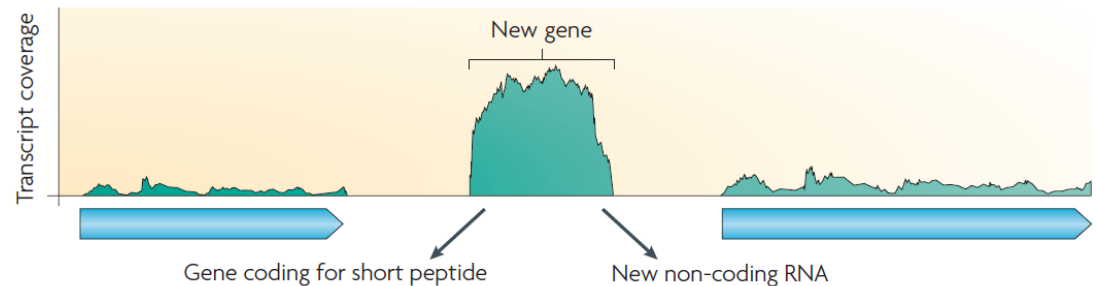


Mäder *et al.* (2010), *Curr. Opin. Biotechnol.*, 22(1):32-41

- ❖ **Use of RNA-seq data to discover new genes and detect operon structure**

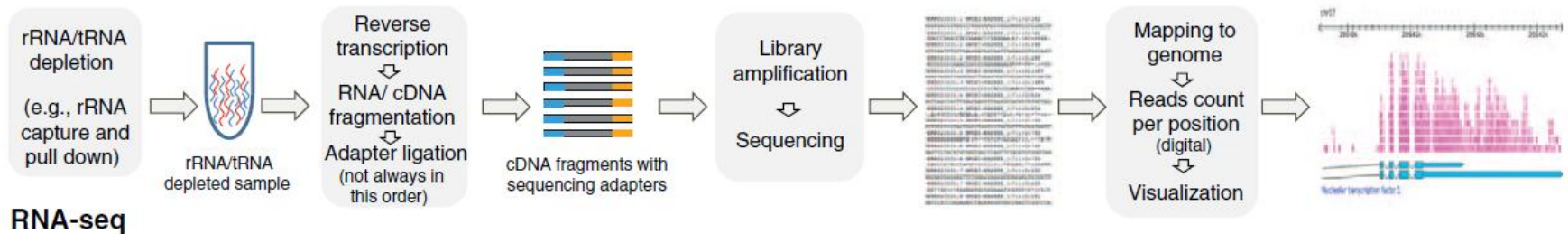
Sorek and Cossart (2010), *Nat. Rev. Genet.*, 11(1):9-16

a Discovery of new genes



RNA sequencing

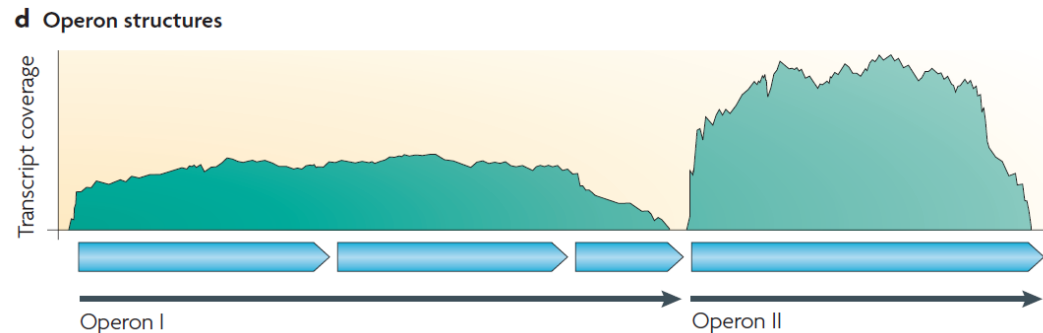
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Mäder *et al.* (2010), *Curr. Opin. Biotechnol.*, 22(1):32-41

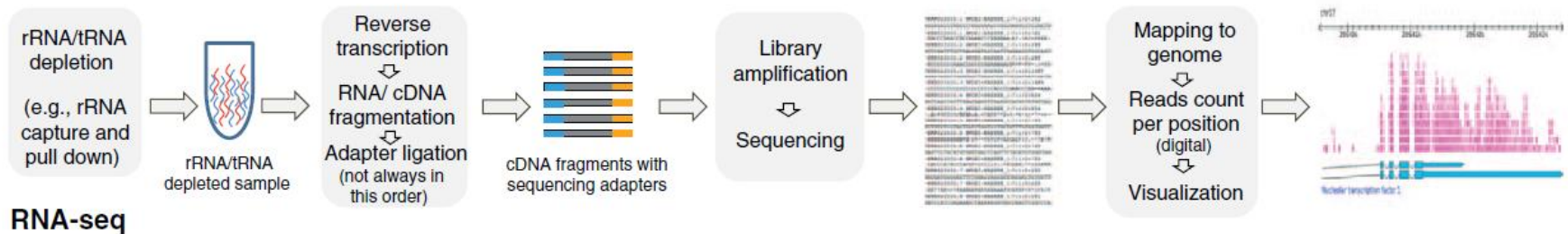
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Sorek and Cossart (2010), *Nat. Rev. Genet.*, 11(1):9-16



RNA sequencing

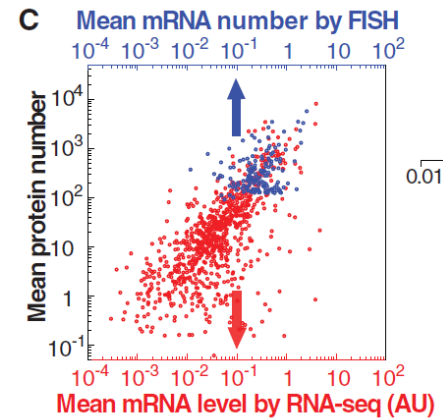
- ❖ **RNA sequencing (RNA-seq)** exploits new generation of sequencing technologies for quantifying RNA levels



Mäder *et al.* (2010), *Curr. Opin. Biotechnol.*, 22(1):32-41

- ❖ Use of RNA-seq to determine correlation between mean RNA and mean protein levels

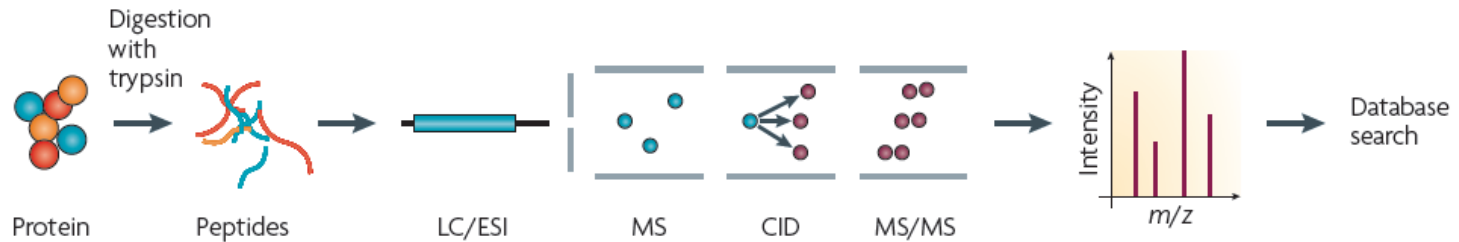
Taniguchi *et al.* (2010), *Science*, 329(5991):533-9



Quantitative proteomics

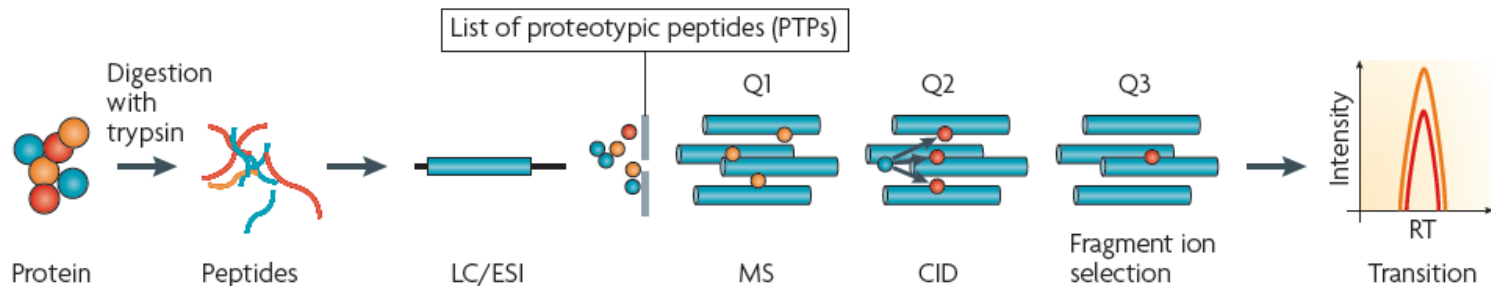
- ❖ Measurement of protein abundance using mass-spectrometry-based techniques (**quantitative proteomics**)

Use of calibration standards to achieve absolute quantification



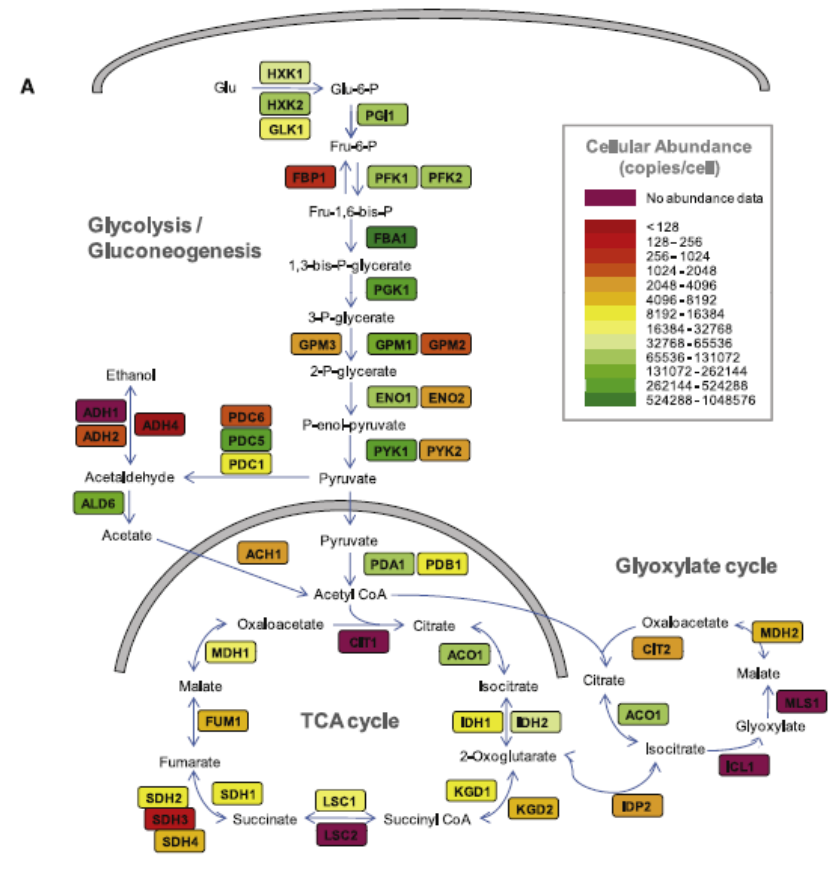
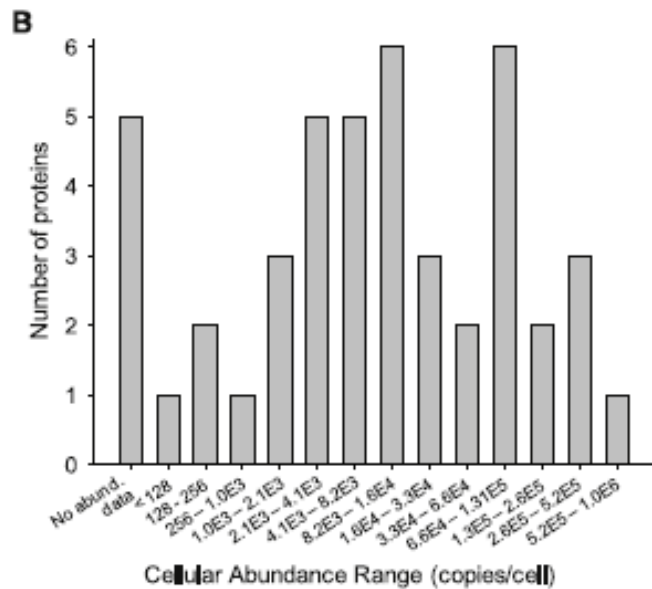
Gstaiger and Aebersold (2009), *Nat. Rev. Genet.*, 10:617-27

- ❖ Several targeted proteomics techniques developed to improve quantification of low-abundance proteins



Quantitative proteomics

❖ Absolute quantification of proteins in yeast carbon metabolism by means of selected reaction monitoring (SRM)



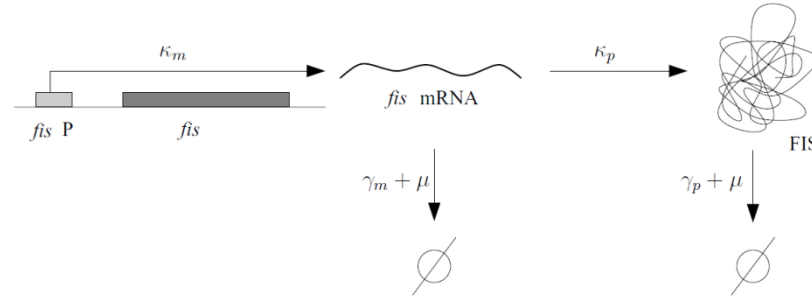
Picotti *et al.* (2009), *Cell*, 138:795-806

Systems biology

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- ❖ **No global view of functioning** of network available, despite abundant knowledge on network components
 - Understanding of dynamics requires **experimental tools** for monitoring gene expression over time
 - Understanding of dynamics requires **mathematical modeling** and **computer analysis and simulation**
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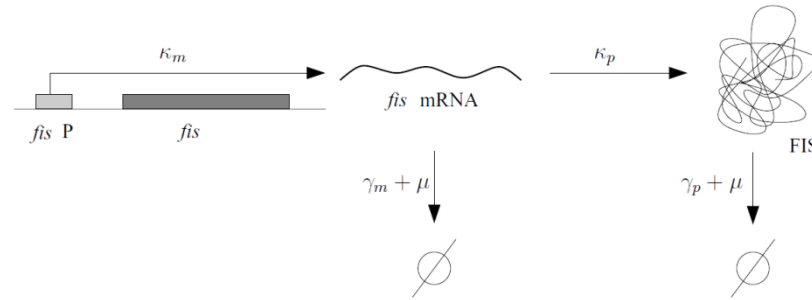
Alon (2007), *An Introduction to Systems Biology*, Chapman & Hall/CRC Press

Modeling of gene regulatory networks



- ❖ Modeling of gene regulatory networks amount to modeling of gene expression and regulation of gene expression
- ❖ Possible **aims** of modeling of gene regulatory networks:
 - Understanding role of individual components and interactions
 - Suggesting missing components and interactions
- ❖ Advantages of **mathematical and computer tools**:
 - Precise and unambiguous description of network
 - Systematic derivation of predictions of network behavior

Modeling of gene regulatory networks



- ❖ Modeling of gene regulatory networks amount to modeling of gene expression and regulation of gene expression
- ❖ First models of gene regulatory networks date back to early days of molecular biology

Feedback circuits and oscillators

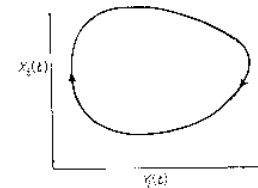
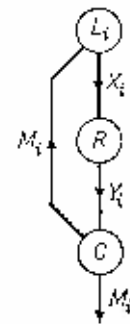
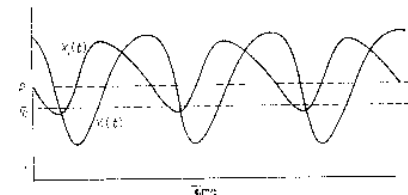


Figure 3.



Goodwin (1963), *Temporal Organization in Cells*

Modeling of gene regulatory networks

- ❖ Different modeling formalisms exist, describing gene expression on different levels of detail



Stochastic master equations

Ordinary differential equations (ODEs)

Boolean networks

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