Modeling and simulation of gene regulatory networks 1

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October 3, 2018

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://team.inria.fr/ibis

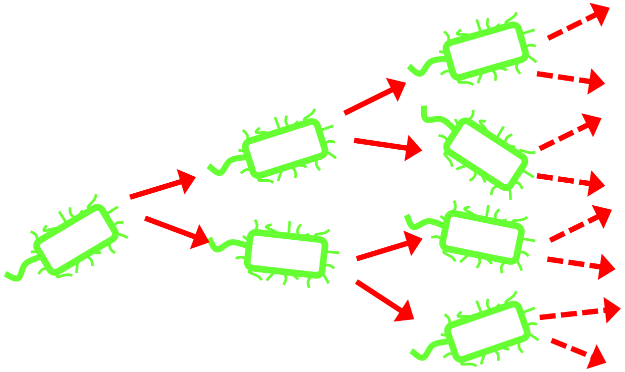
Overview

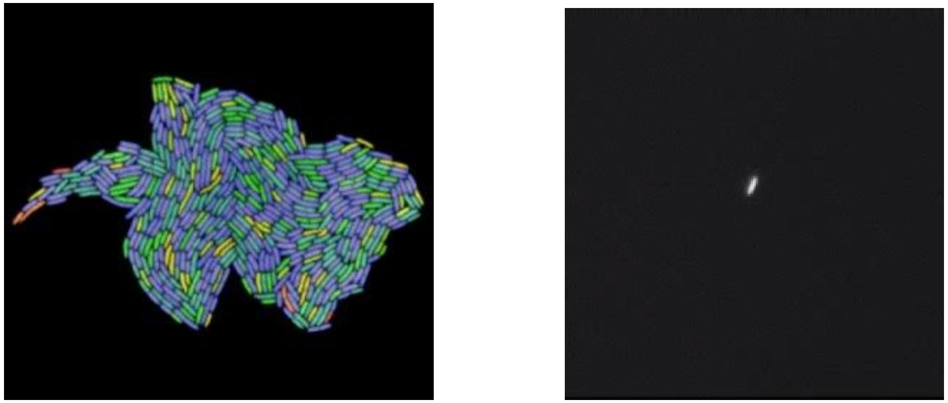
1. **Gene regulatory networks in bacteria**
2. Quantitative modeling of gene regulatory networks
3. Qualitative modeling of gene regulatory networks
4. Identification of gene regulatory networks
5. Towards integrated models of the cell

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

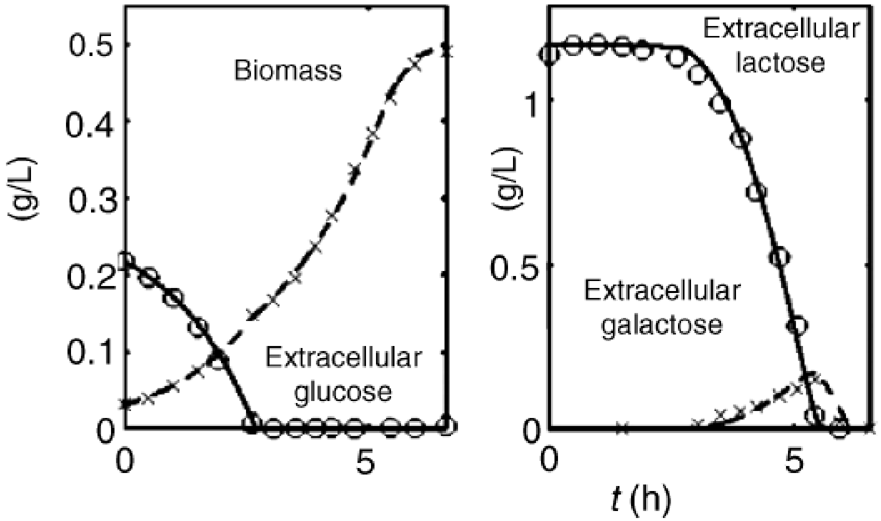
- **Metabolism** fuels growth by production of energy and building blocks for macromolecules, using nutriments from environment

  ATP, amino acids, nucleotides, …

Bacterial growth and metabolism

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

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

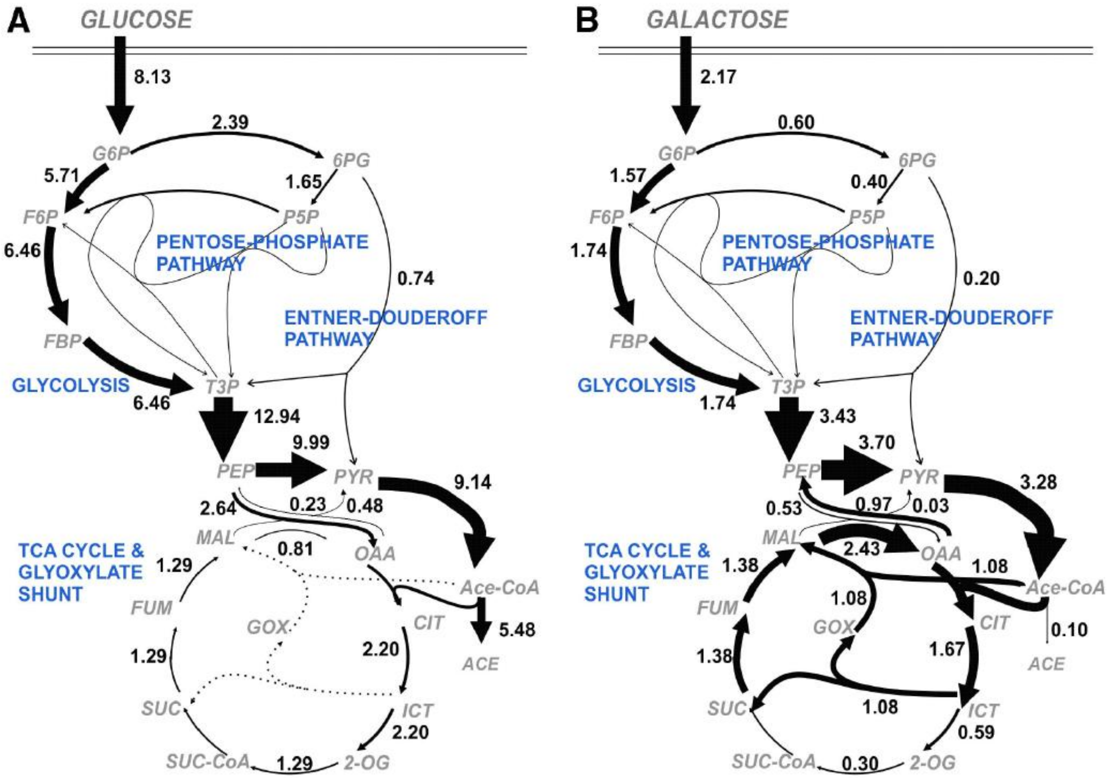


Bettenbrock *et al.* (2006), *J. Biol. Chem.*, 281(5):2578-84

- Adaptation of bacterial physiology to different carbon sources

Growth transition and metabolism

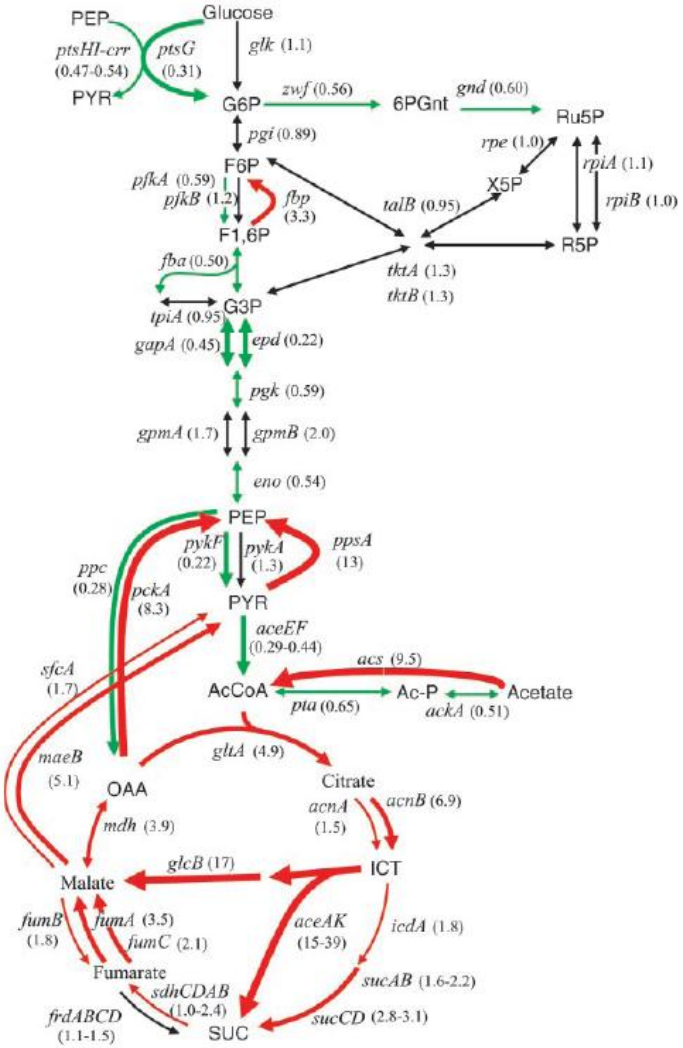
- Adaptation to different carbon source involves changes in **metabolic fluxes**
  - Different flux distribution in central metabolism of *E. coli* during growth on glucose and galactose



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

Growth transition and gene expression

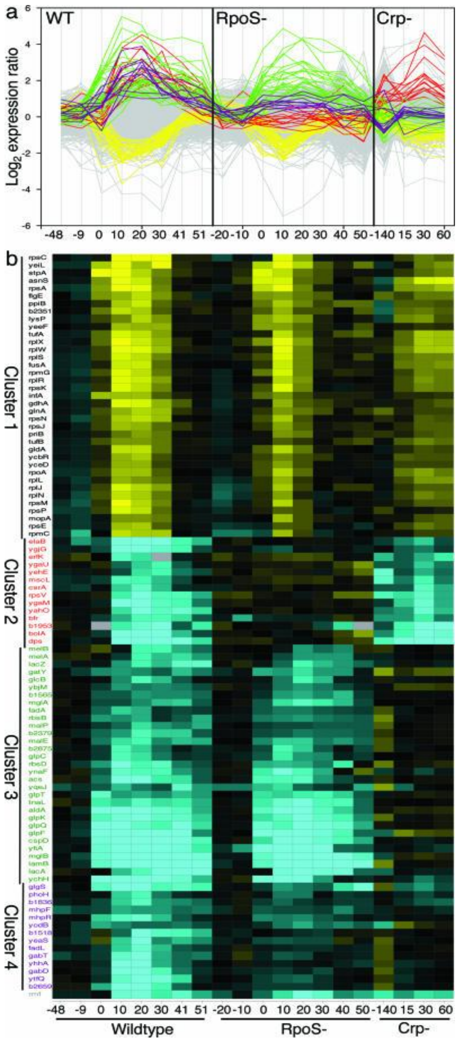
- Adaptation to different carbon source involves adjustment of **expression of enzymatic genes**
  - Difference in expression levels of genes encoding enzymes in central metabolism of *E. coli* during growth on glucose and acetate



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

Growth transition and gene expression

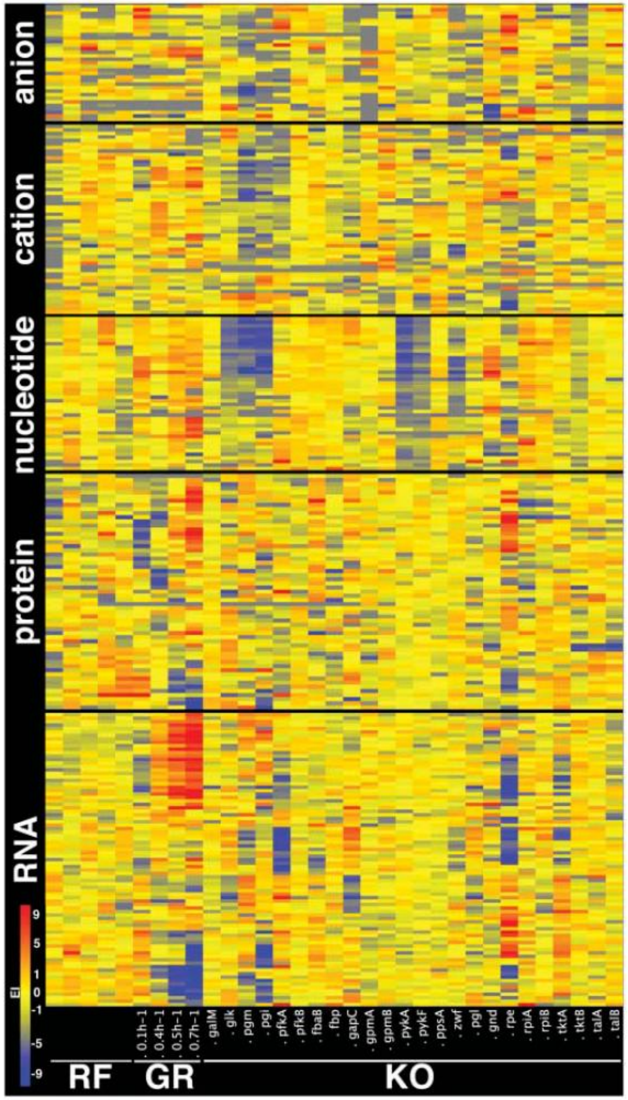
- Adaptation to different carbon source involves **genome-wide reorganisation of gene expression**
  - Gene expression during glucose-lactose shift in *E. coli*



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

Adaptation on multiple levels

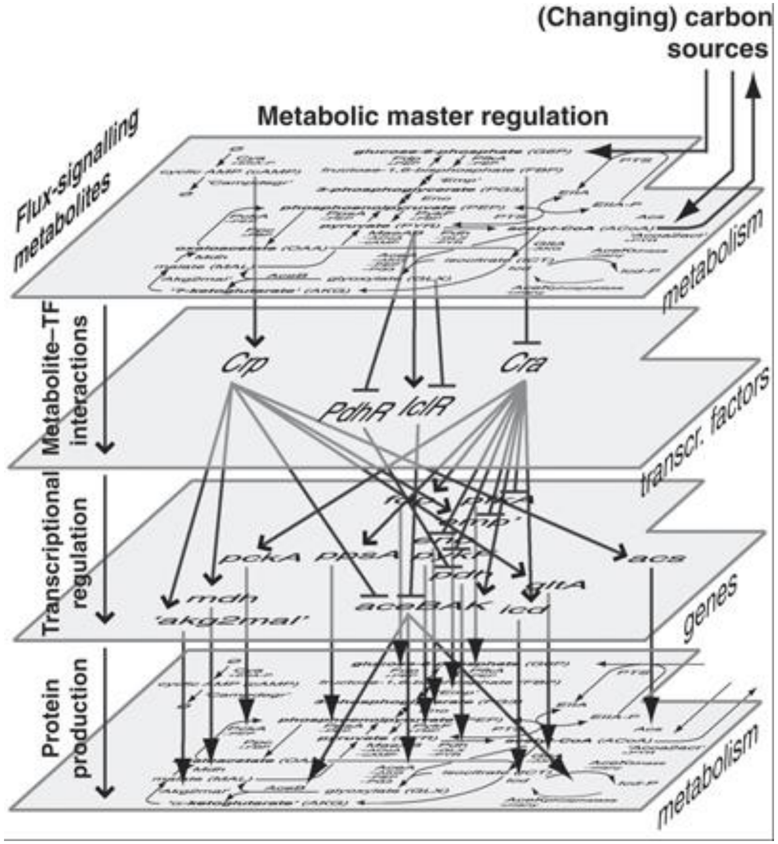
- Adaptation to different carbon source involves **adjustments on multiple levels** at the same time!
  - Parallel measurement of enzyme and metabolite concentrations, and metabolic fluxes in a variety of experimental conditions



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

Molecular bases of adaptative responses

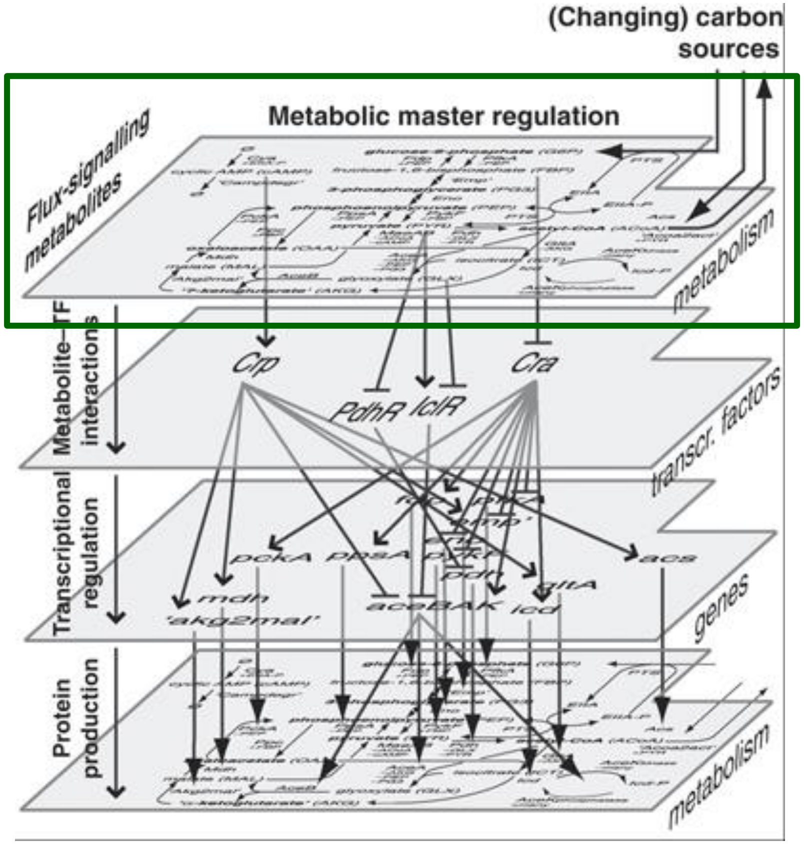
- Adaptative responses of bacterial cell brought about by **large and complex networks** of biochemical reactions
  - Variety of reaction mechanisms…
  - … operating on different time-scales…
  - … involving numerous feedback loops across levels



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

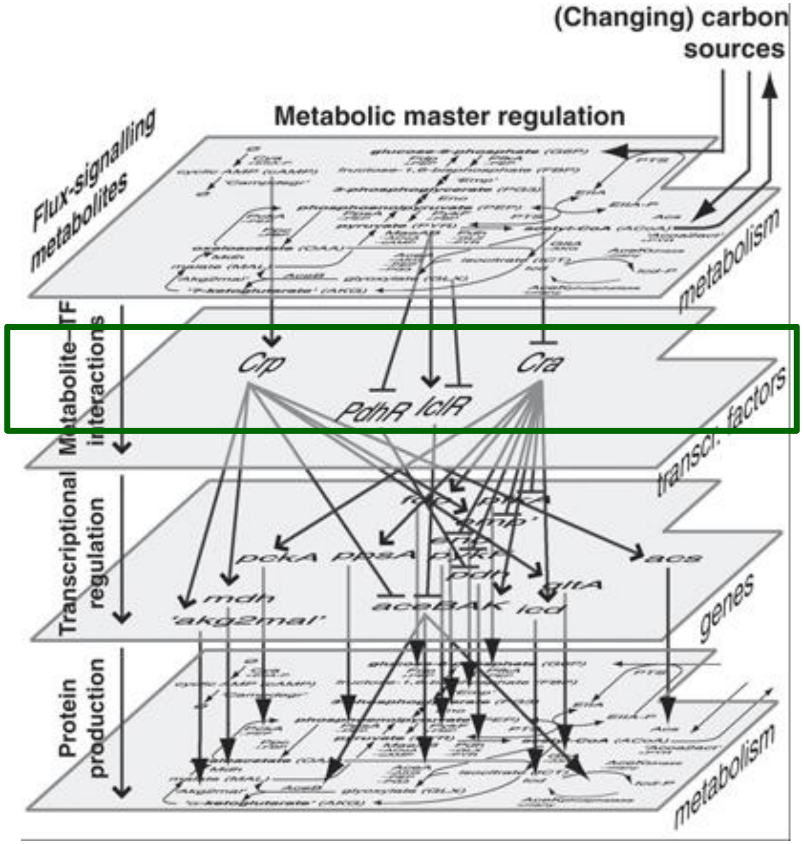
Metabolic, signalling, gene networks

- Different types of networks distinguished by function, types of biochemical reactions and time-scale of dynamics
  - **Metabolic networks**: conversion of substrate into precursors and energy
  - Enzymatic reactions and regulation of enzyme activity
  - Time-scale: ms to s



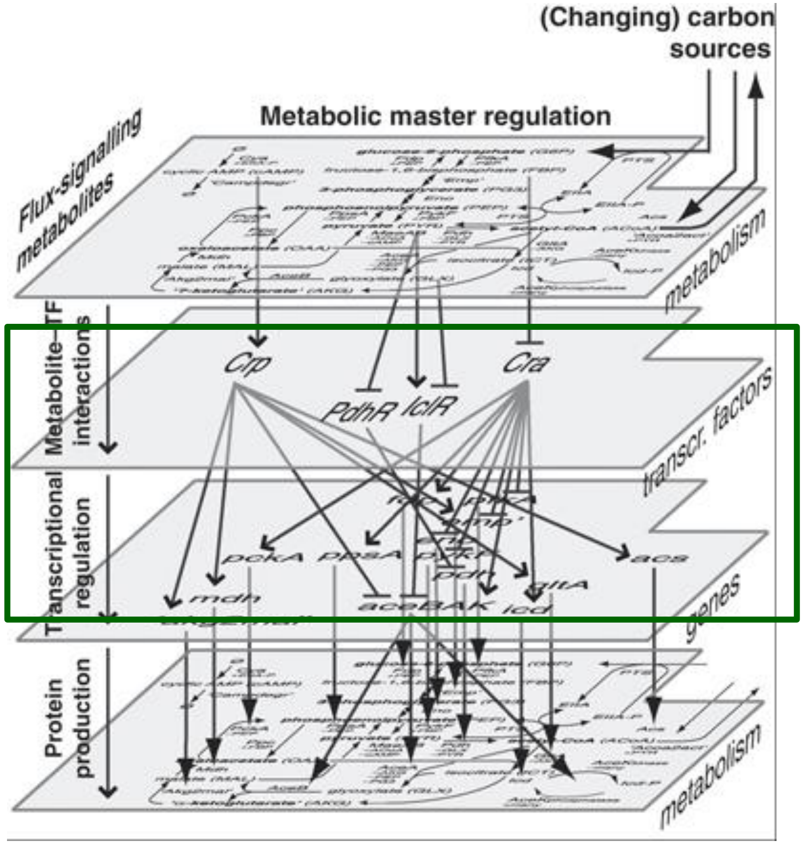
Metabolic, signalling, gene networks

- Different types of networks distinguished by function, types of biochemical reactions and time-scale of dynamics
  - **Signalling networks**: transmission of information on state of environment and cellular physiology
  - Reactions involved in protein (enzyme) modification and their regulation
  - Time-scale: µs



Metabolic, signalling, gene networks

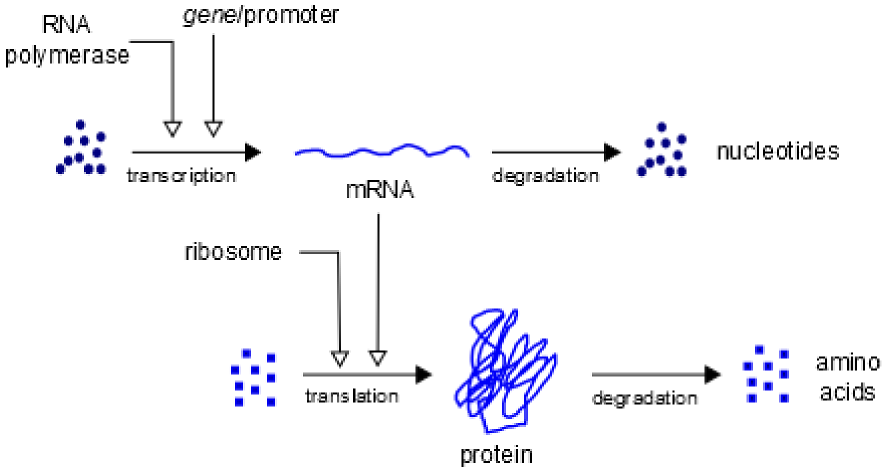
- Different types of networks distinguished by function, types of biochemical reactions and time-scale of dynamics
  - **Gene regulatory networks**: gene expression in response to changes in environment and cellular physiology
  - Reactions involved in transcription and translation and their regulation
  - Time-scale: min (mRNA) to h (proteins)



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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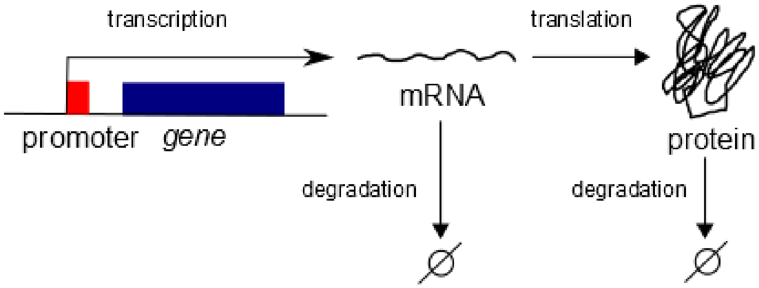
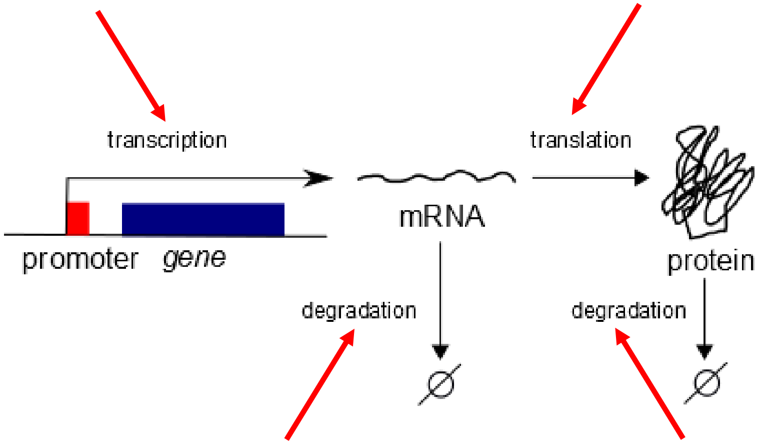
Simplified view:



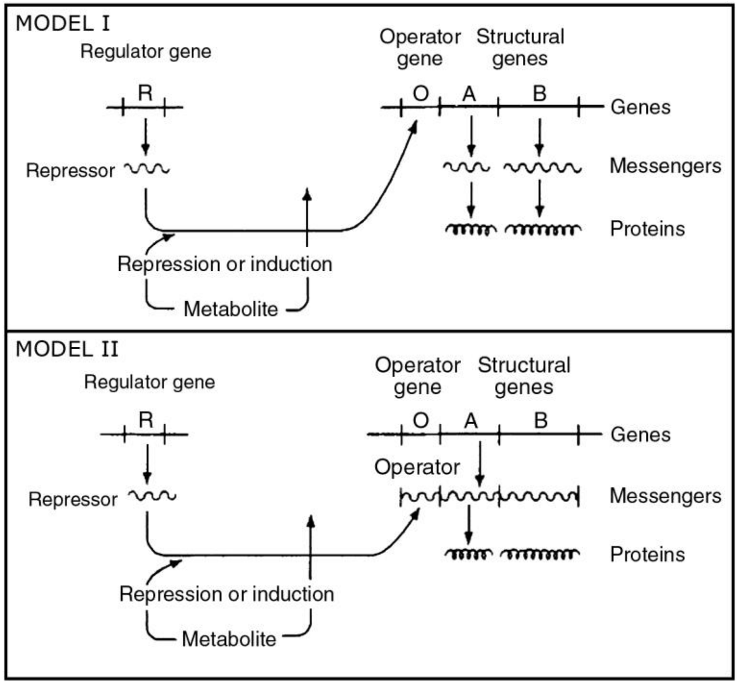
Regulation of gene expression

- 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 expression levels in response to environmental perturbations



Original *lac* operon model

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

Gene regulatory networks

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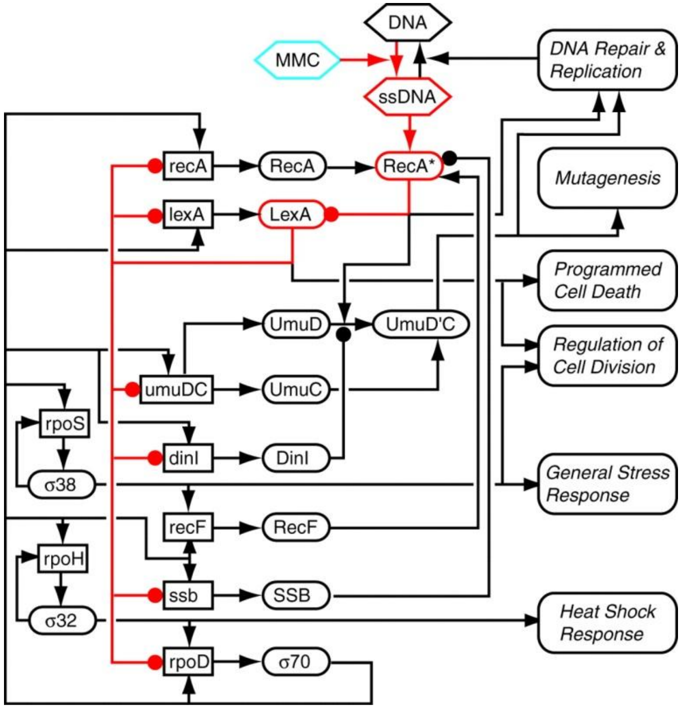

SOS response network in *E. coli*

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

Gene regulatory networks

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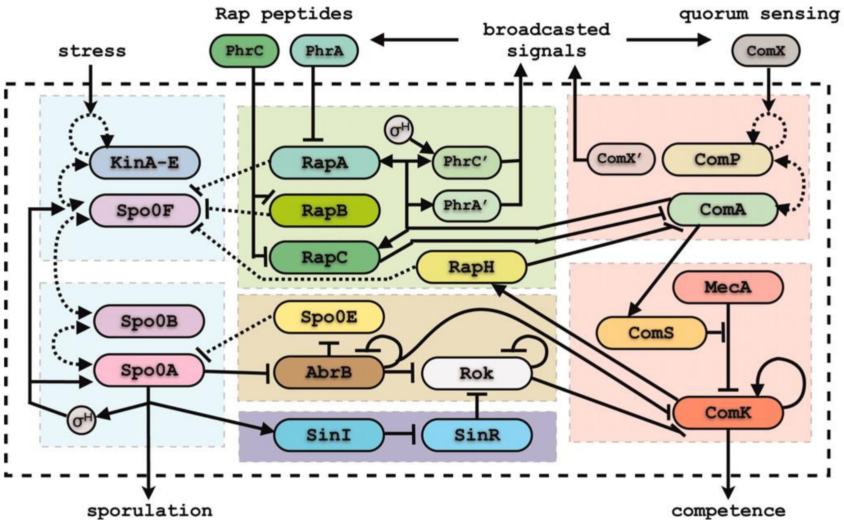
Sporulation and competence network in *B. subtilis*

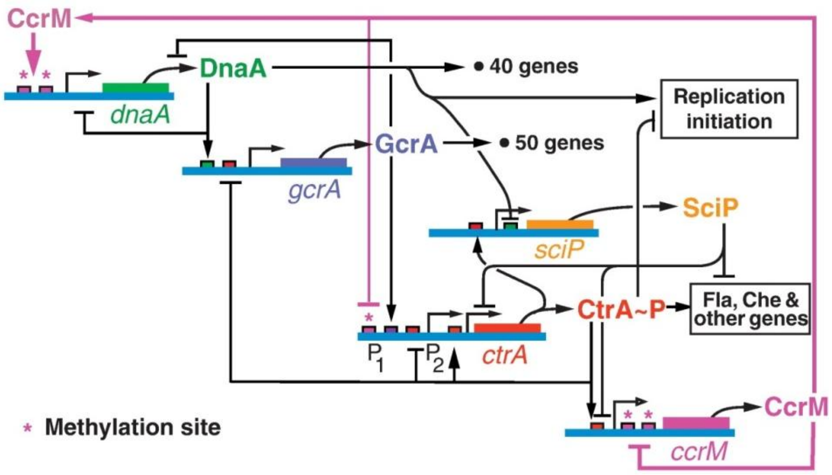


Schultz *et al.* (1961), *Proc. Natl. Acad. Sci. USA*, 106(50):21027-34

Gene regulatory networks

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

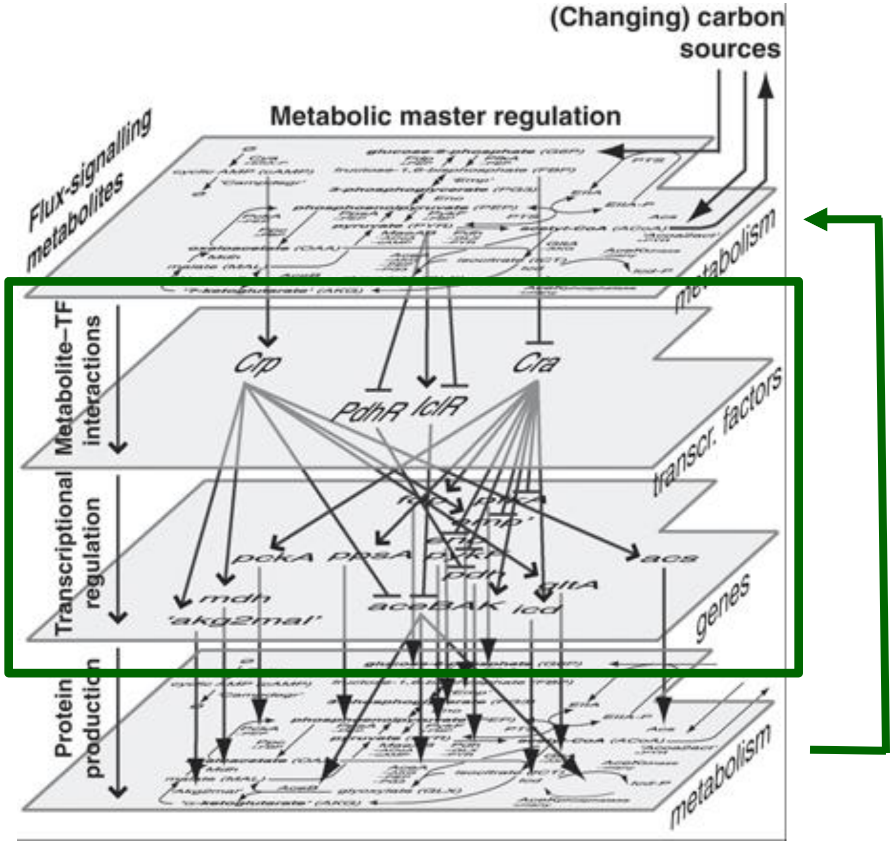
Cauleobacter cell cycle network



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

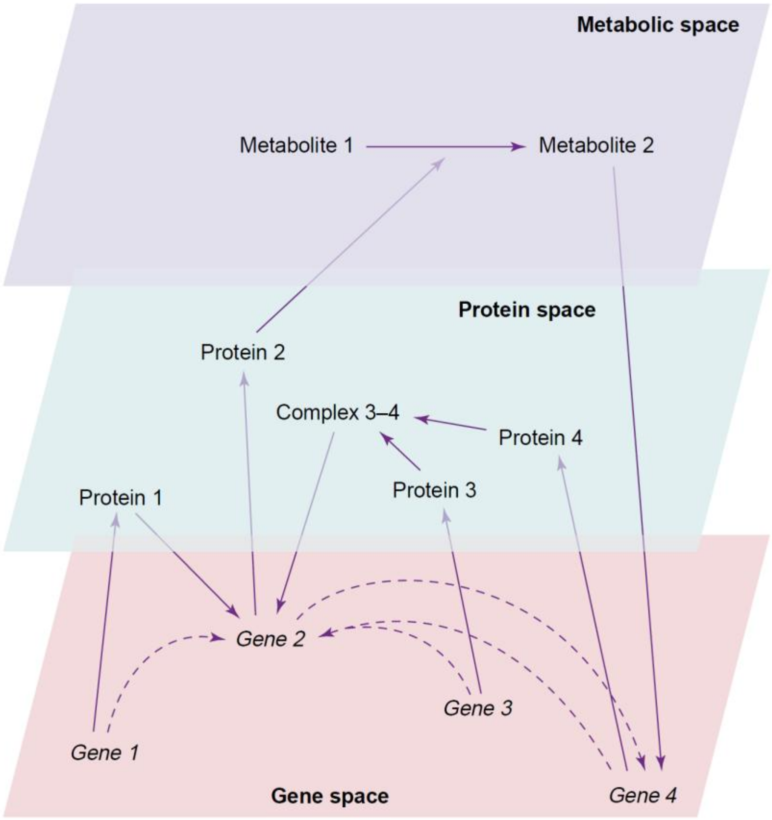


- **But**: adaptation of gene expression leads to changes in metabolism which feed back into regulatory network
- Indirect regulatory interactions: **metabolic coupling**

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

Broader view on gene regulatory networks

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

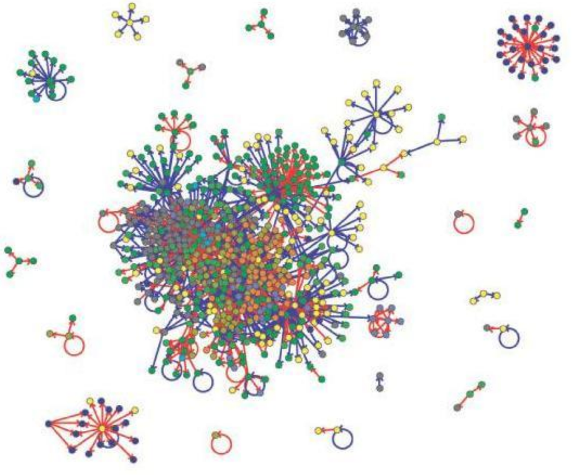


- **But**: adaptation of gene expression leads to changes in metabolism which feed back into regulatory network
- Indirect regulatory interactions: **metabolic coupling**

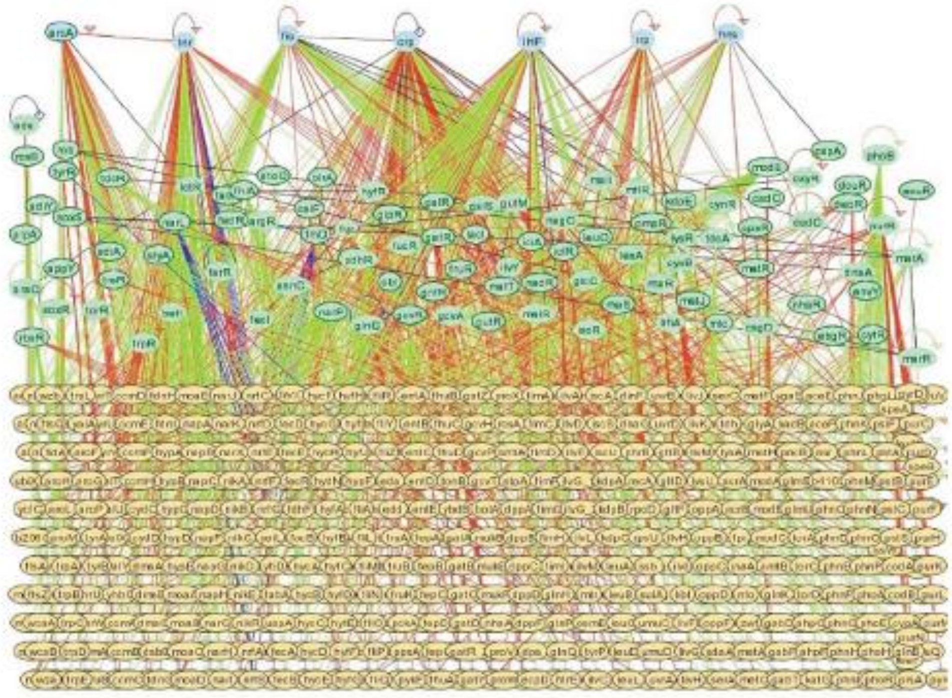
Braznik *et al.* (2002), *Trends Biotechnol.*, 20(11):467-71

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**

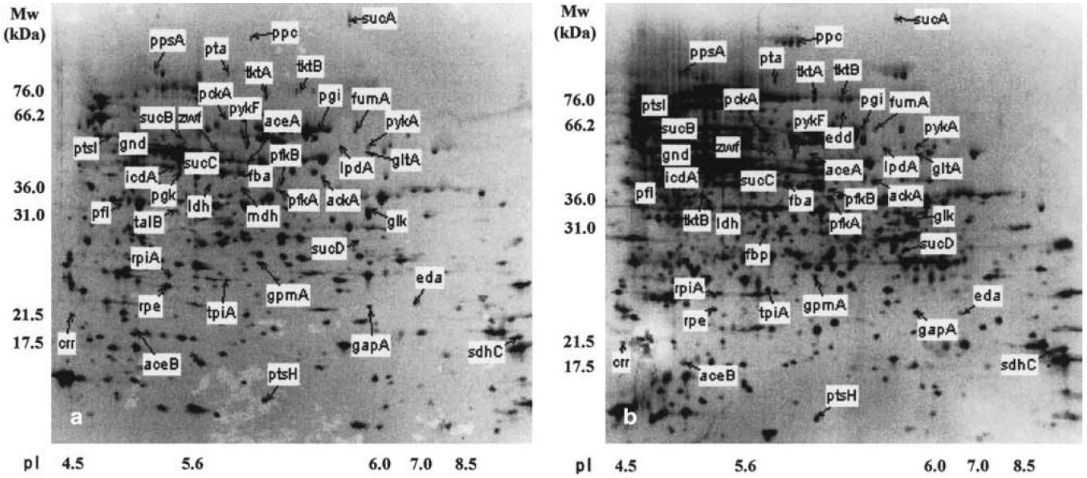
Alon (2007), *An Introduction to Systems Biology*, Chapman & Hall/CRC Press

Experimental tools

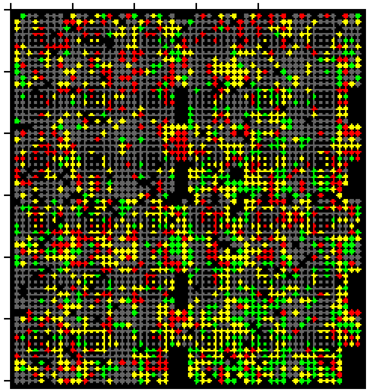
- A variety of experimental tools allow gene expression to be measured, by quantifying mRNA and protein abundancies

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

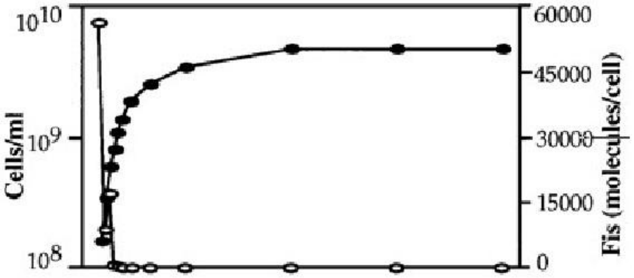
2D gels



DNA
microarrays



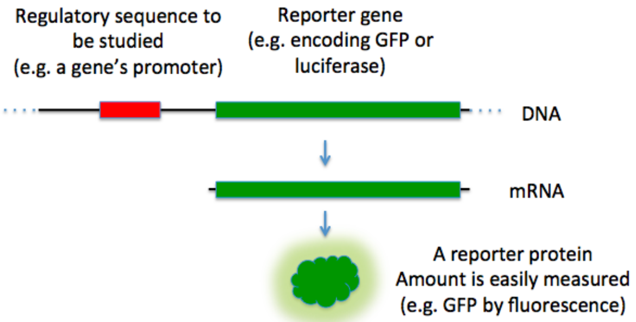
Western blots



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



Regulatory sequence to be studied (e.g. a gene's promoter)

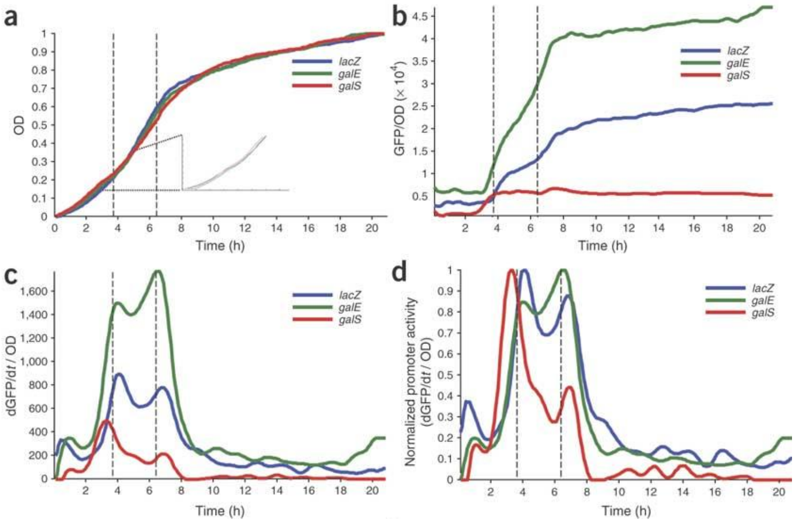
Reporter gene (e.g. encoding GFP or luciferase)

DNA

mRNA

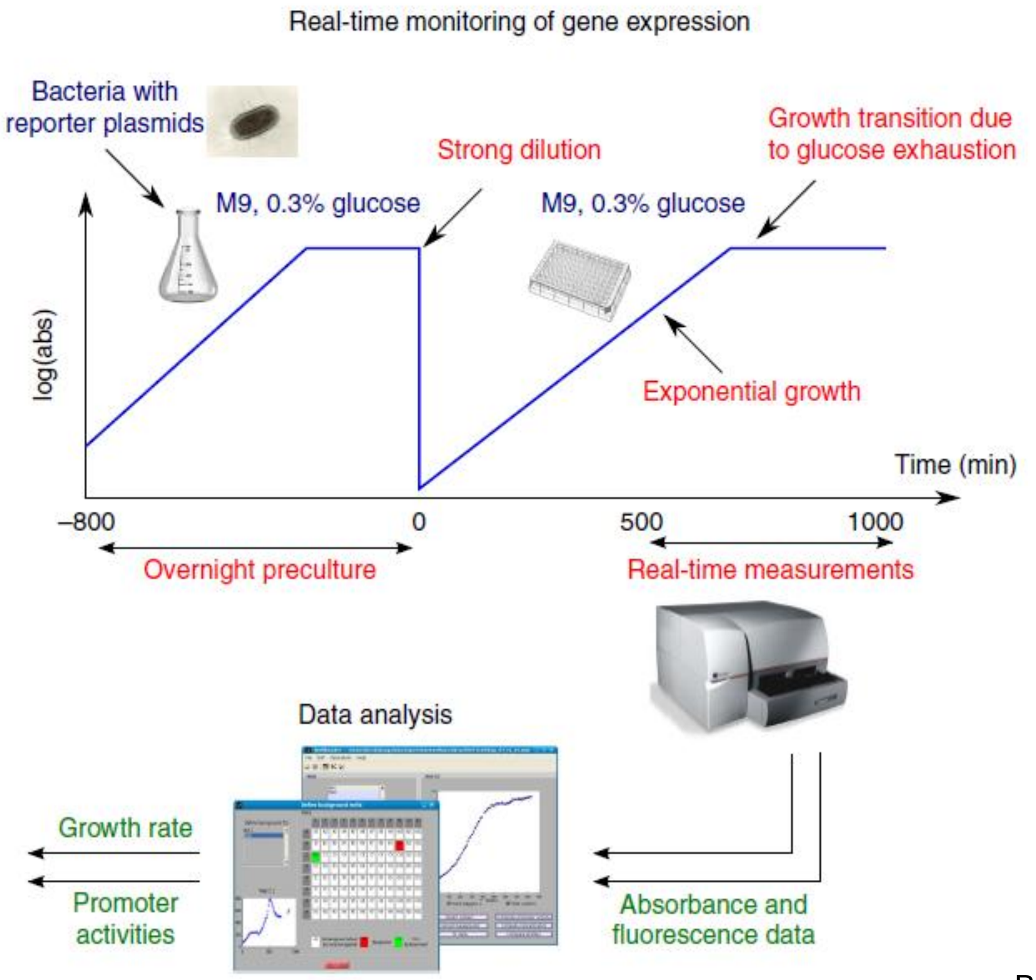
A reporter protein
Amount is easily measured
(e.g. GFP by fluorescence)

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



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

Microplate readers



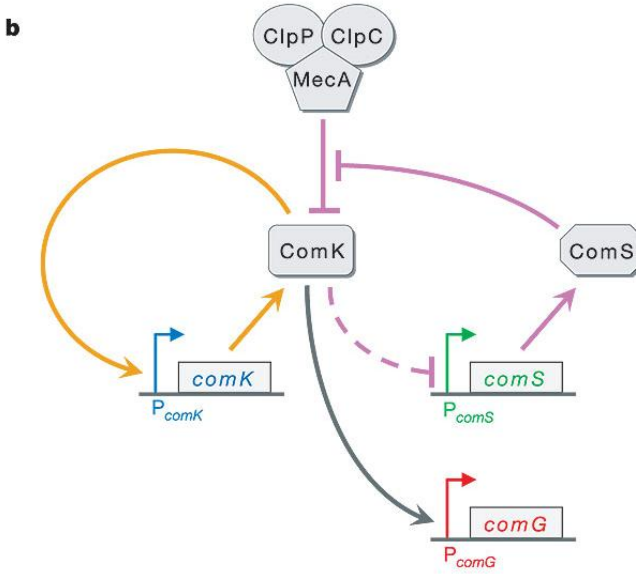
- Monitoring of gene expression on population level using fluorescent reporters and **automated microplate readers**

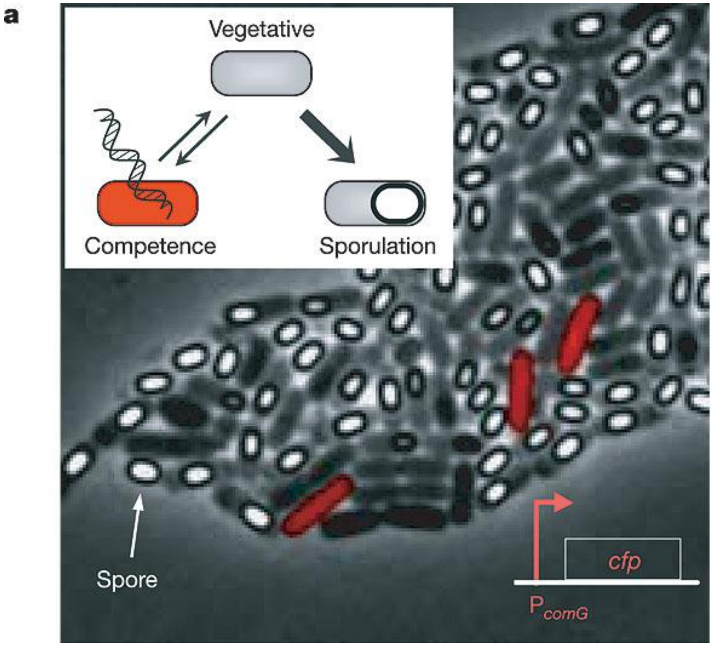
Berthoumieux *et al.* (2013), *Mol. Syst. Biol.*, 9:634

Single-cell microscopy

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

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





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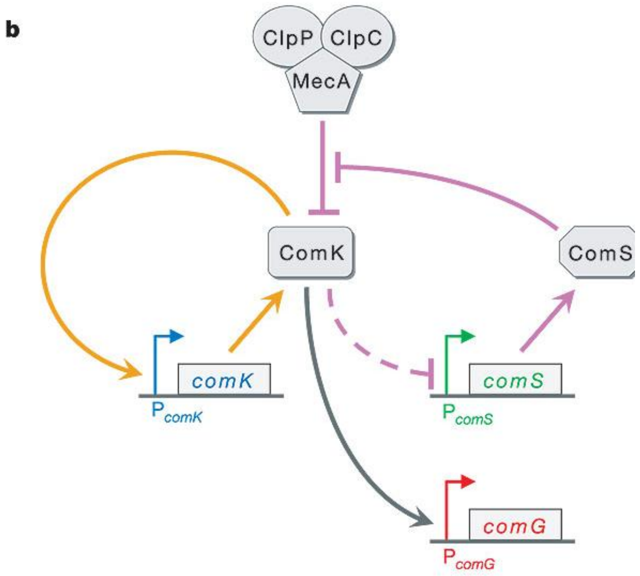
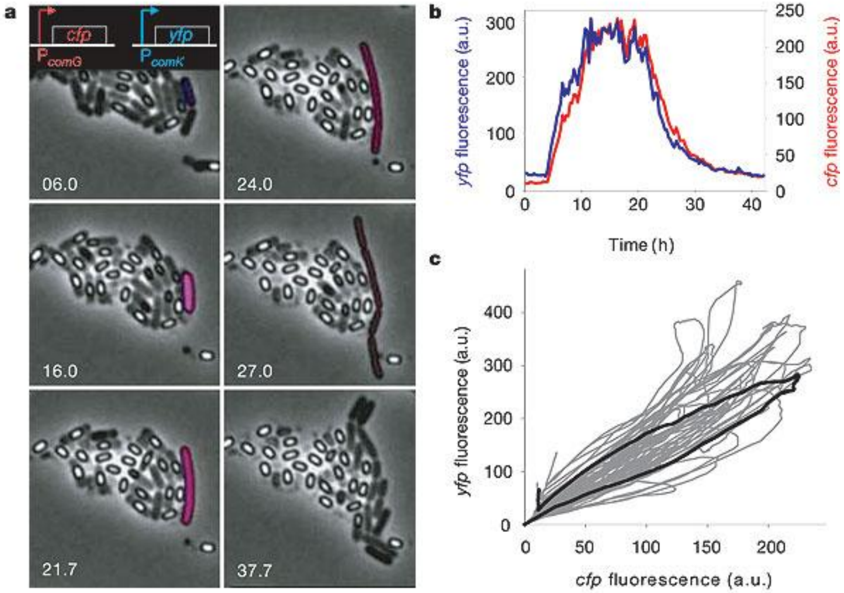
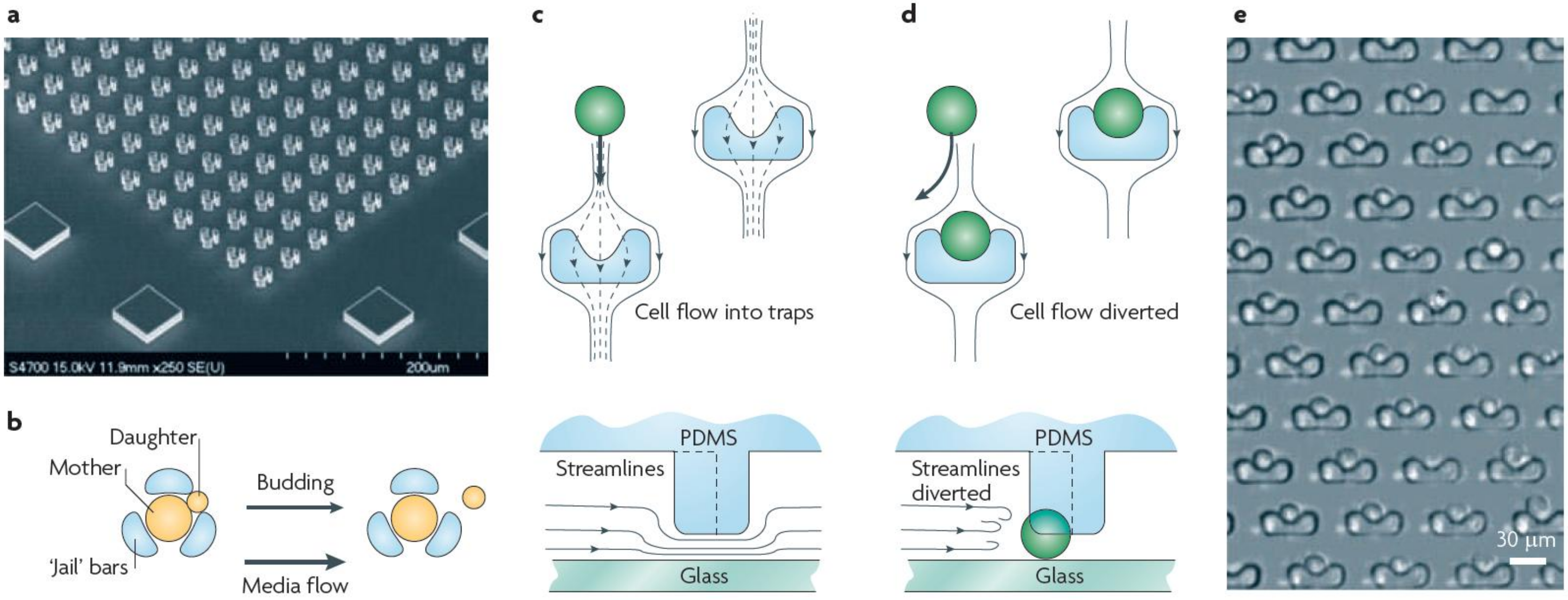




Single-cell microscopy and microfluidics

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



- Microfluidic devices allow tight control of environmental perturbations

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

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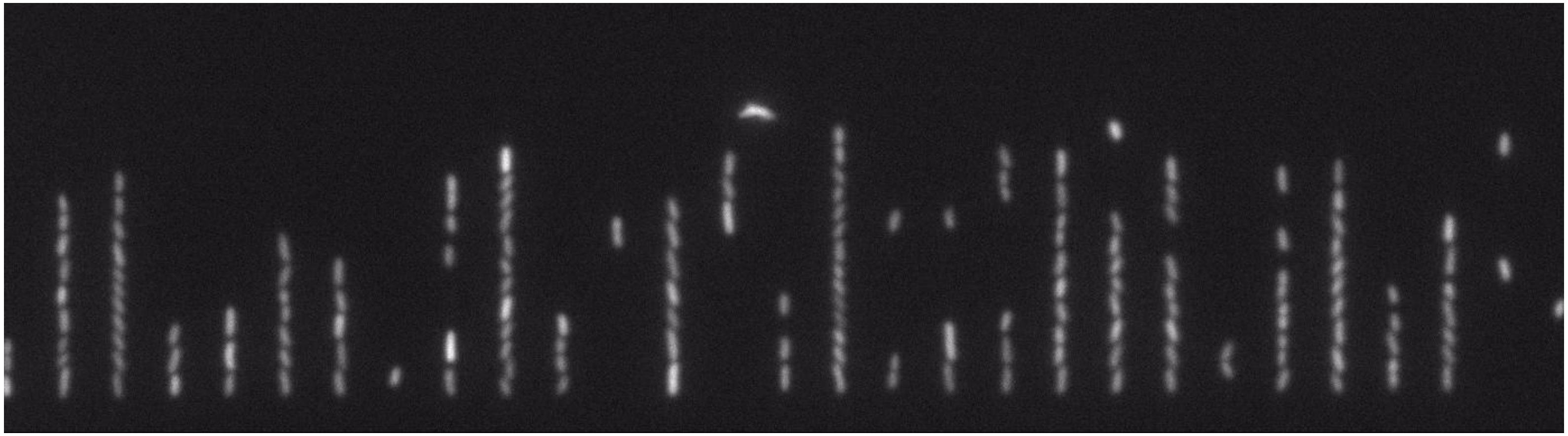

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Izard, Gomez Balderas *et al.* (2015), *Mol. Syst. Biol.*, 11:840

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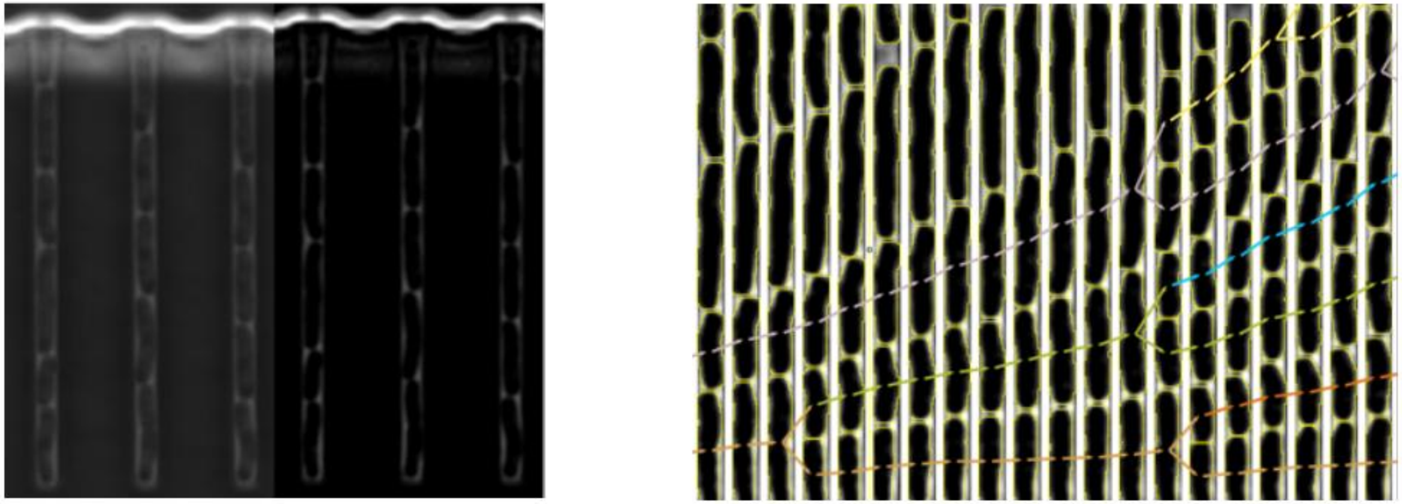

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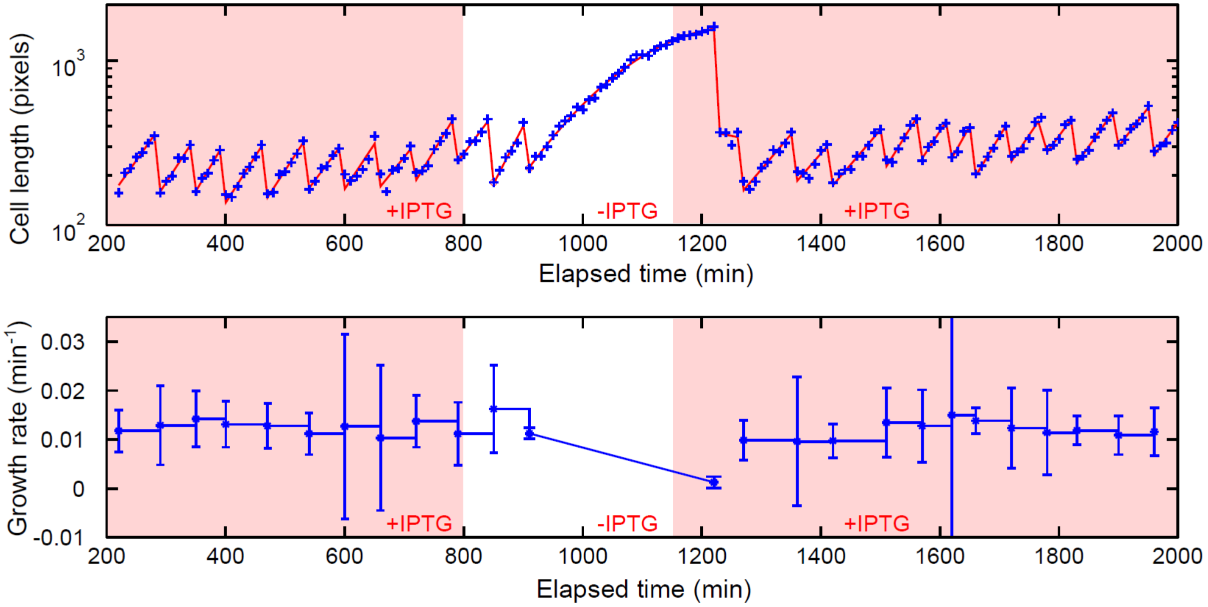

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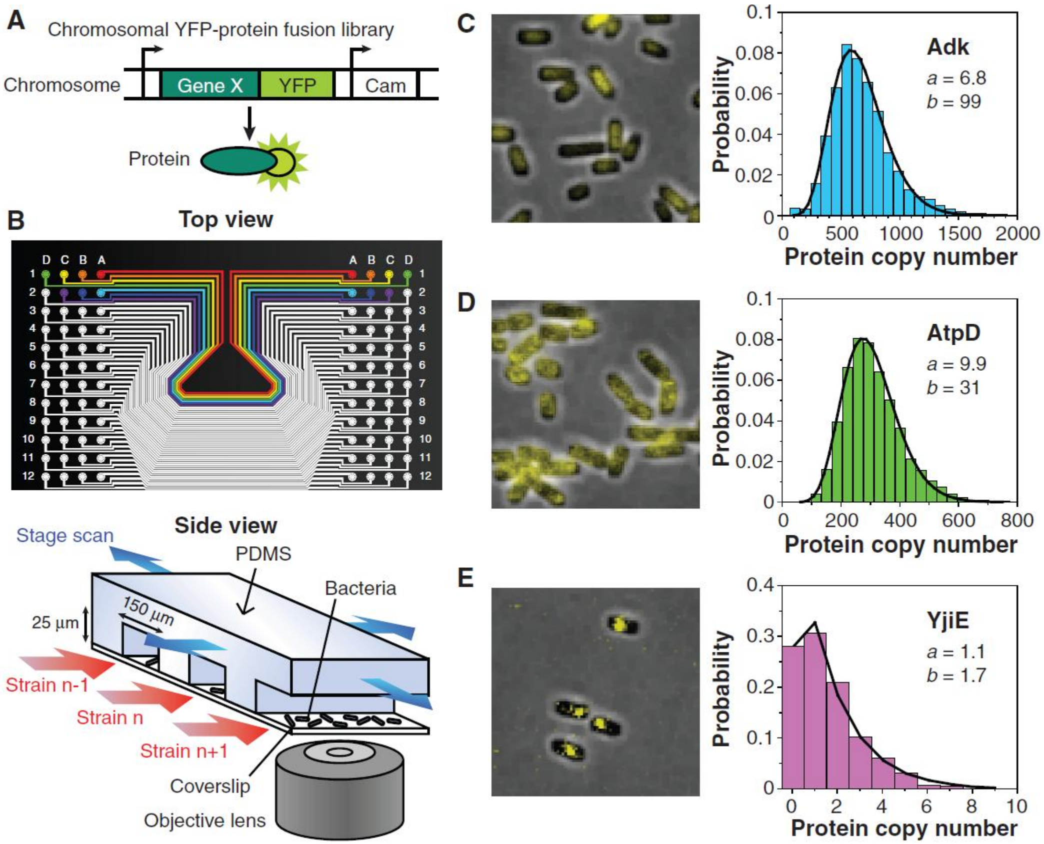
Single-molecule quantification

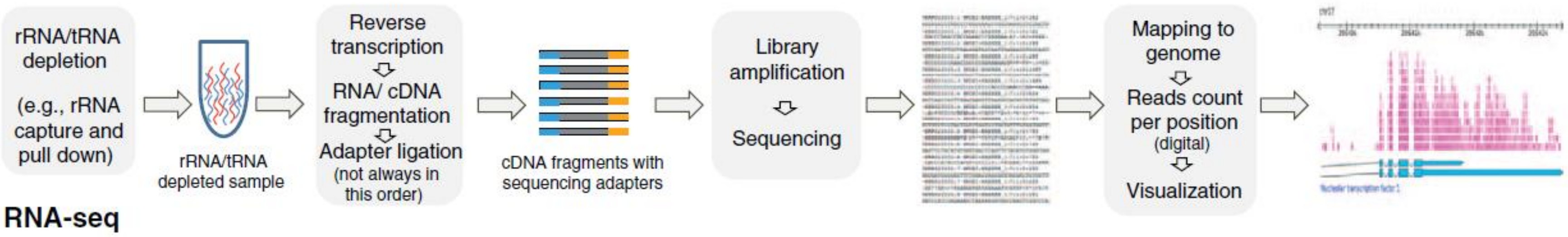
- Measurement of gene expression at **single-molecule level** using fluorescence reporter genes, microfluidic device, fluorescence microscopy, and calibration
- Measurement of expression of thousand *E. coli* genes using YFP-tagged chromosomal reporters



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



rRNA/tRNA depletion

(e.g., rRNA capture and pull down)

rRNA/tRNA depleted sample

Reverse transcription ⇩ RNA/ cDNA fragmentation ⇩ Adapter ligation (not always in this order)

cDNA fragments with sequencing adapters

Library amplification ⇩ Sequencing

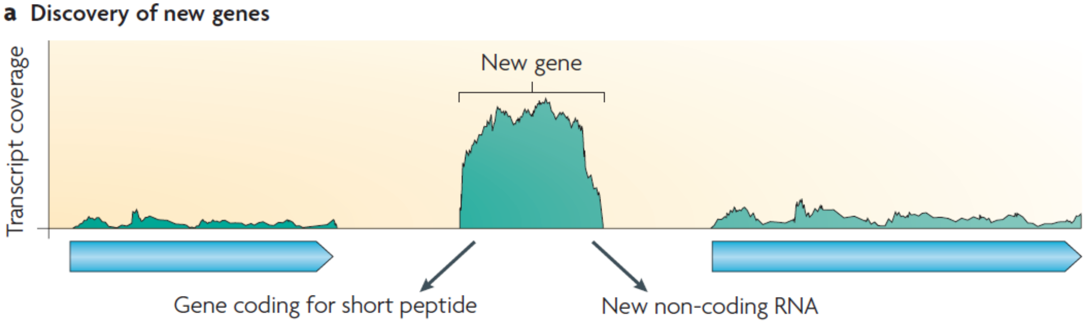
Mapping to genome ⇩ Reads count per position (digital) ⇩ Visualization

RNA-seq

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

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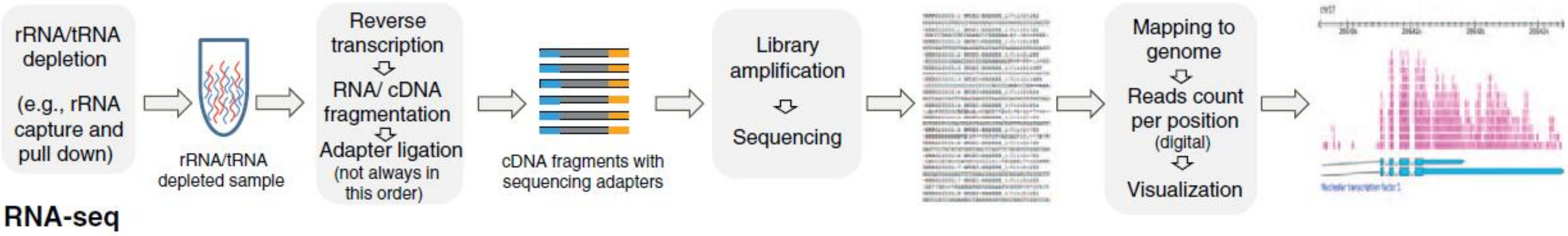

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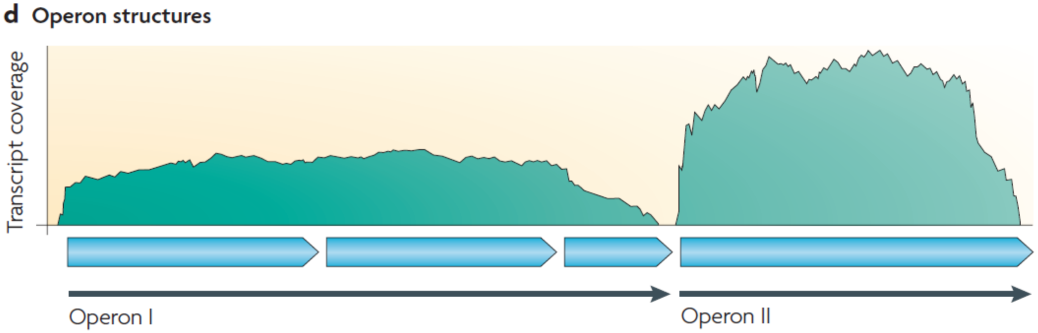

d Operon structures

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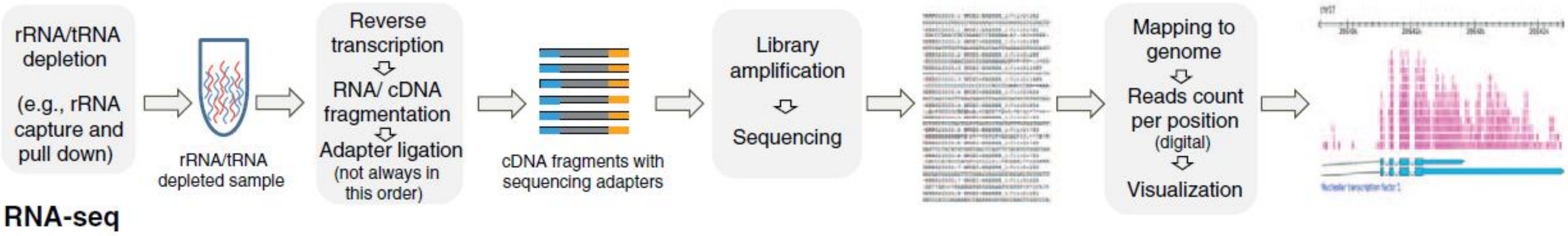


rRNA/tRNA depletion

(e.g., rRNA capture and pull down)

rRNA/tRNA depleted sample

Reverse transcription

⇩

RNA/ cDNA fragmentation

⇩

Adapter ligation

(not always in this order)

cDNA fragments with sequencing adapters

Library amplification

⇩

Sequencing

Mapping to genome

⇩

Reads count per position

(digital)

⇩

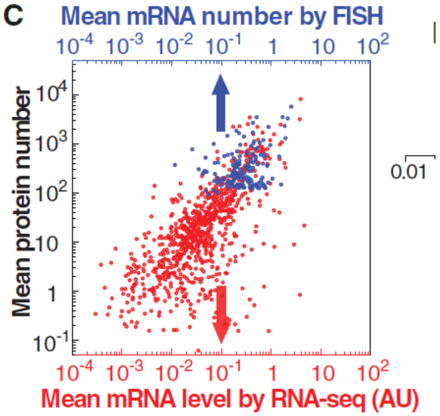
Visualization

RNA-seq

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

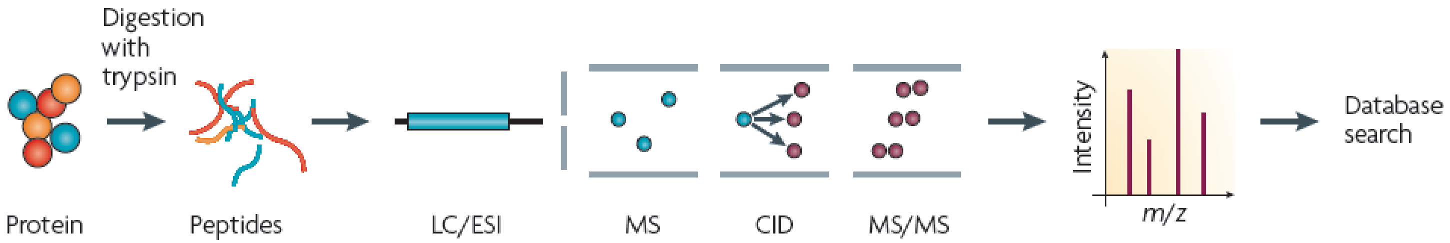
- Use of RNA-seq to determine correlation between mean RNA and mean protein levels

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



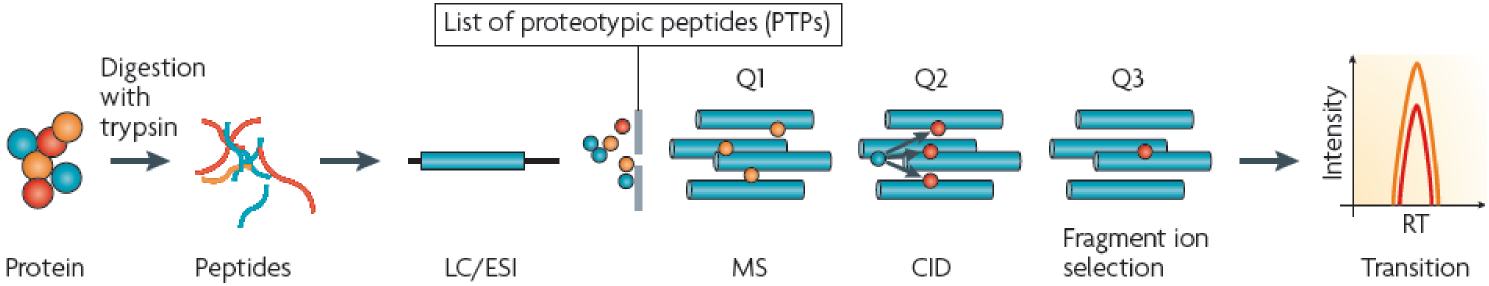
Quantitative proteomics

- Measurement of protein abundance using mass-spectrometry-based techniques (**quantitative proteomics**)
    - Use of calibration standards to achieve absolute quantification



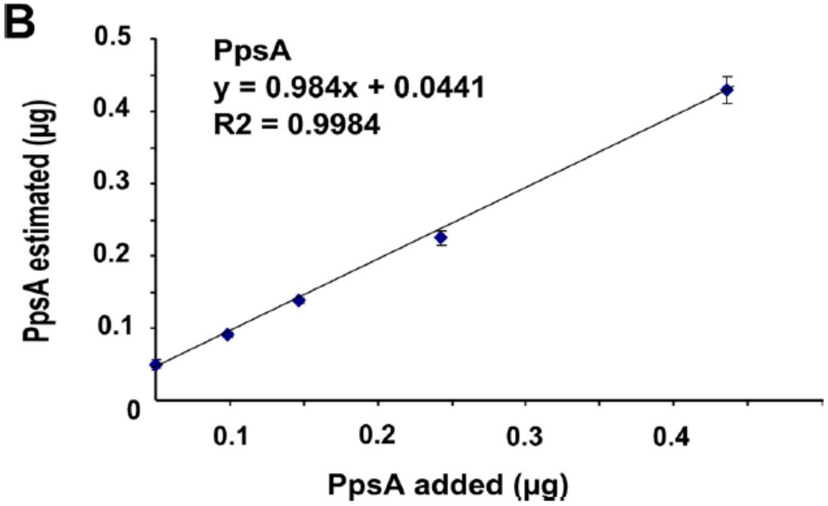
Gstaiger and Aebersold (2009), *Nat. Rev. Genet.*, 10:617-27

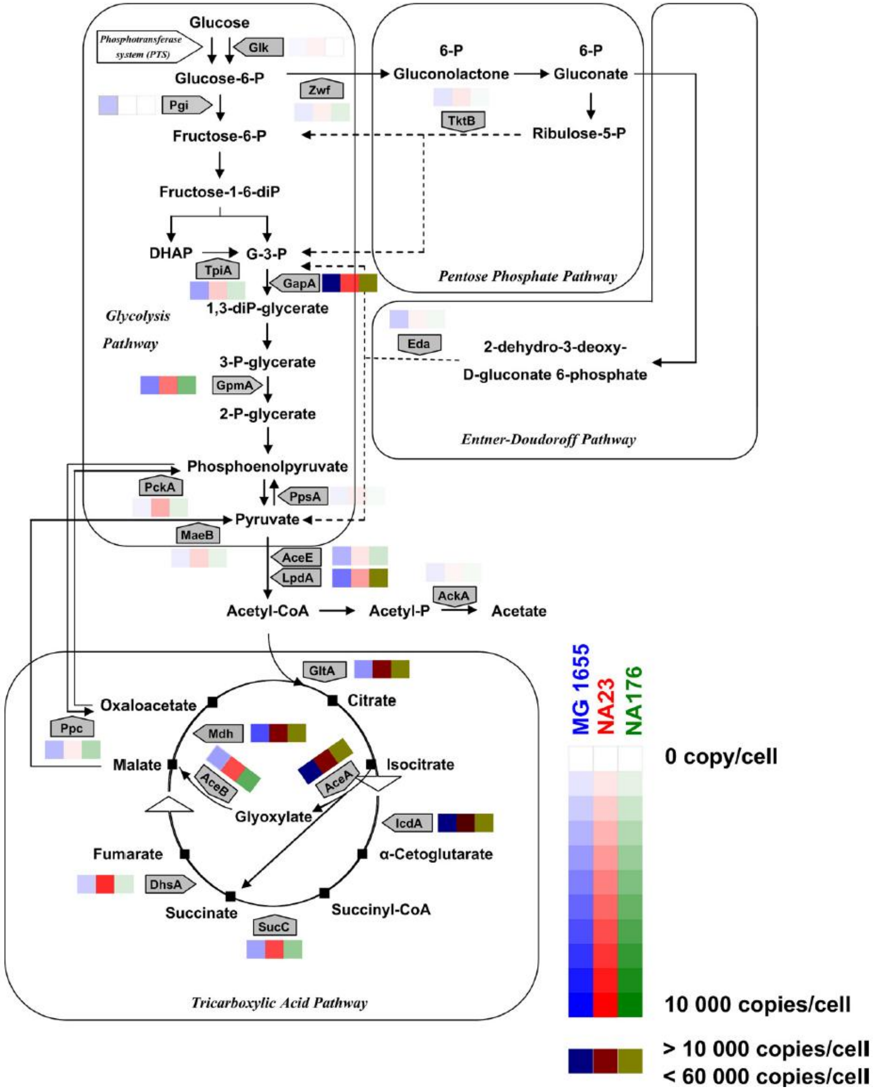
- Several targeted proteomics techniques developed to improve quantification of low-abundance proteins



Quantitative proteomics

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





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

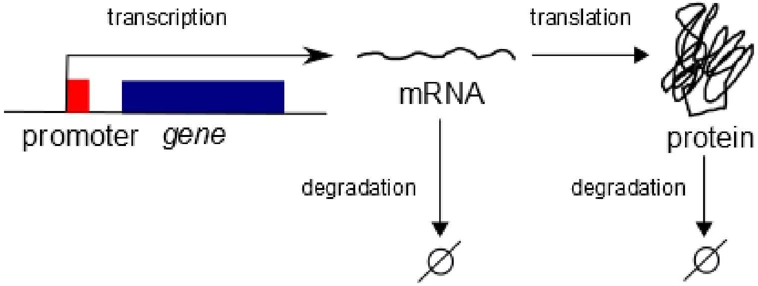
Systems biology

- 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

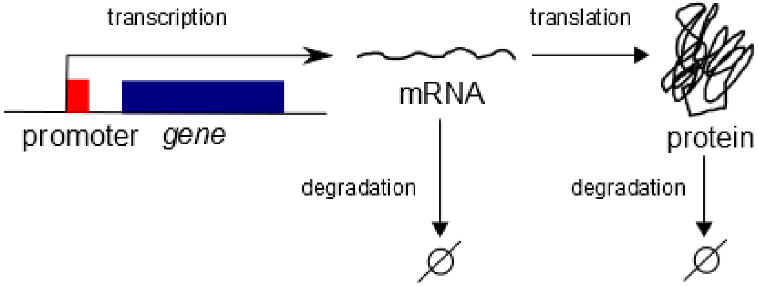
- Modeling of gene regulatory networks amount to modeling of gene expression and regulation of gene expression



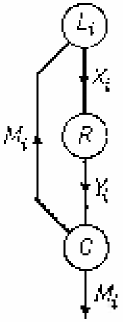
- Possible **aims** of modeling of gene regulatory networks:
  - Understanding role of individual components and interactions
  - Suggesting missing components and interactions
- Advantages of **mathematical and computer tools**:
  - Precise and unambiguous description of network
  - Systematic derivation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



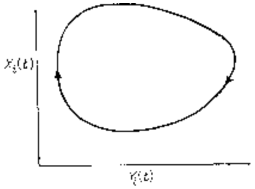
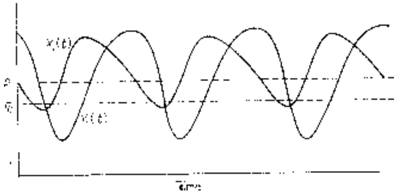


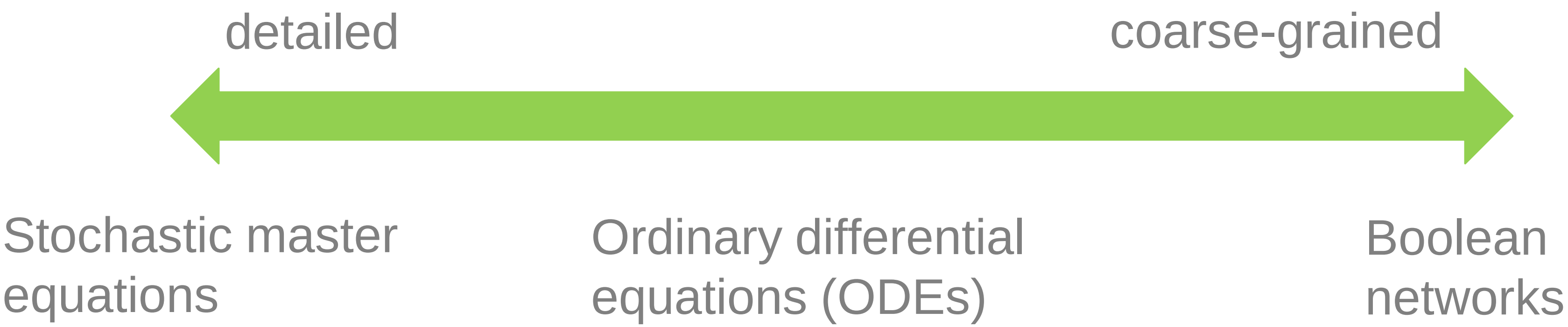
FIGURE 3.



Goodwin (1963), *Temporal Organization in Cells*

Modeling of gene regulatory networks

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



detailed

coarse-grained

Stochastic master equations

Ordinary differential equations (ODEs)

Boolean networks

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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