

# Modeling and simulation of gene regulatory networks 1

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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

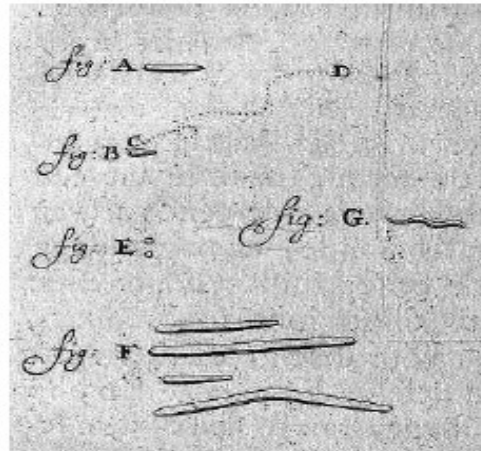
1. **Gene regulatory networks in bacteria**
2. Deterministic modeling of gene regulatory networks
3. Qualitative modeling of gene regulatory networks
4. Stochastic modeling of gene regulatory networks
5. Some current issues and perspectives

# Bacteria

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



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



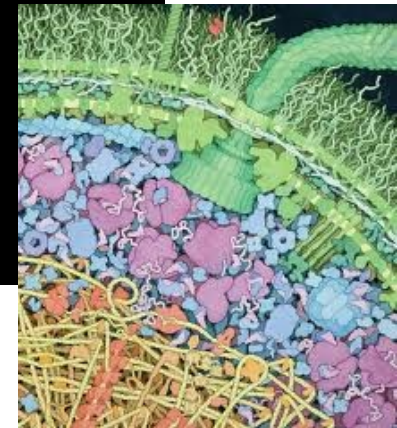
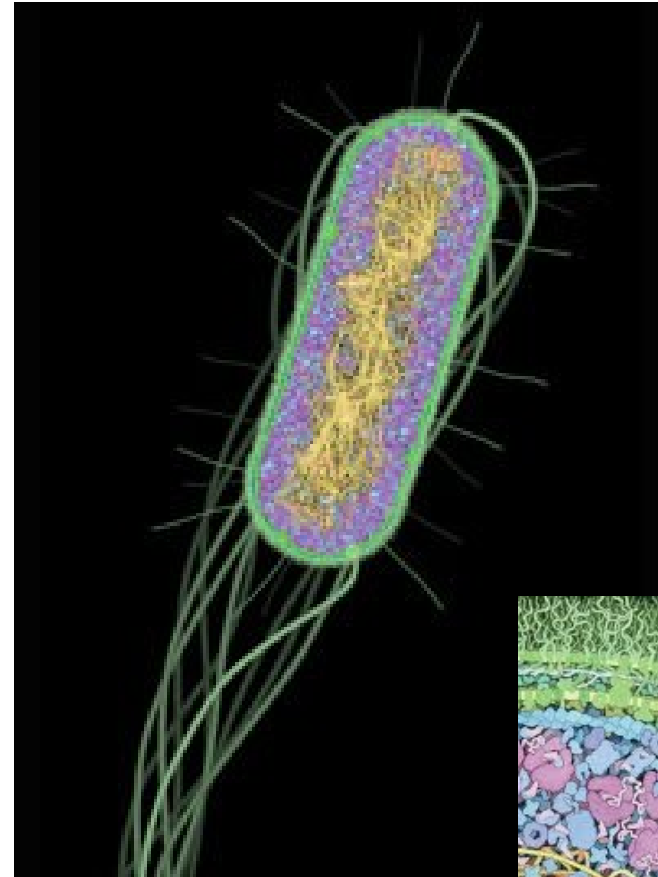
[www.euronet.nl/users/wamar/leeuwenhoek.html](http://www.euronet.nl/users/wamar/leeuwenhoek.html)

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

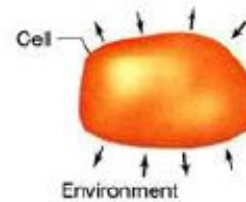
- Bacterial cells are complex biochemical and biophysical machines
  - Wide range of shapes, typically 0.5-5  $\mu\text{m}$  in length
  - $10^6$  bacterial cells in 1 ml of fresh water
  - 10 times as much bacterial cells as human cells in human body



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



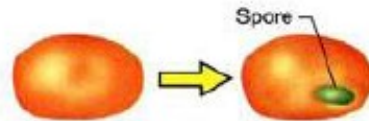
## 1. Metabolism

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.



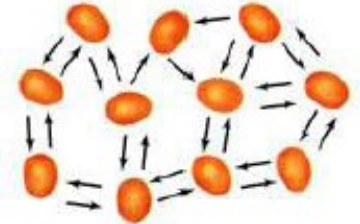
## 2. Reproduction (growth)

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



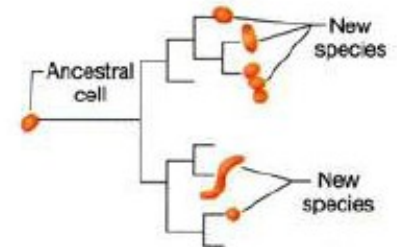
## 3. Differentiation

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.

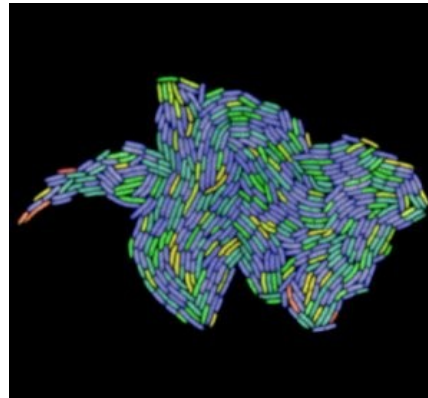
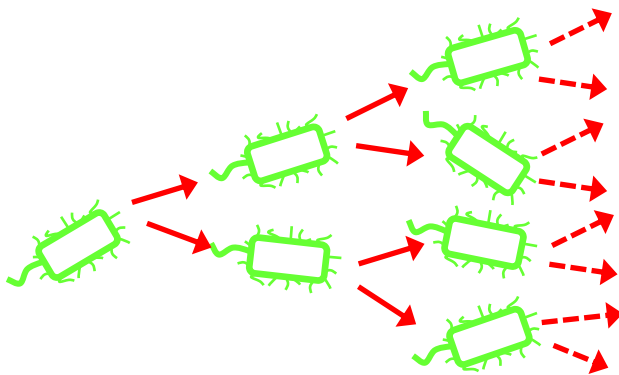
Madigan et al. (2003), *Brock Biology of Microorganisms*, Prentice Hall, 10th ed.



# 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

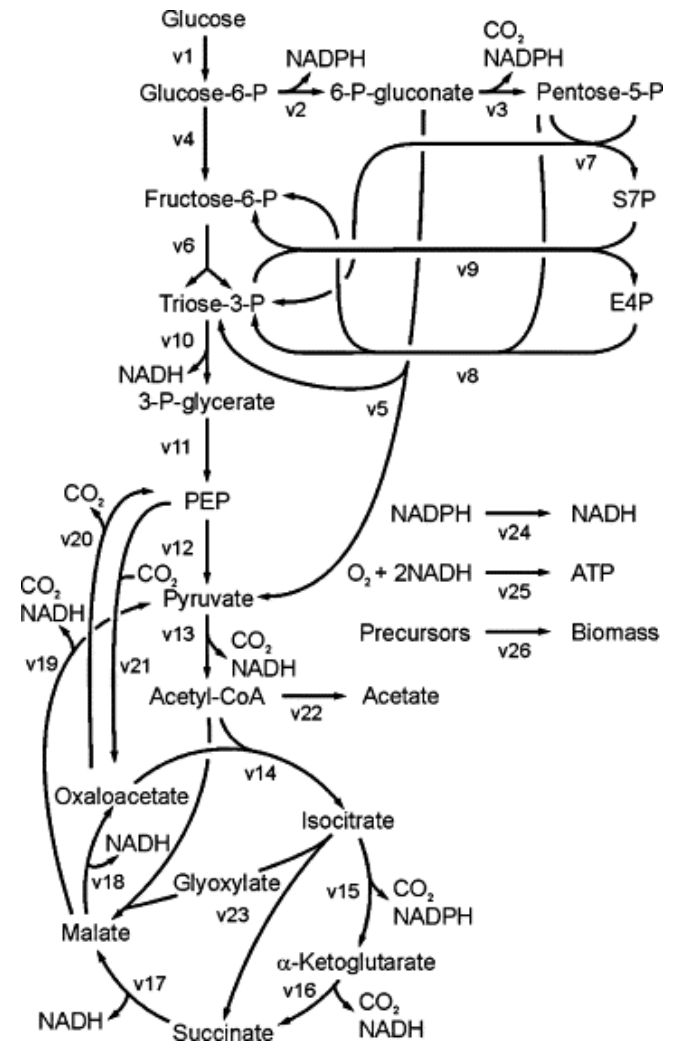
- **Metabolism** fuels growth by production of energy and building blocks for macromolecules, using nutriment from environment

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



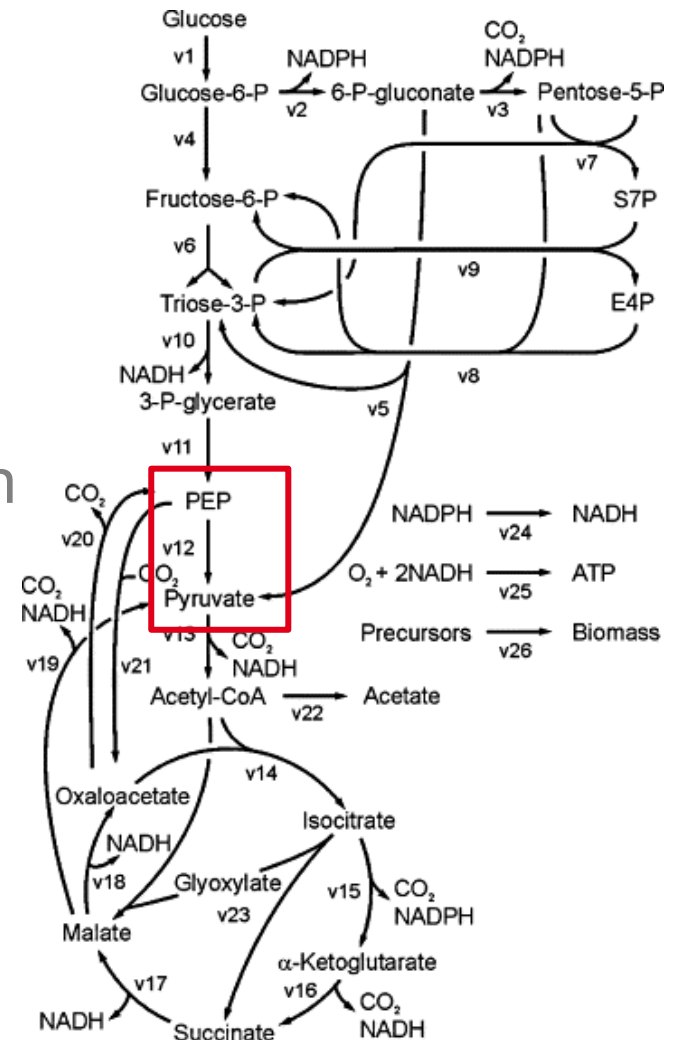
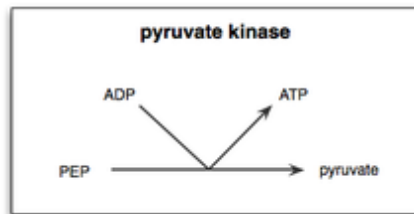
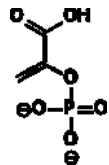
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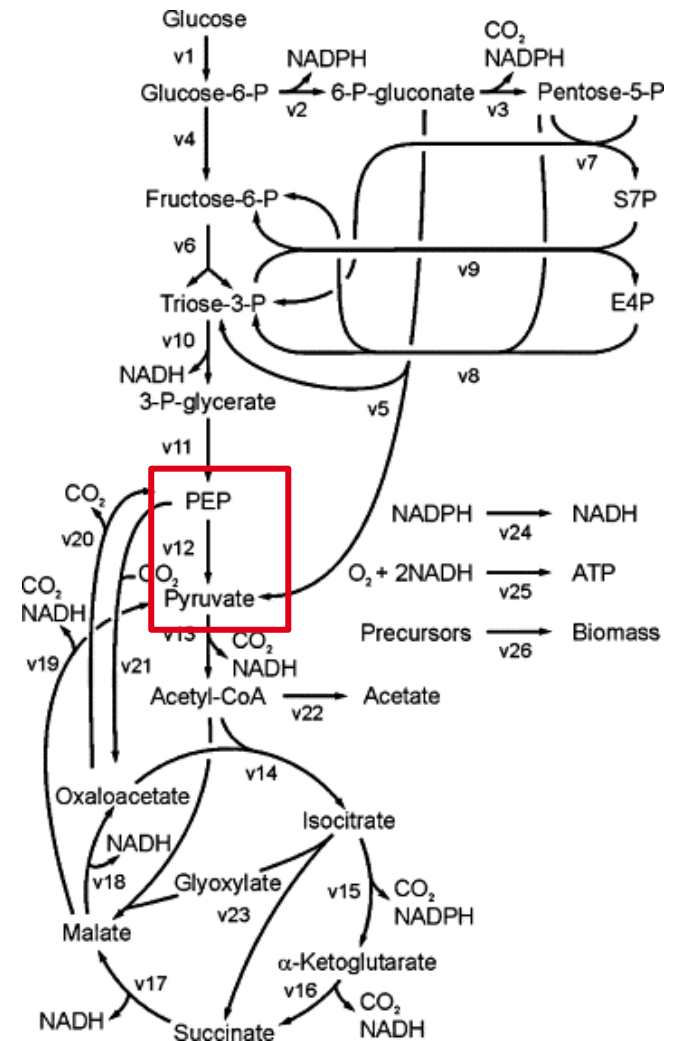
- Enzymes** catalyse individual steps in metabolic network

Pyruvate kinase transforms phosphoenolpyruvate (PEP) into pyruvate



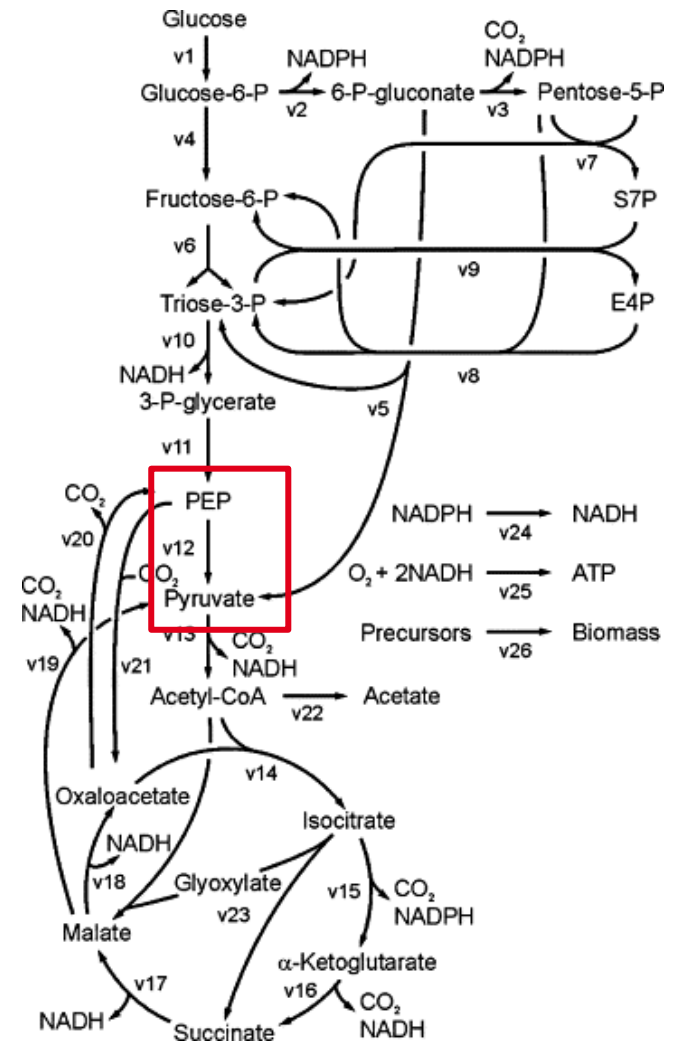
# Bacterial growth and metabolism

- 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

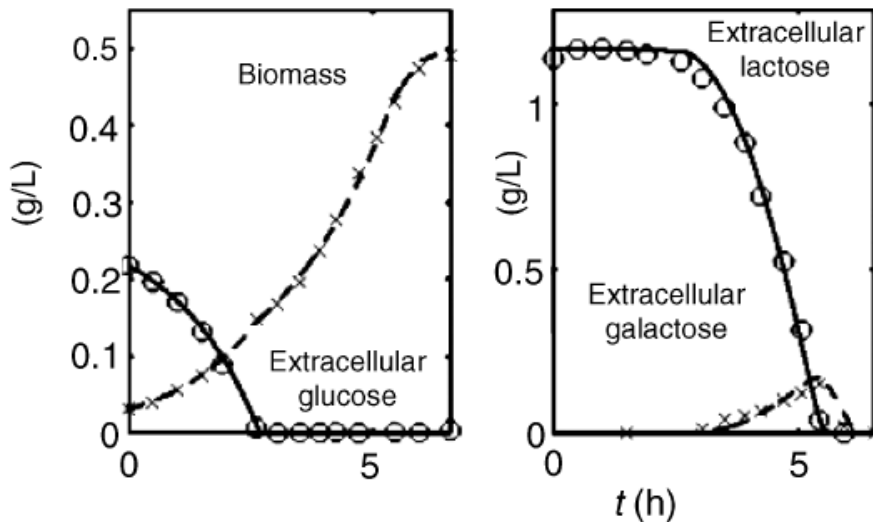
- Central **carbon metabolism** breaks down carbon sources for energy production and macromolecular synthesis
  - Glucose, acetate, lactose, ...
- Enzymes produced from information encoded in **genes**
  - pykF* is gene encoding pyruvate kinase
  - Expression of *pykF* regulated by transcription factor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



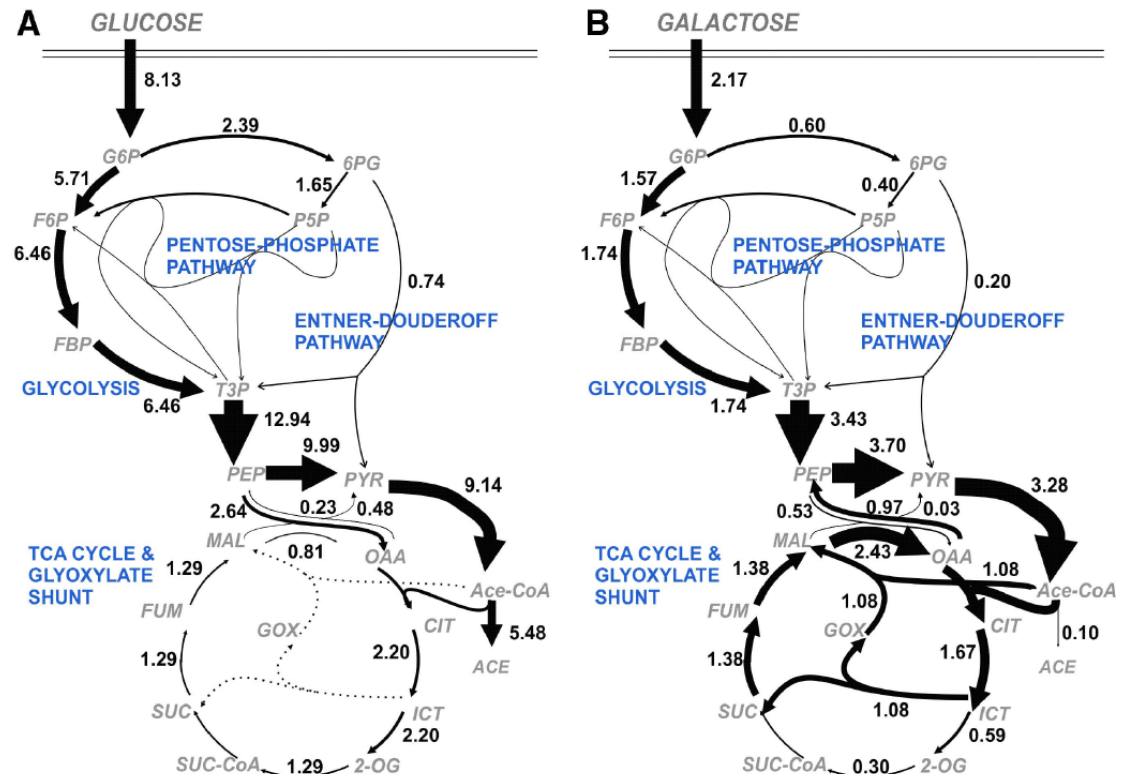
Bettenbrock *et al.* (2006), *J. Biol. Chem.*, 281(5):2578-84

- 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

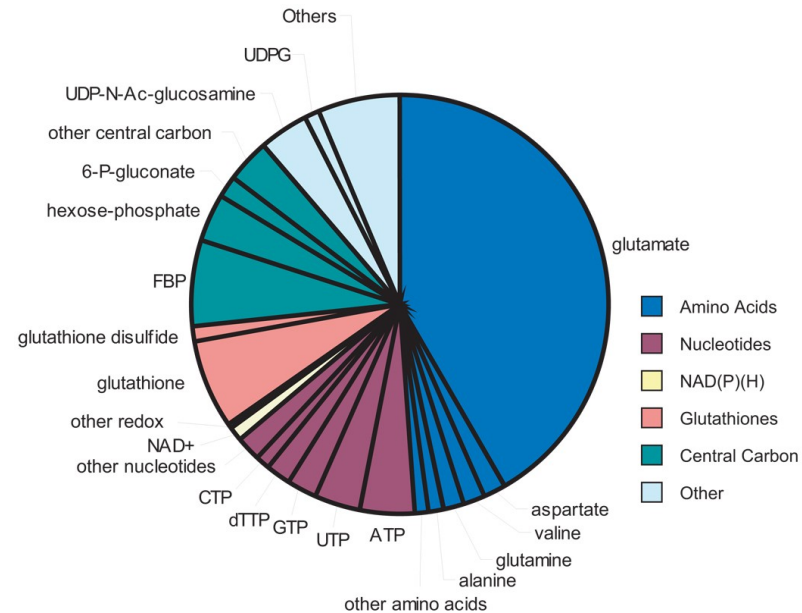


Haverkorn van Rijsewijk *et al.* (2011), *Mol. Syst. Biol.*, 7:477

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

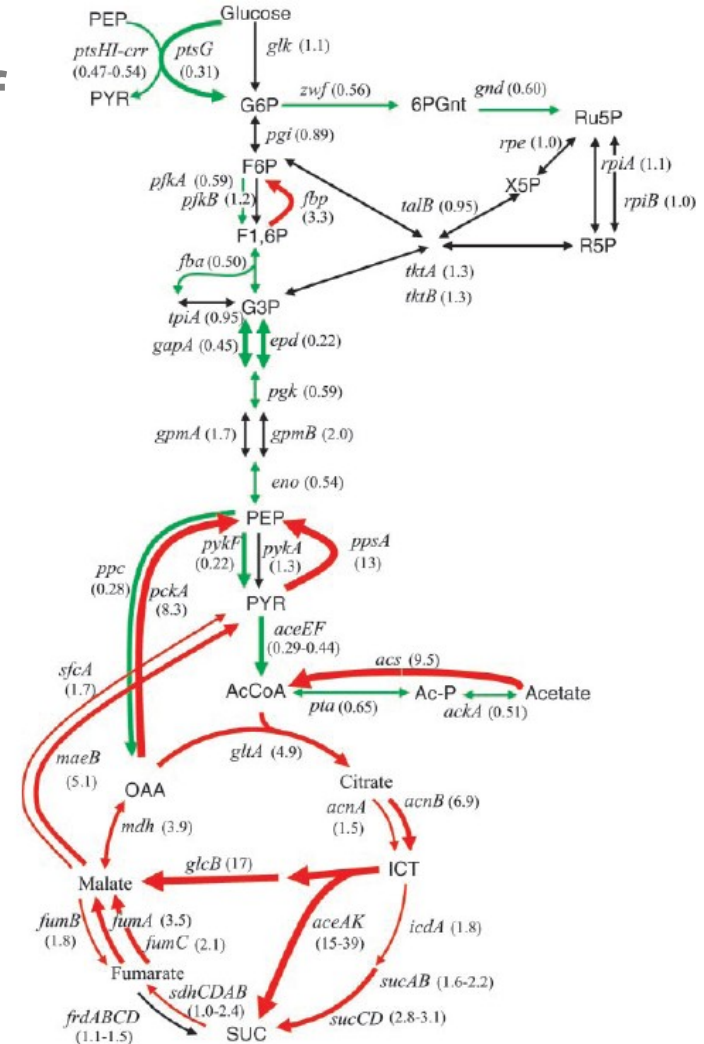
Bennett *et al.* (2009), *Nat. Chem. Biol.*, 5(8):593-9



# 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

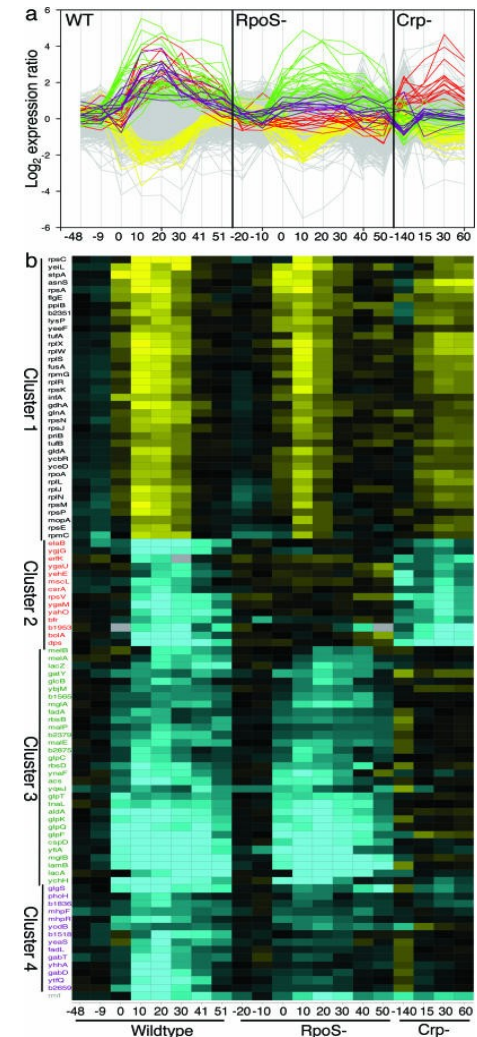


Oh *et al.* (2002), *J. Biol. Chem.*, 277(15):13175–83

# 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*



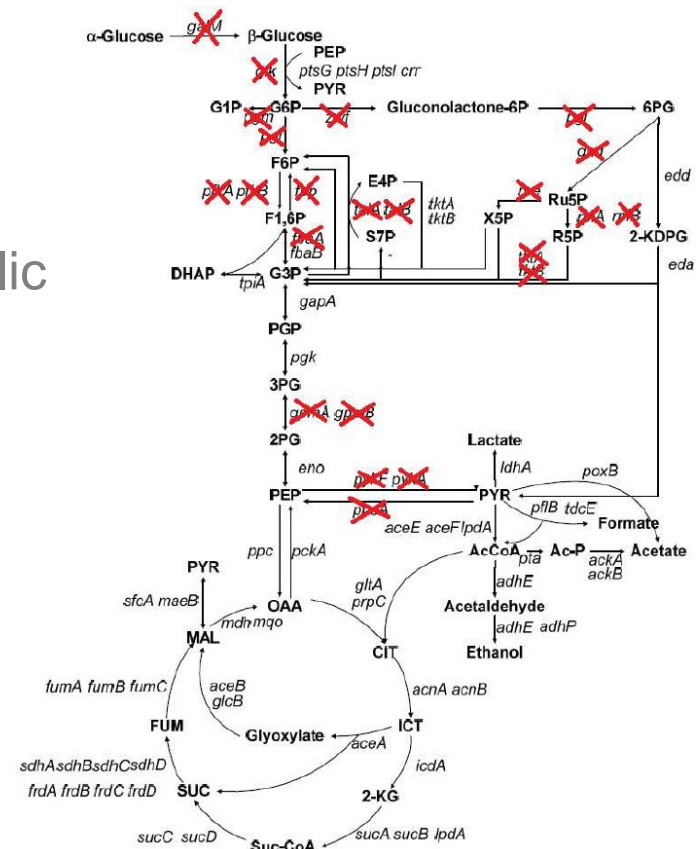
Traxler *et al.* (2006), *Proc. Natl. Acad. Sci. USA*, 103(7):2374–9

# 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

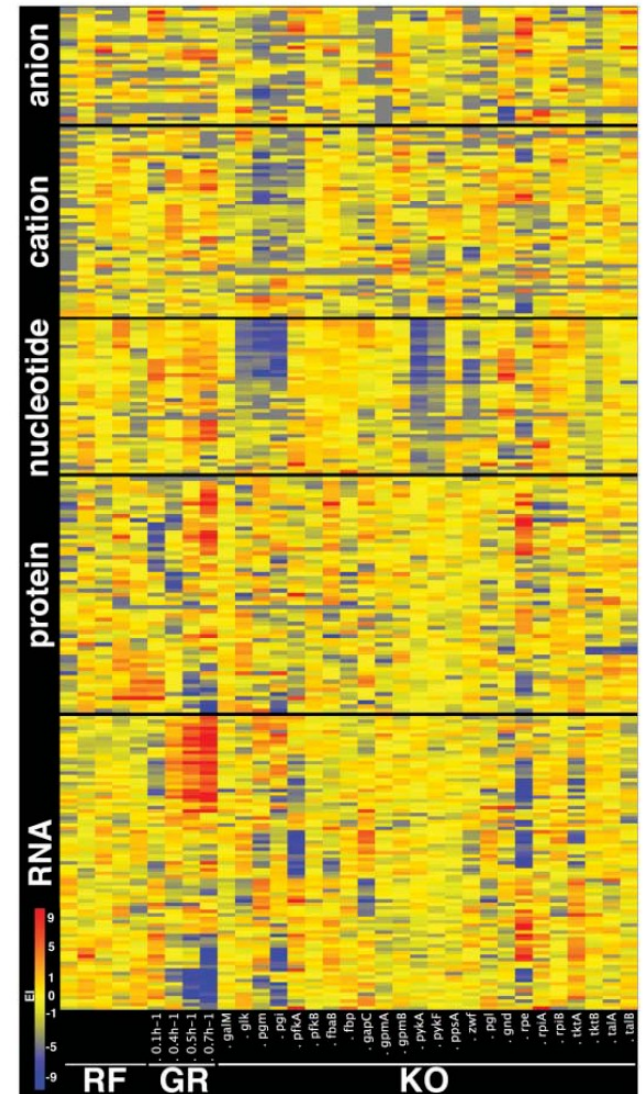


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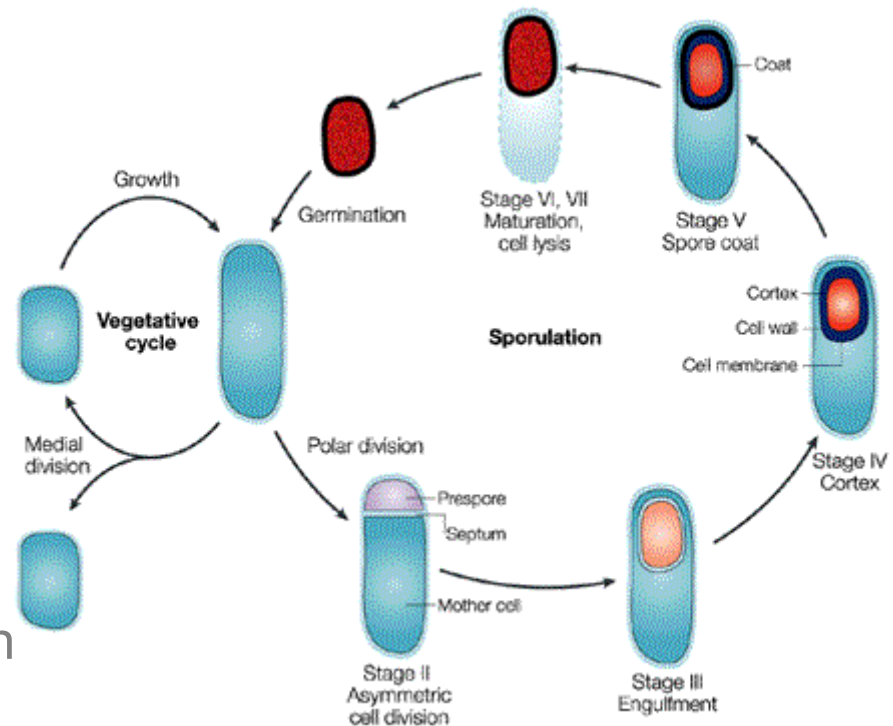


# Sporulation and cell differentiation

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

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

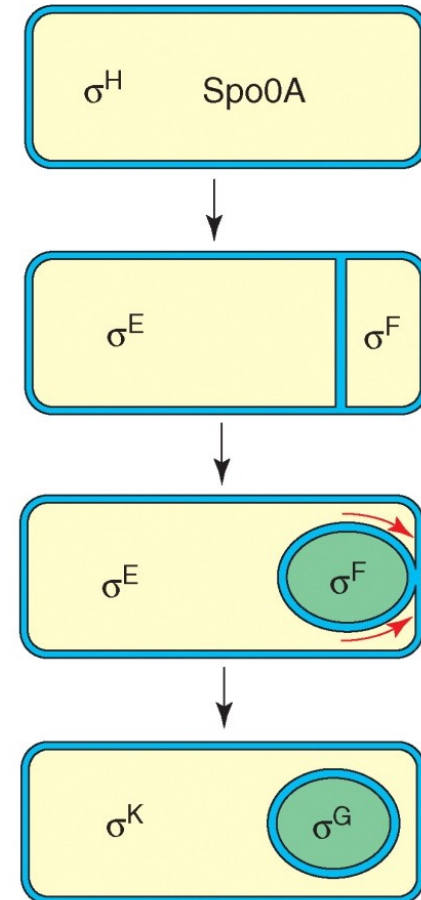
- Asymmetric cell division produces smaller forespore cell and larger mother cell  
Prototype of cell differentiation and intercellular signalling



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



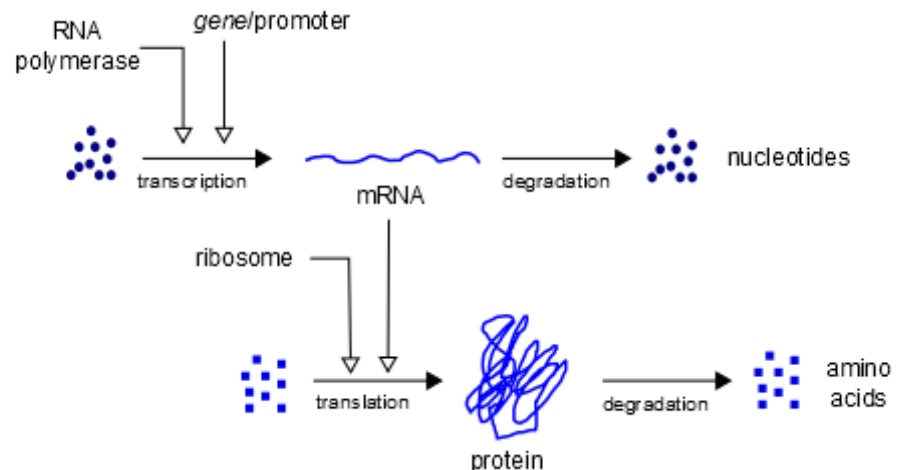
# General questions on cellular adaptation

- 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?
- Changes in protein concentrations involve changes in gene expression

# Gene expression

- Typically, and simplifying quite a bit, **gene expression** in bacteria involves:
  - Transcription by RNA polymerase (mRNA)
  - Translation by ribosomes (proteins)
  - Degradation of mRNA and protein

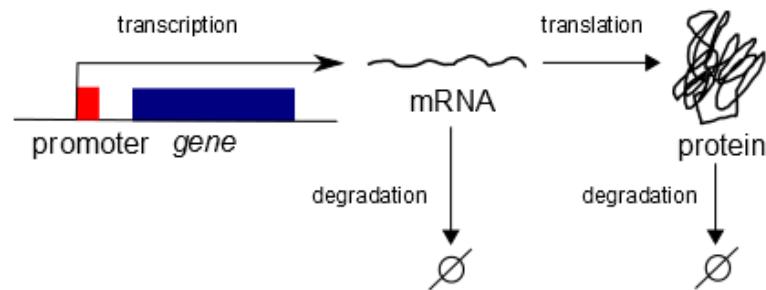
## Biochemical view:



# Gene expression

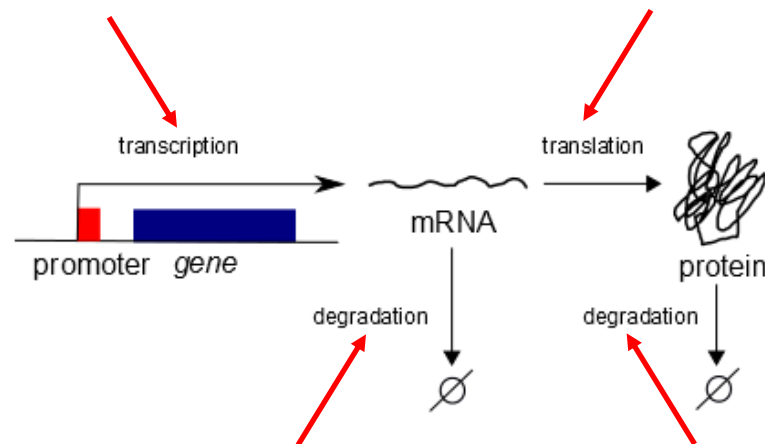
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## Simplified view:



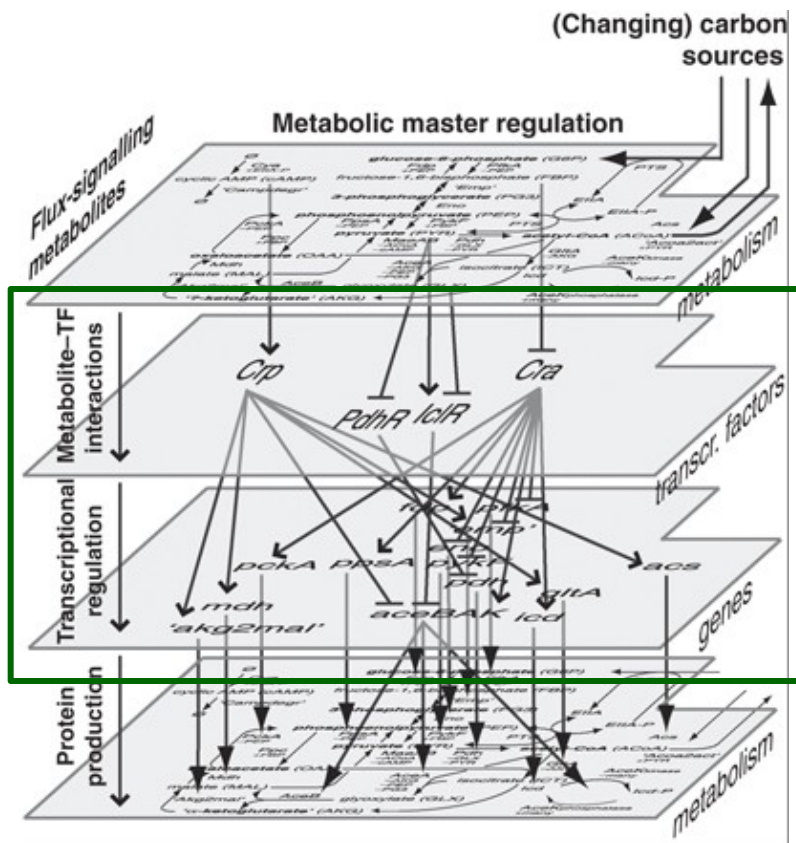
# Regulation of gene expression

- Typically, and simplifying quite a bit, **regulation of gene expression** in bacteria involves:
  - Transcription regulation by transcription factors
  - Translation 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



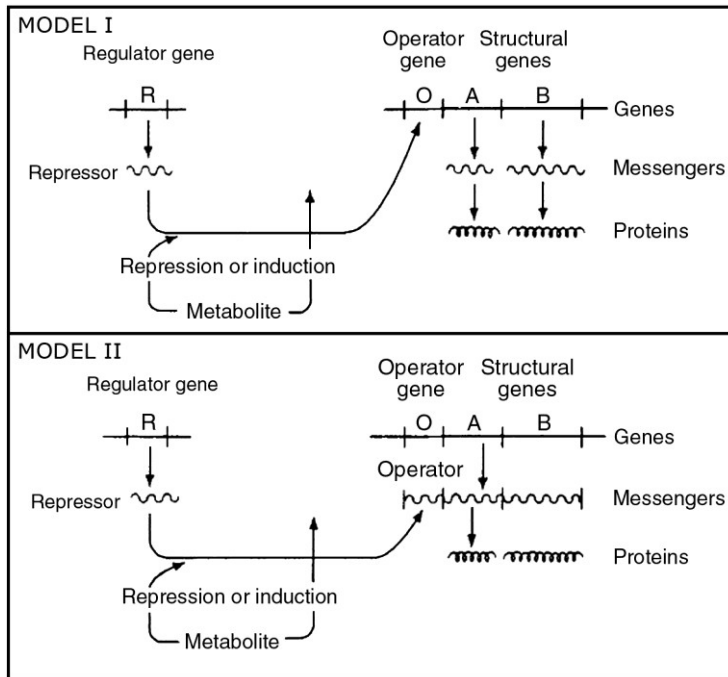
- Gene regulatory networks consist of genes, gene products, signalling metabolites, and their mutual regulatory interactions

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

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

# Gene regulatory networks

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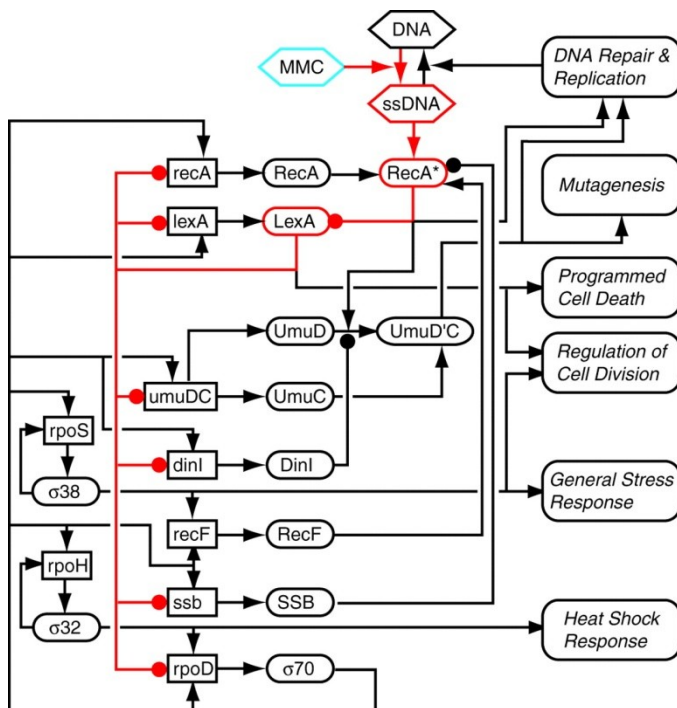
Original *lac* operon model

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*

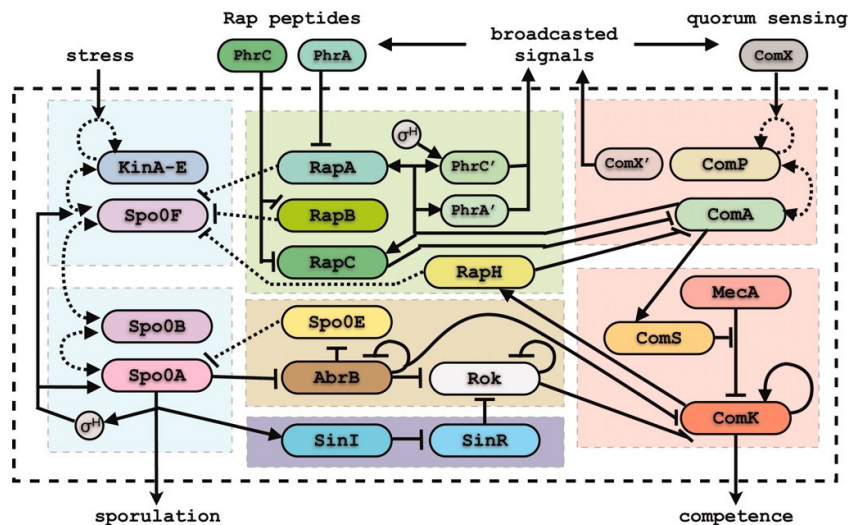
Gardner *et al.* (2011), *Science*, 301(5629):102-5

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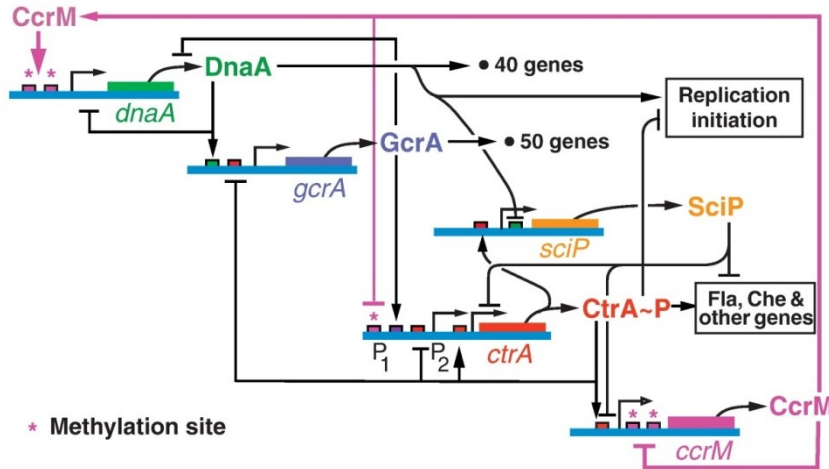
Sporulation and competence network in *B. subtilis*



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

# Gene regulatory networks

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



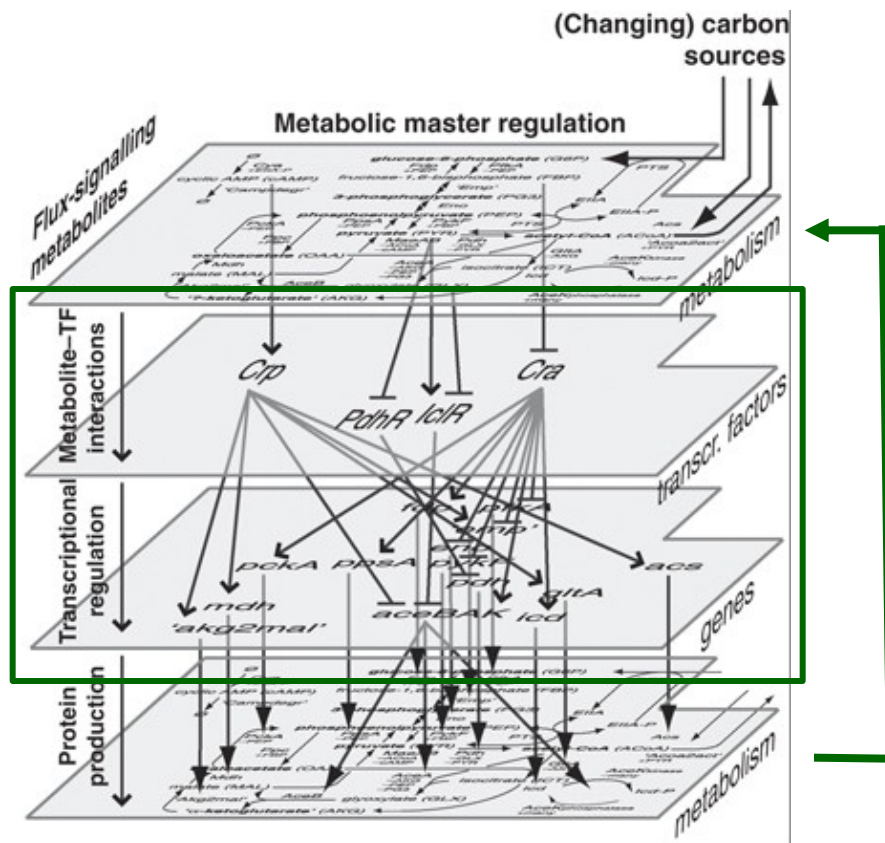
- Gene regulatory networks consist of genes, gene products, signalling metabolites, and their mutual regulatory interactions

*Cauleobacter* cell cycle network

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

# Broader view on gene regulatory networks

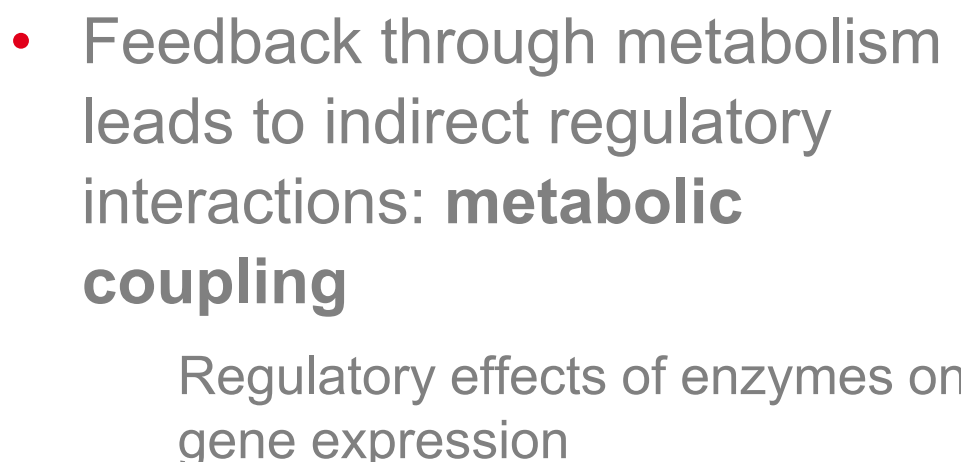
- Gene regulatory networks control changes in expression levels in response to environmental perturbations



- 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

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



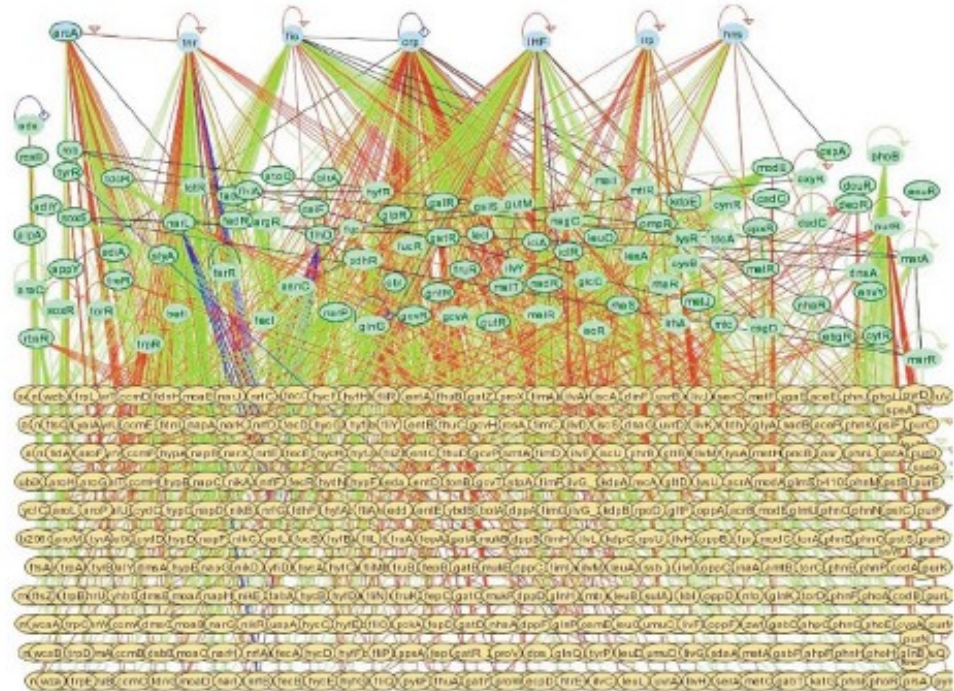
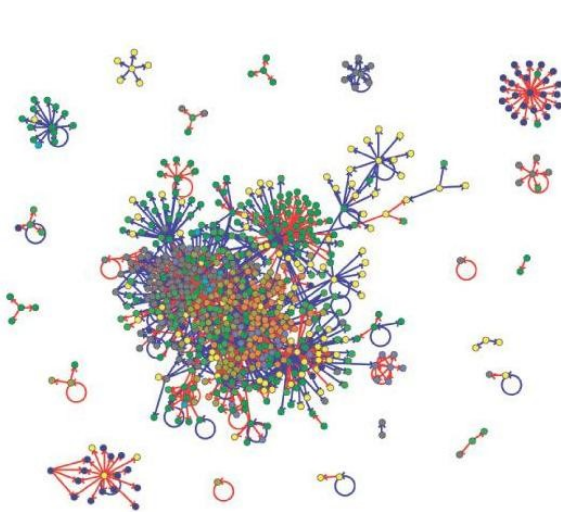
Baldazzi *et al.* (2010), *PLoS Comput. Biol.*, 6(6):e1000812



# 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

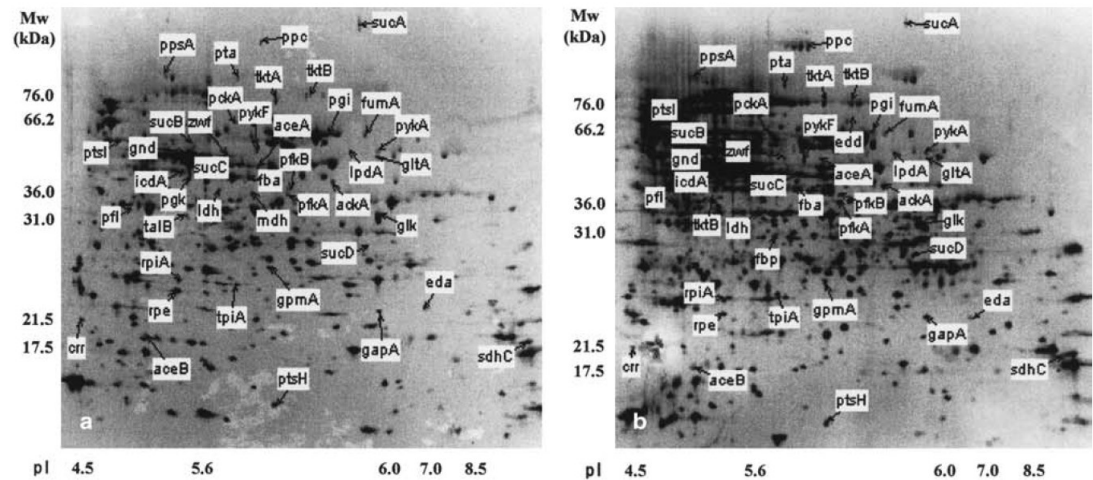
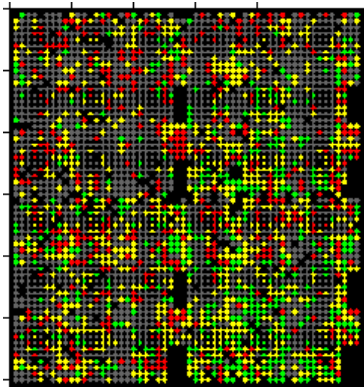
# Experimental tools

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

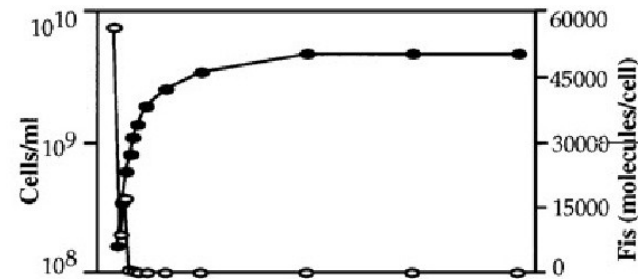
Peng and Shimizu (2003),  
*App. Microbiol. Biotechnol.*, 61:163-78

## 2D gels

## DNA microarrays



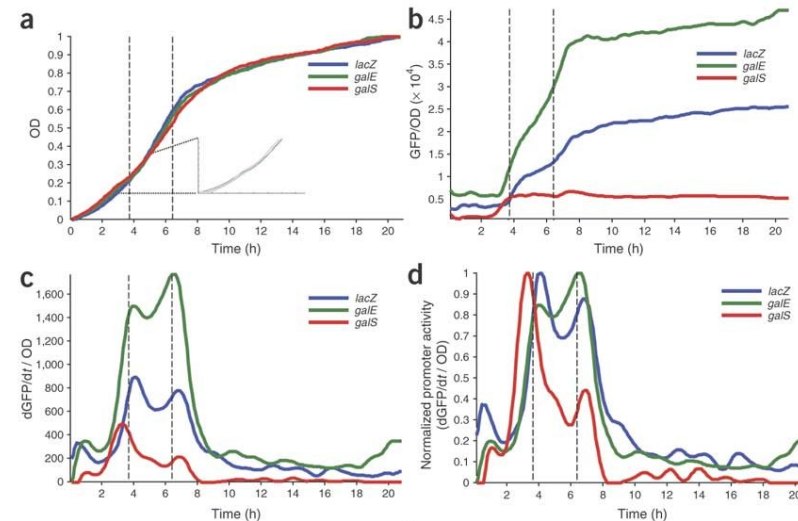
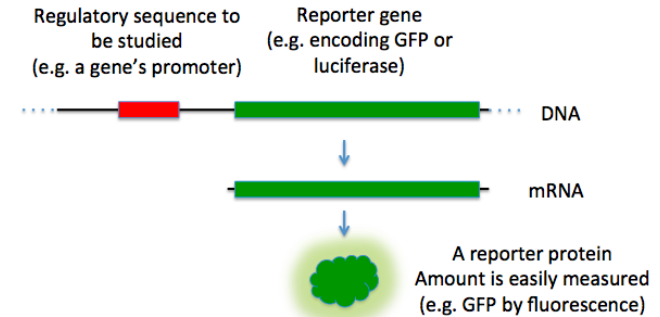
## Western blots



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*

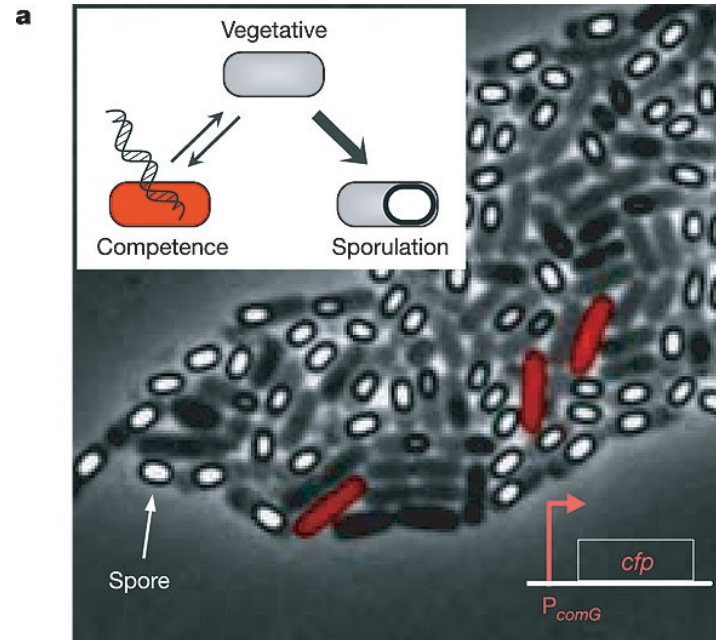
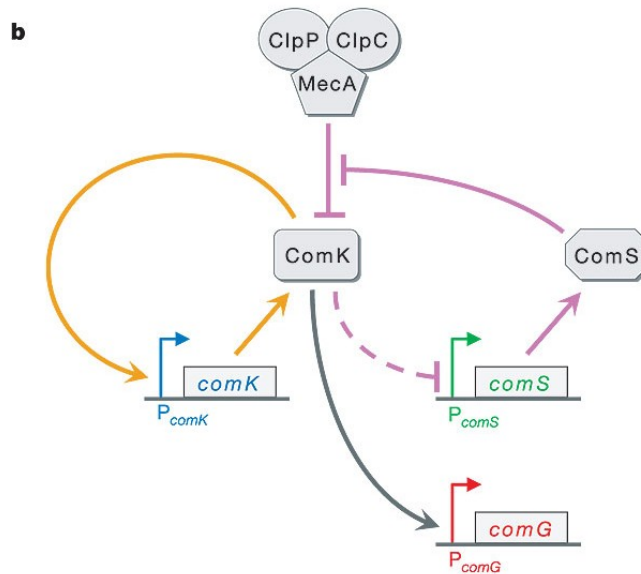


Zaslaver *et al.* (2006), *Nat. Methods*, 3(8):623-8

# 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*

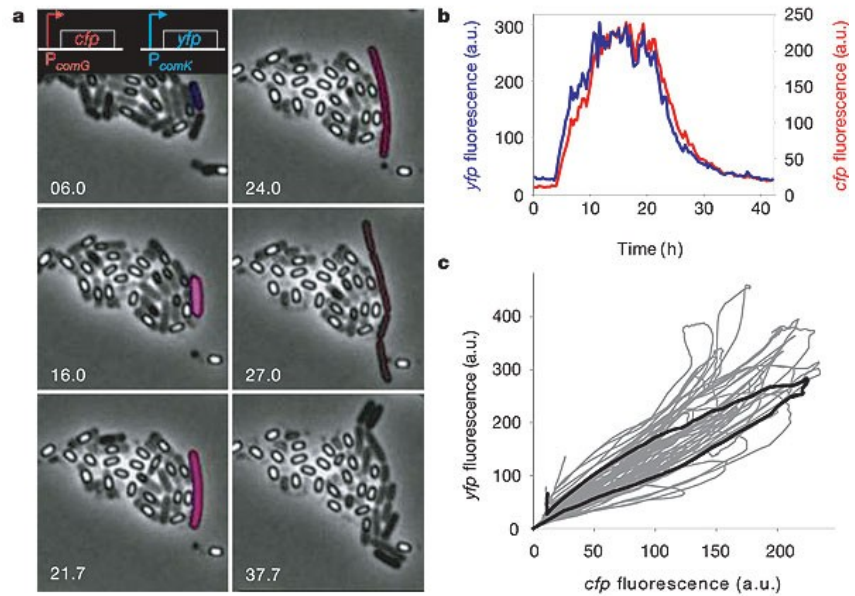
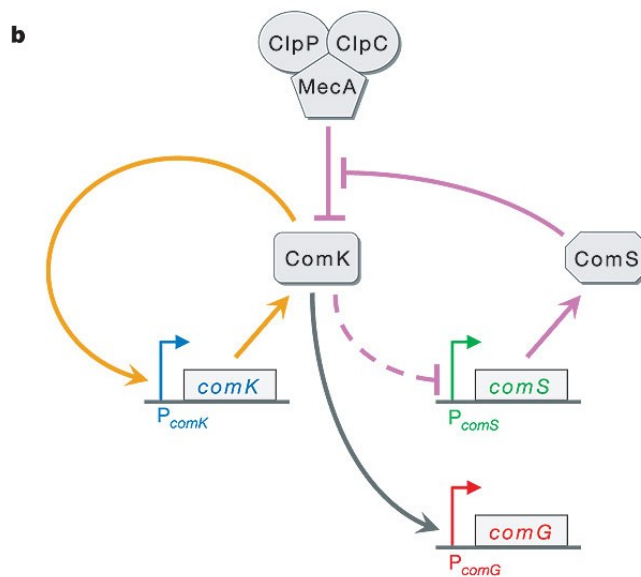
Süel *et al.* (2006), *Nature*, 440:545-50



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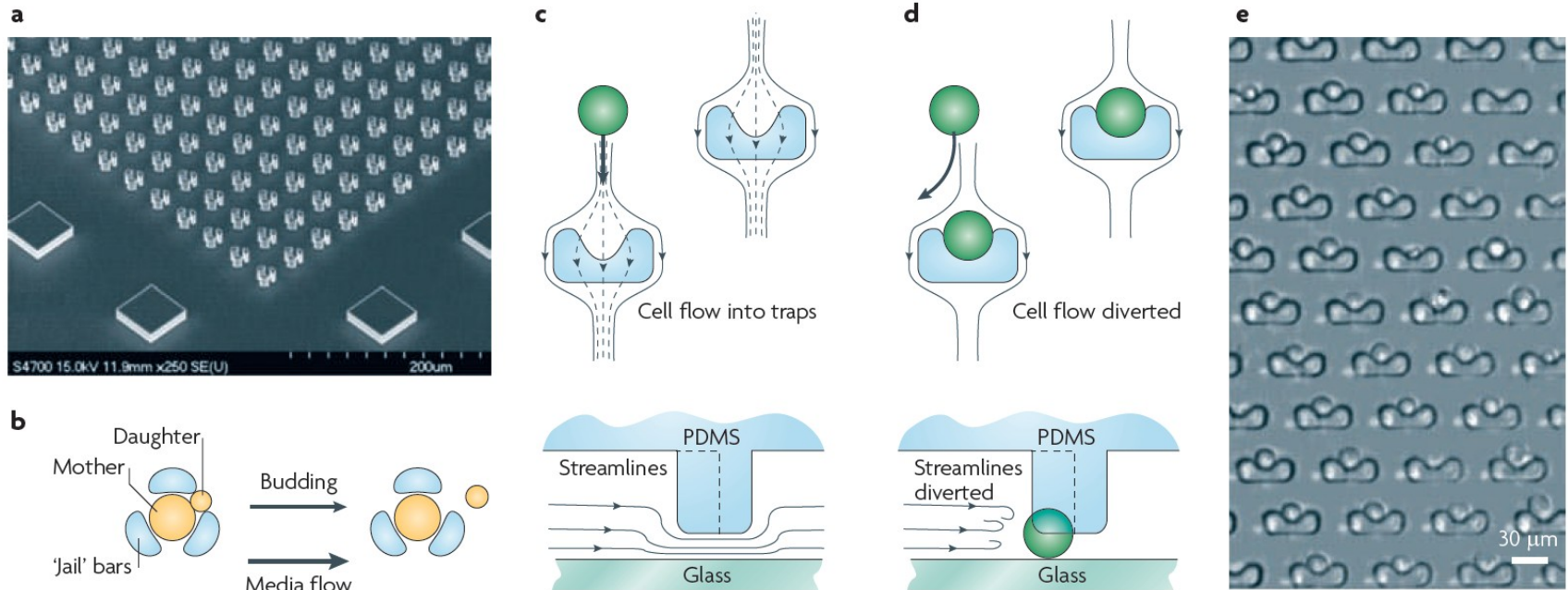
Süel *et al.* (2006), *Nature*, 440:545-50





# Single-cell microscopy and microfluidics

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

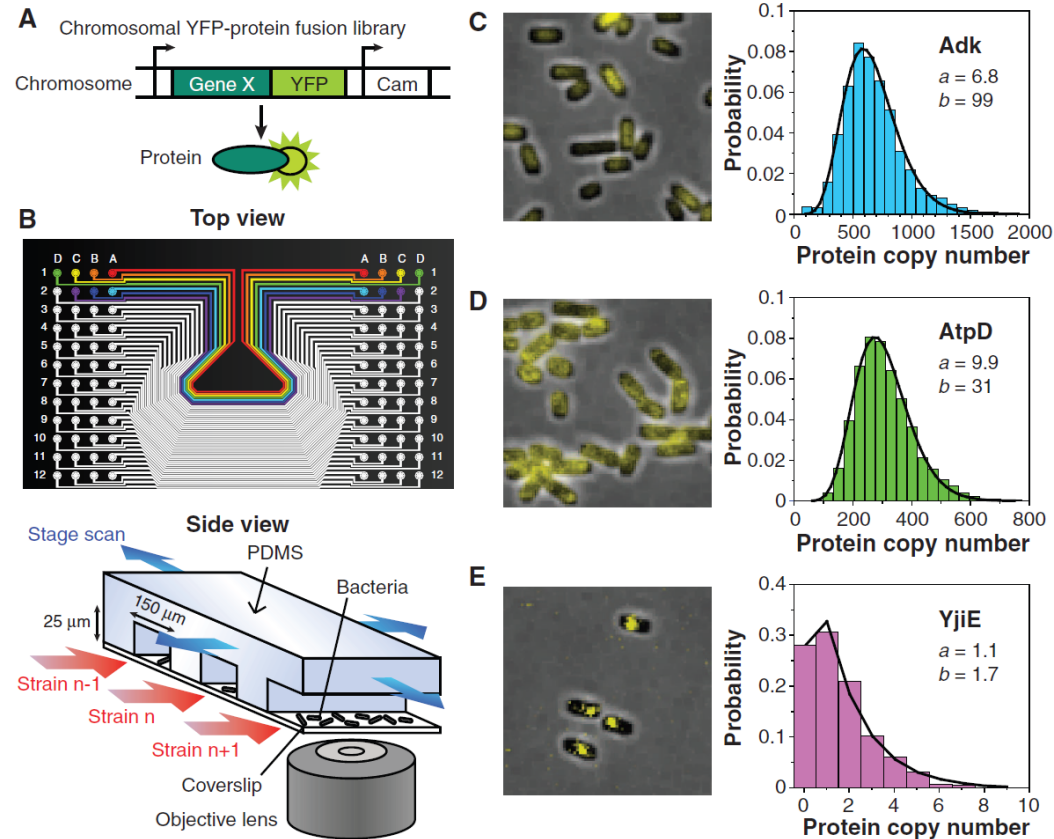


- Microfluidic devices allow tight control of environmental perturbations

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

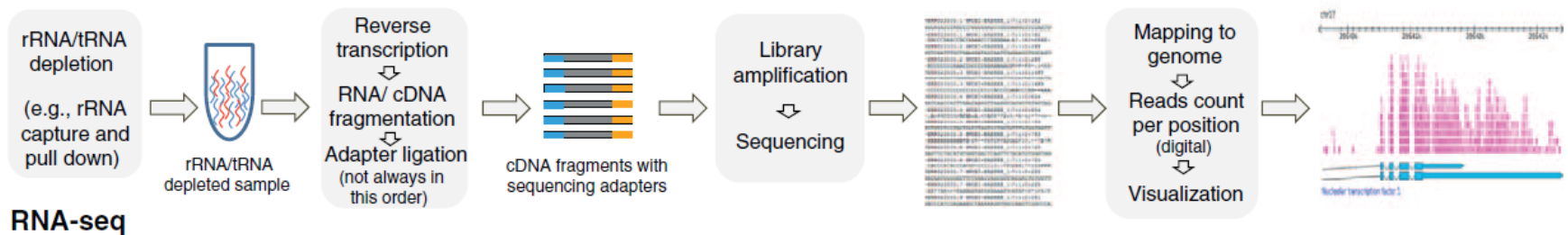


Taniguchi *et al.* (2010), *Science*, 329(5991):533-9



# RNA sequencing

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

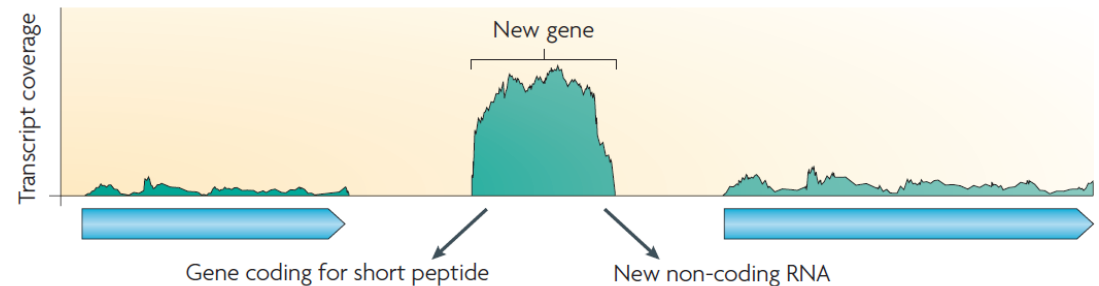


Mäder et al. (2010), *Curr. Opin. Biotechnol.*, 22(1):32-41

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

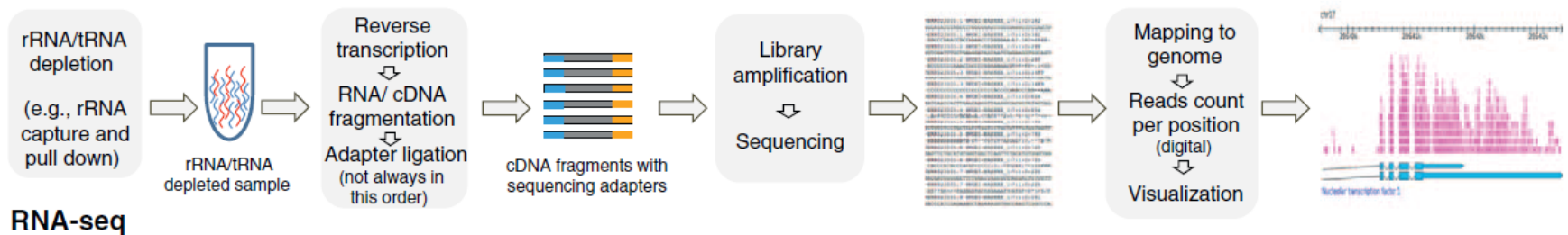
Sorek and Cossart (2010), *Nat. Rev. Genet.*, 11(1):9-16

a Discovery of new genes



# RNA sequencing

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

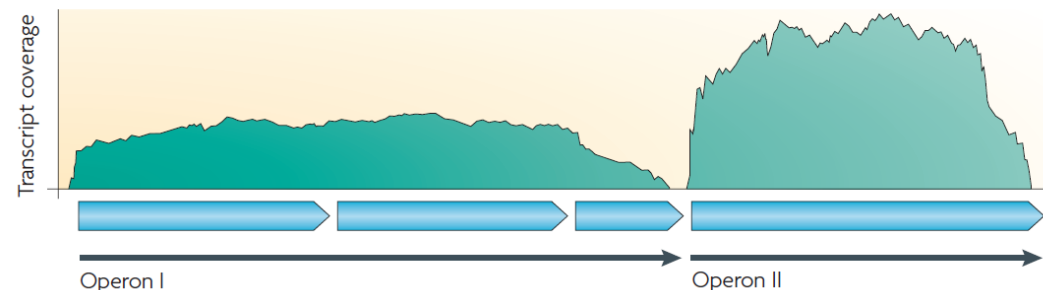


Mäder et al. (2010), *Curr. Opin. Biotechnol.*, 22(1):32-41

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

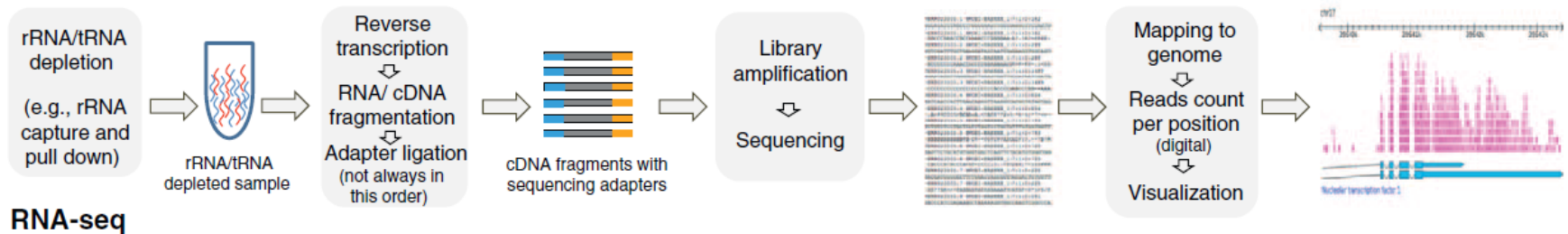
Sorek and Cossart (2010), *Nat. Rev. Genet.*, 11(1):9-16

d Operon structures



# RNA sequencing

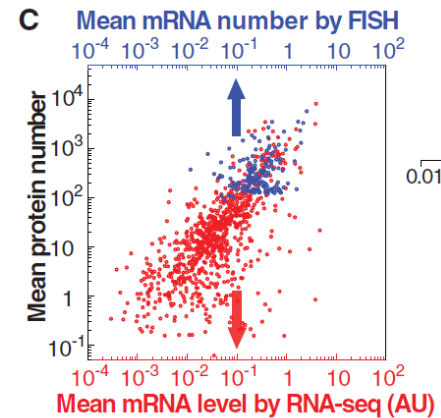
- RNA sequencing (RNA-seq) exploits new generation of sequencing technologies for quantifying RNA levels



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

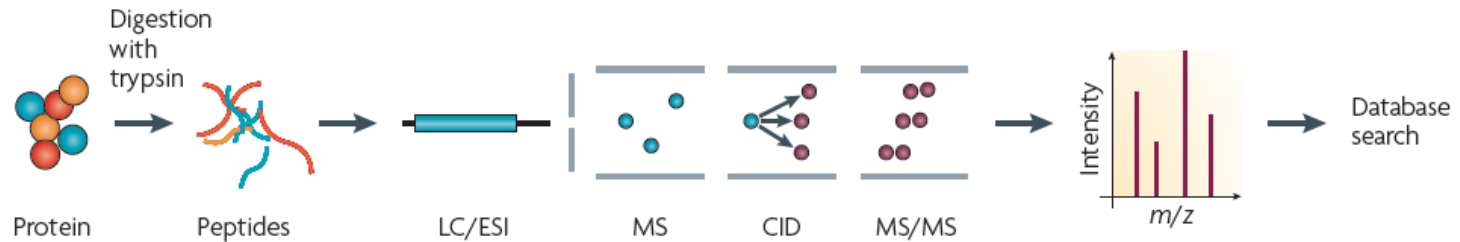
Taniguchi *et al.* (2010), *Science*, 329(5991):533-9



# Quantitative proteomics

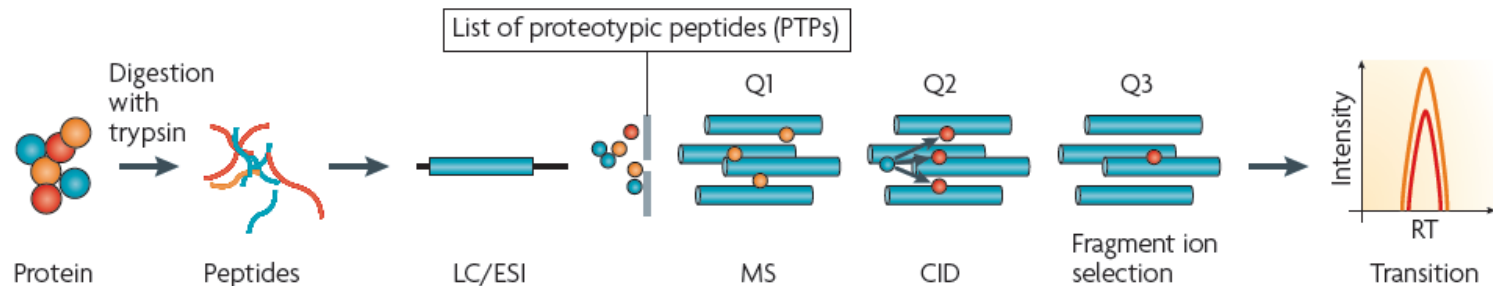
- Measurement of protein abundance using mass-spectrometry-based techniques (**quantitative proteomics**)

Use of calibration standards to achieve absolute quantification



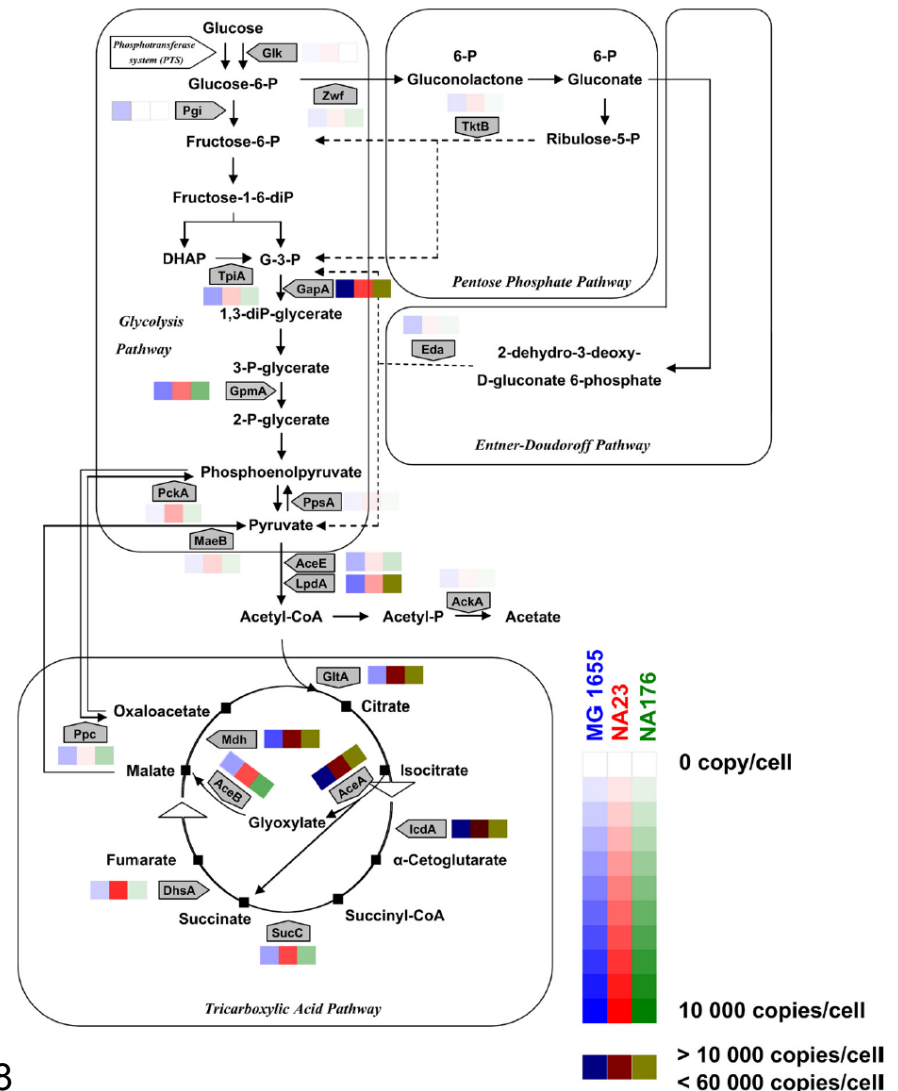
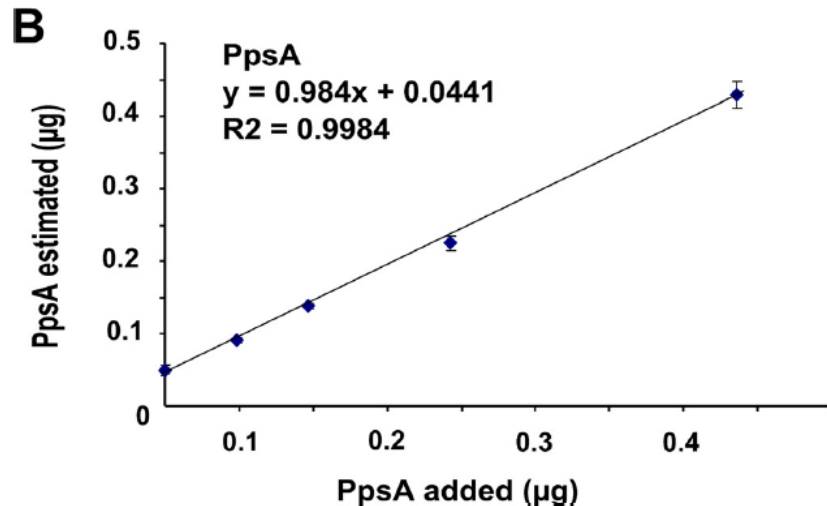
Gstaiger and Aebersold (2009), *Nat. Rev. Genet.*, 10:617-27

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



# Quantitative proteomics

- Absolute quantification of proteins in central metabolism in *E. coli*



Trachessec et al. (2014), *Mol. Cell. Proteom.*, 13(4):954-68

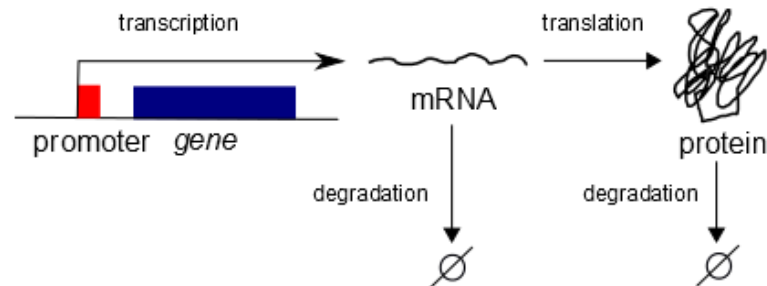
# Systems biology

- 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

# Modeling of gene regulatory networks

- Modeling of gene regulatory networks amount to modeling of gene expression and regulation of gene expression

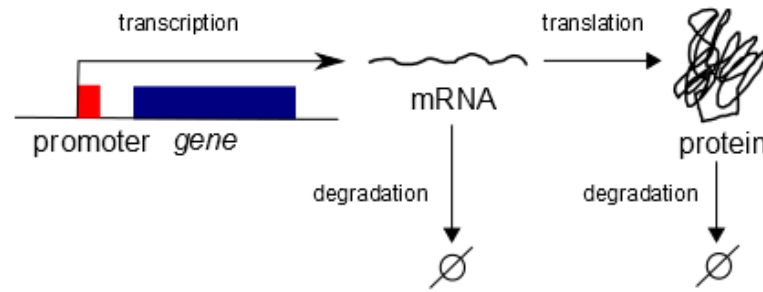


- Possible **aims** of modeling of gene regulatory networks:
  - Understanding role of individual components and interactions
  - Suggesting missing components and interactions
- Advantages of **mathematical and computer tools**:
  - Precise and unambiguous description of network
  - Systematic derivation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



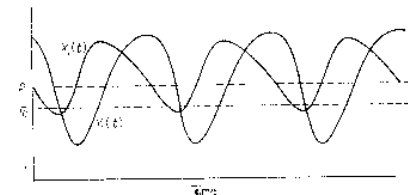
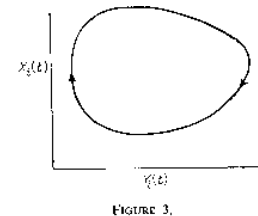
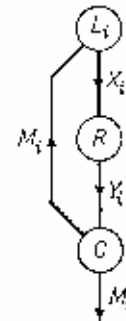
# Modeling of gene regulatory networks

- 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

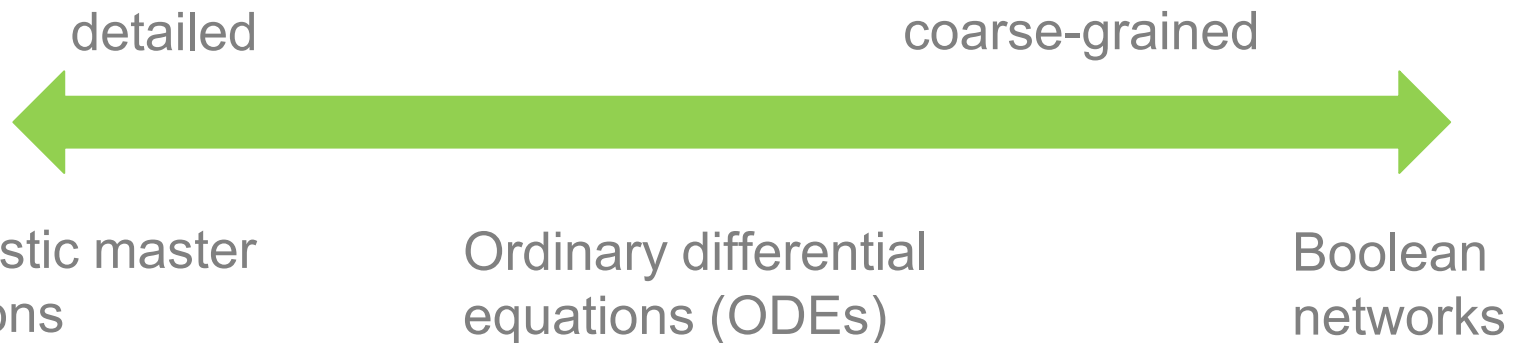
Feedback circuits and oscillators



Goodwin (1963), *Temporal Organization in Cells*

# Modeling of gene regulatory networks

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



Smolen *et al.* (2000), *Bull. Math. Biol.*, 62(2):247-292

Hasty *et al.* (2001), *Nat. Rev. Genet.*, 2(4):268-279

de Jong (2002), *J. Comput. Biol.*, 9(1): 69-105

Szallassi *et al.* (2006), *System Modeling in Cellular Biology*, MIT Press

Bolouri (2008), *Computational Modeling of Gene Regulatory Networks*, Imperial College Press

Karleback and Shamir (2008), *Nat. Rev. Mol. Cell Biol.*, 9(10):770-80

# Conclusions

- Gene regulatory networks control adaptive response of bacteria to changes in environment
- Gene regulatory networks are intertwined with metabolic and signaling networks
- Technology for measuring gene expression over time, and thus functioning of gene regulatory networks, are rapidly developing
- Modeling necessary for understanding dynamics of complex networks: systems biology
- A variety of formalisms for modeling gene regulatory networks, in a detailed or coarse-grained way, have been developed

**Merci !**



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