Modeling and simulation of gene regulatory networks 5

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INRIA Grenoble - Rhône-Alpes and IBIS

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

http://team.inria.fr/ibis

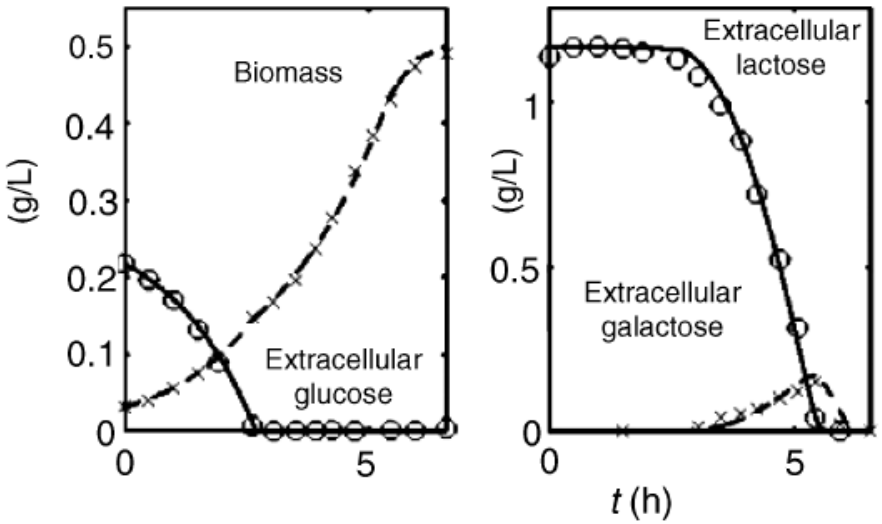
Overview

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. **Integrated models of the bacterial cell**

Bacterial growth and metabolism

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

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



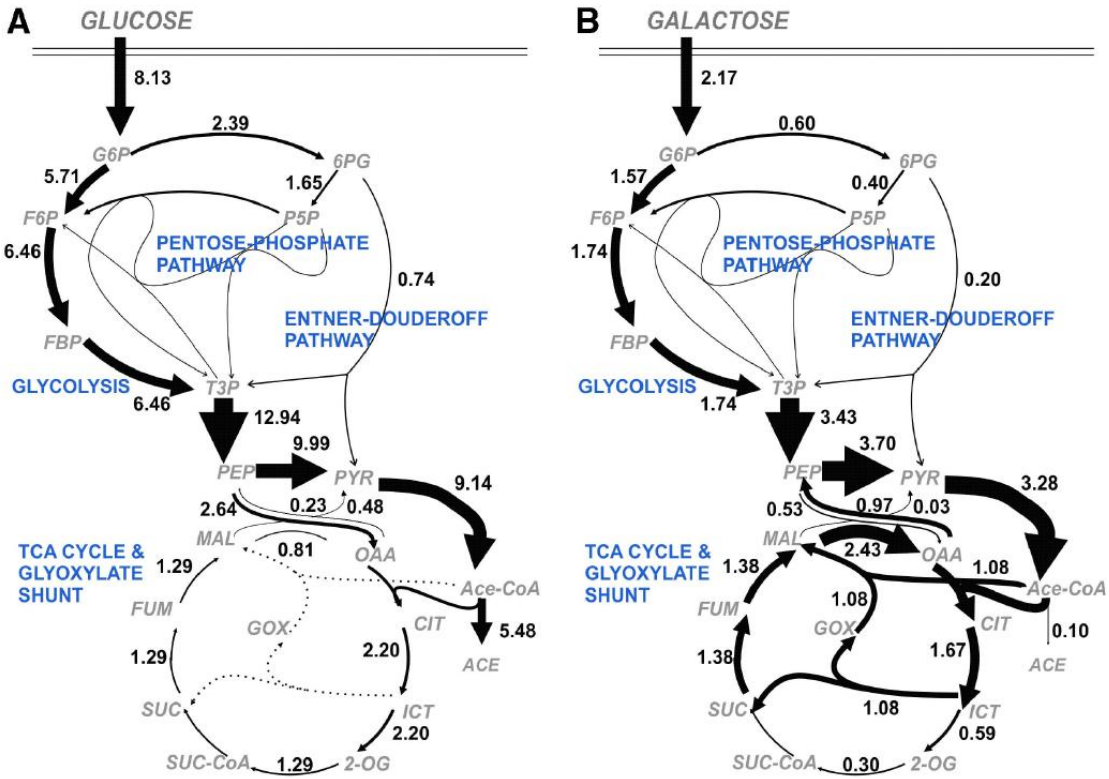
Bettenbrock *et al.* (2006), *J. Biol. Chem.*, 281(5):2578-84

- 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

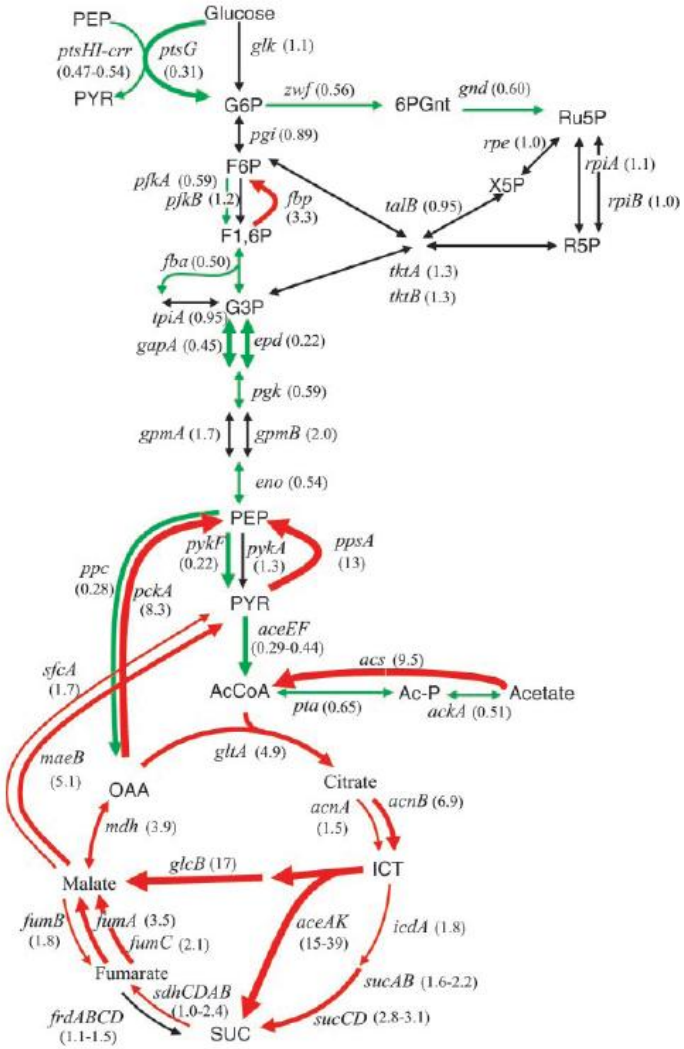


Haverkorn van Rijsewijk *et al.* (2011), *Mol. Syst. Biol.*, 7:477

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

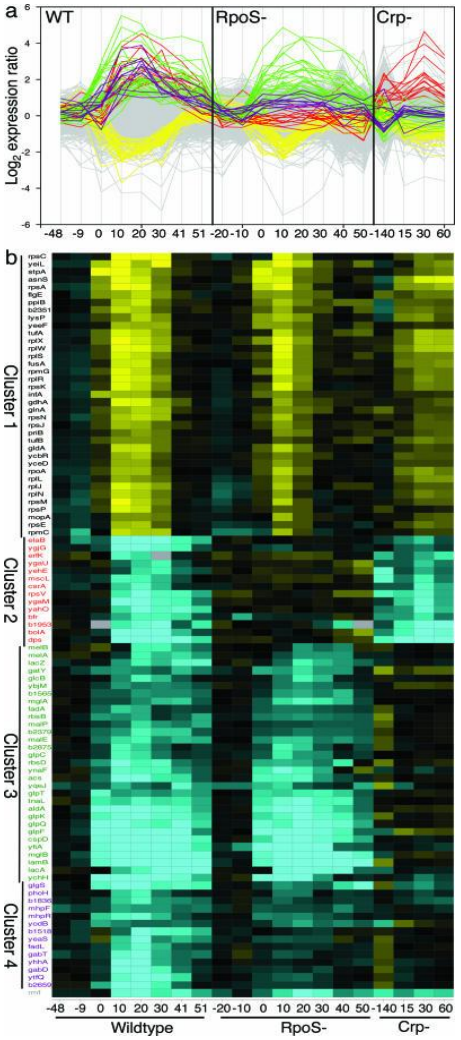


Oh *et al.* (2002), *J. Biol. Chem.*, 277(15):13175–83

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*

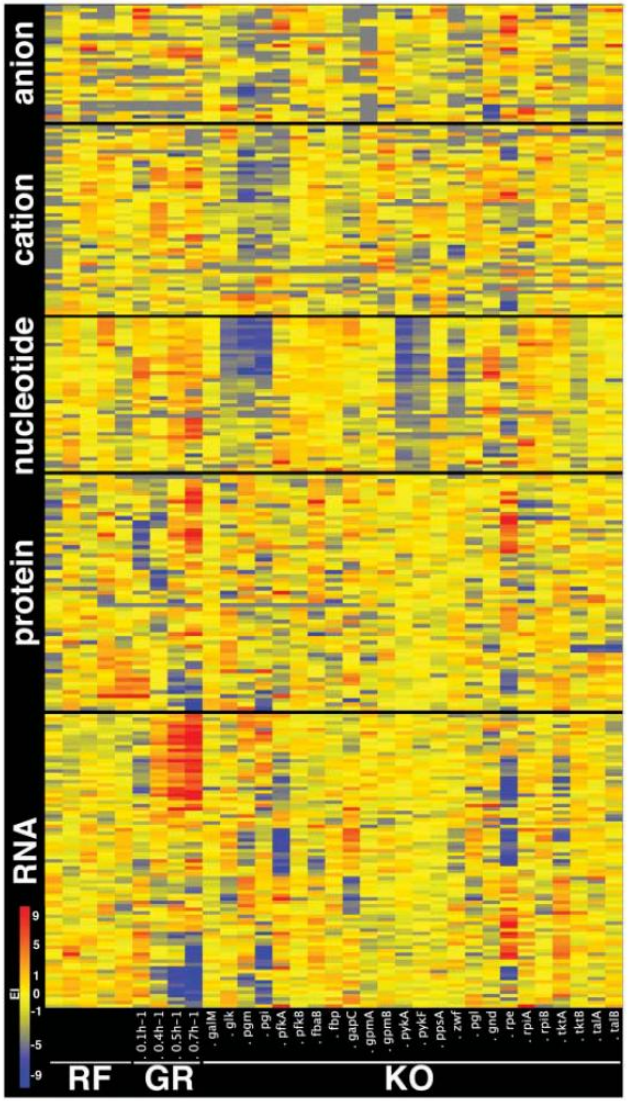


Traxler *et al.* (2006), *Proc. Natl. Acad. Sci. USA*, 103(7):2374–9

Adaptation on multiple levels

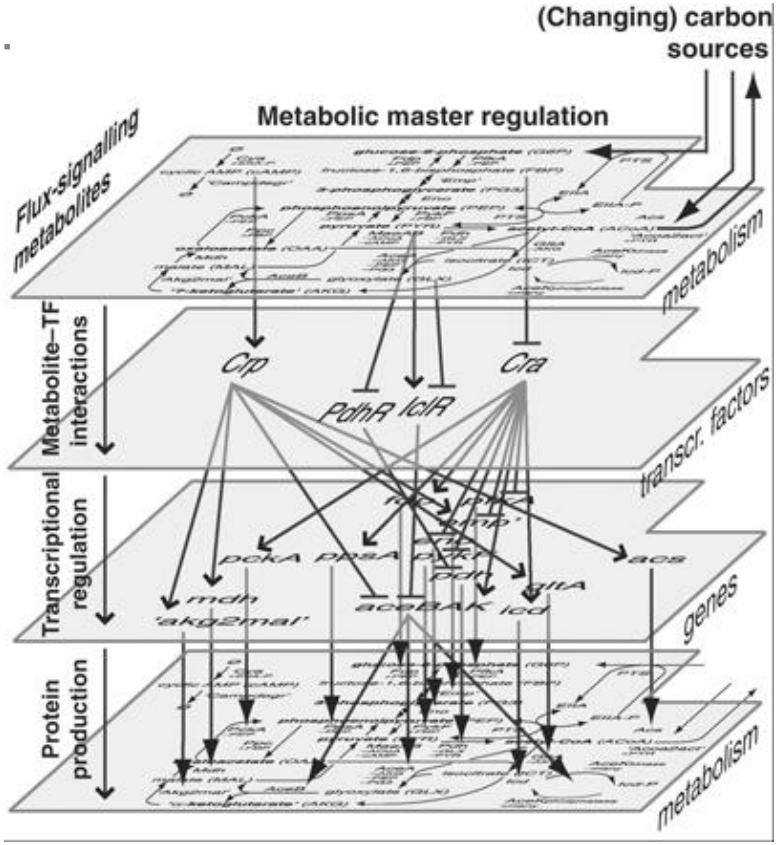
- Adaptation to different carbon source involves **adjustments on multiple levels** at the same time!
  - Parallel measurement of enzyme and metabolite concentrations, and metabolic fluxes in a variety of experimental conditions

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



Coordination of adaptative responses

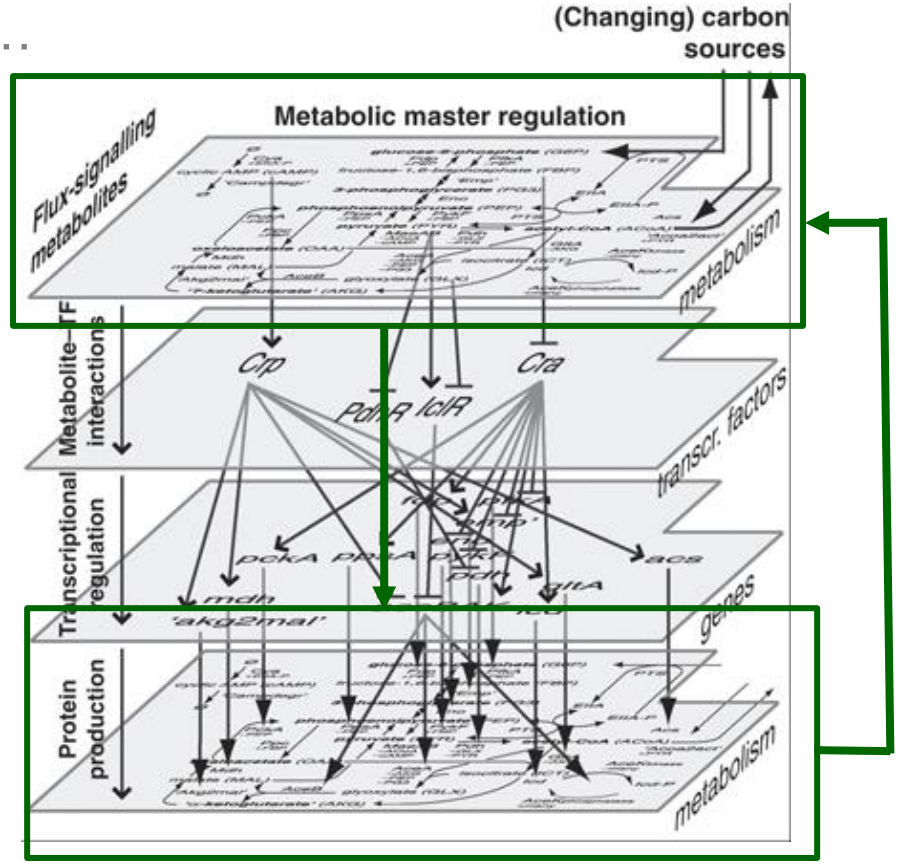
- Coordination of adaptative responses of bacterial cell achieved by **large and complex regulatory networks**
  - Variety of molecular mechanisms…
  - … operating on different time-scales…



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

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
- Use of models to analyse and predict dynamical behaviour of system

  Emergence of new discipline: **systems biology**

Towards integrated models of cell

- Most systems biology studies have focused on isolated, relatively small subsystems
- Increasing awareness that for answering many interesting questions, one needs to consider **integrated models of the cell**:
  - Multiple levels of regulation: metabolism, gene expression, signal transduction,…
  - Relate cellular processes to growth
  - Explicit modelling of interactions with environment and ecosystem
  - …

Towards integrated models of the cell

- Integrated models of the cell are emerging, but some interesting precursors exist
  - Coarse-grained model of an *E. coli* cell

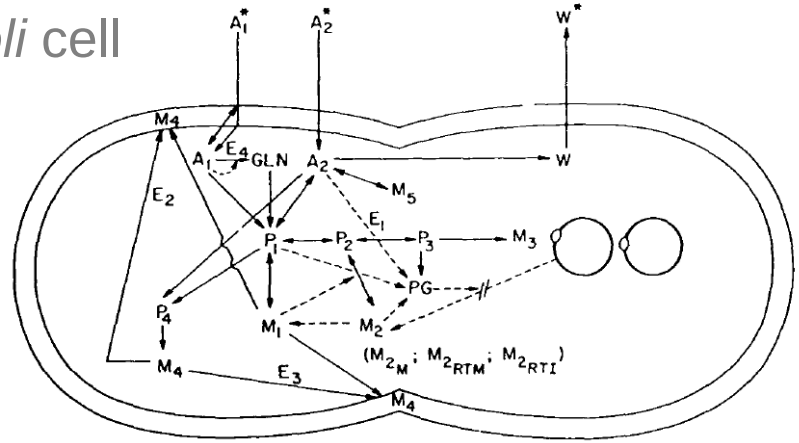


FIGURE 7 An idealized sketch of the model of *E. coli B/rA* growing in a glucose-ammonium salts medium with glucose or ammonia as the limiting nutrient. At the time shown the cell has just completed a round of DNA replication and initiated cross-wall formation and a new round of DNA replication. Solid lines indicate the flow of material, while dashed lines indicate flow of information. Reproduced with permission from Shuler and Domach, 1983.

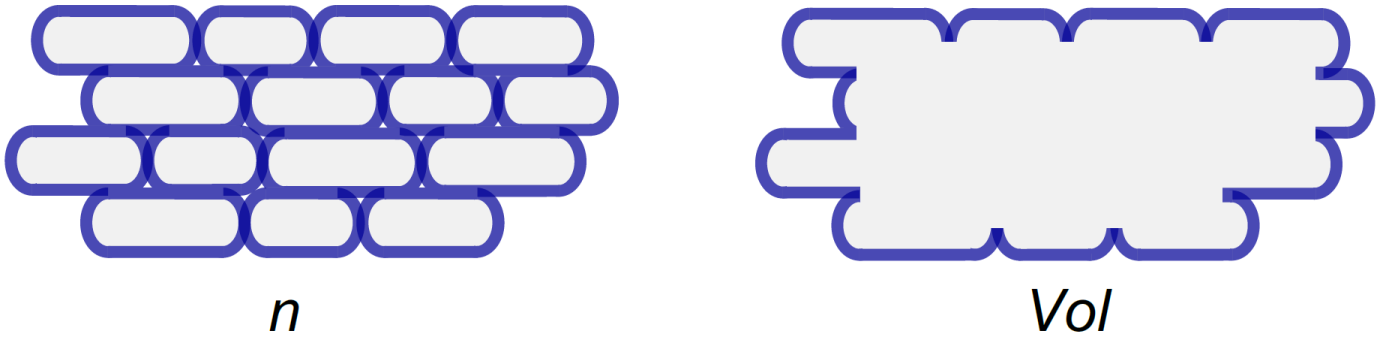
A_1 = ammonium ion
A_2 = glucose (and associated compounds in the cell)
W = waste products (CO_2, H_2O, and acetate) formed from energy metabolism during aerobic growth
P_1 = amino acids
P_2 = ribonucleotides
P_3 = deoxyribonucleotides
P_4 = cell envelope precursors
M_1 = protein (both cytoplasmic and envelope)
$M_{2_{RI}}$ = immature "stable" RNA
$M_{2_{RM}}$ = mature "stable" RNA (*r*-RNA and *r*-RNA—assume 85% *r*-RNA throughout)
M_{2_M} = messenger RNA
M_3 = DNA
M_4 = non-protein part of cell envelope (assume 16.7% peptidoglycan, 47.6% lipid, and 35.7% polysaccharide)
M_5 = glycogen
PG = *ppGpp*
E_2, E_3 = molecules involved in directing cross-wall formation and cell envelope synthesis—the approach used in the prototype model was used here but more recent experimental support is available
GLN = glutamine
E_1 = glutamine synthetase
*—the material is present in the external environment.

Domach *et al.* (1984), *Biotechnol. Bioeng.*, 26(3):203-16

Growth of microbial populations

- Growth can be considered on the level of number of **individual cells** or **aggregated volume of growing population** Vol [L]

  Segregated *vs* nonsegregated models



de Jong *et al.* (2017), *J. Roy. Soc. Interface*, 14(136):20170502

Growth of microbial populations

- Ordinary differential equation (ODE) model of the growth of a population of microorganisms

  Growth rate $\mu\ [\mathrm{h}^{-1}]$

$$\dot{Vol} = \mu \cdot Vol$$

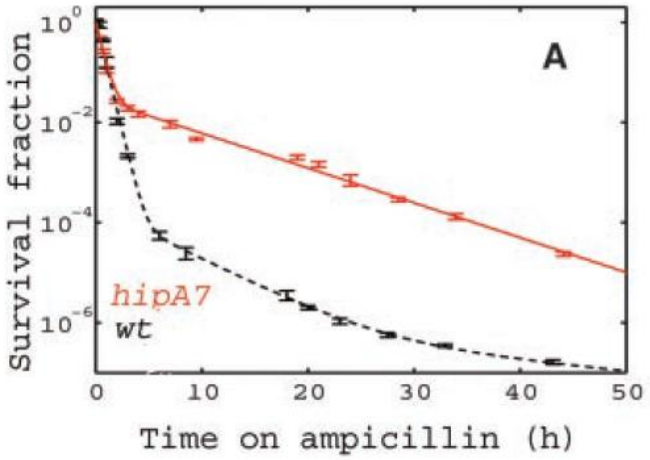
- Solution of growth model for constant growth rate $\mu = \mu^*$

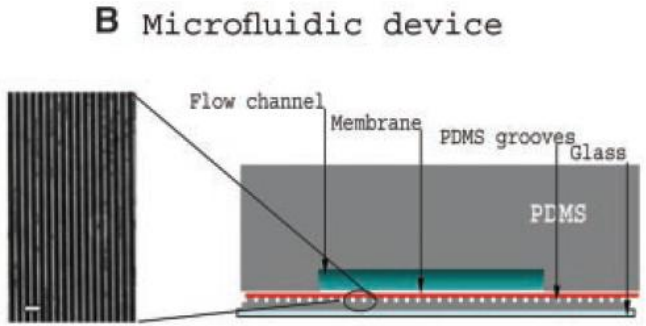
$$Vol(t) = Vol(0) \cdot e^{\mu^* \cdot t}$$

  Half-life $t_{1/2} = \ln 2 / \mu^*$

Growth of microbial populations

- If all cells have same growth rate, segregated and nonsegregated models are identical
- But: growth rate of cells in population may be heterogeneous
  - Bacterial persistence after antibiotics treatment





Balaban *et al.* (2004), *Science*, 305(5690):1622-5

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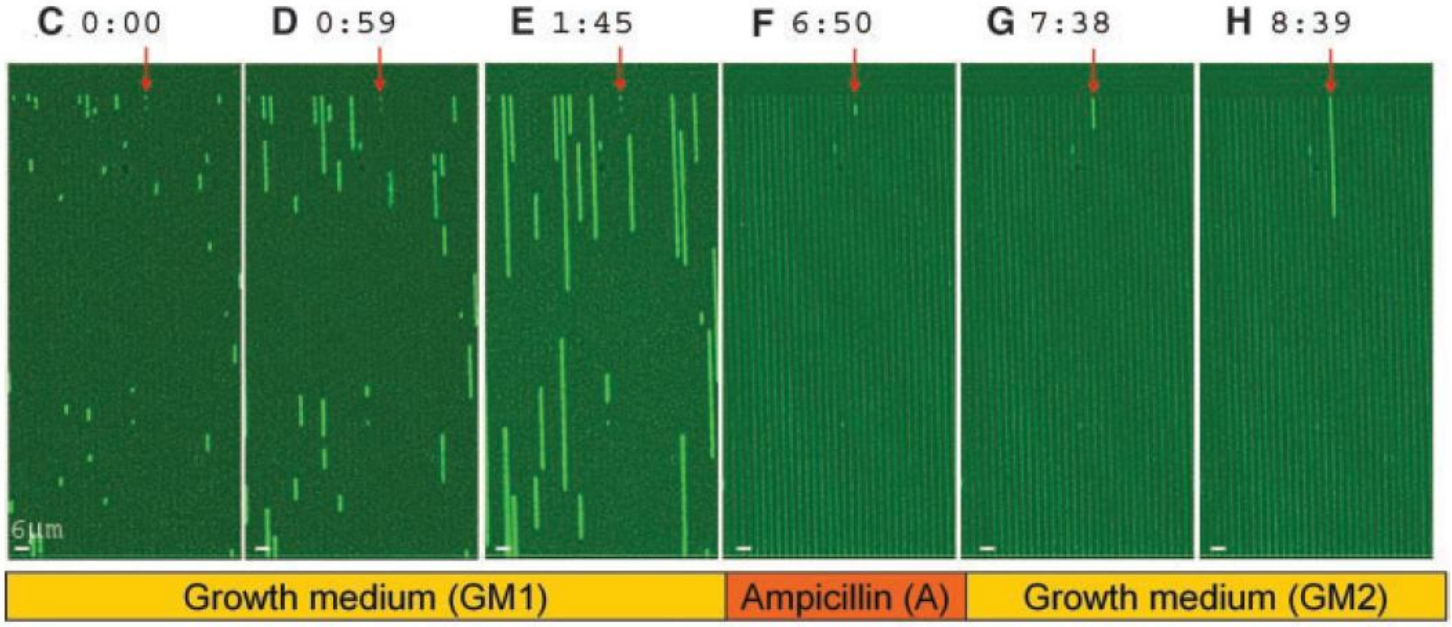
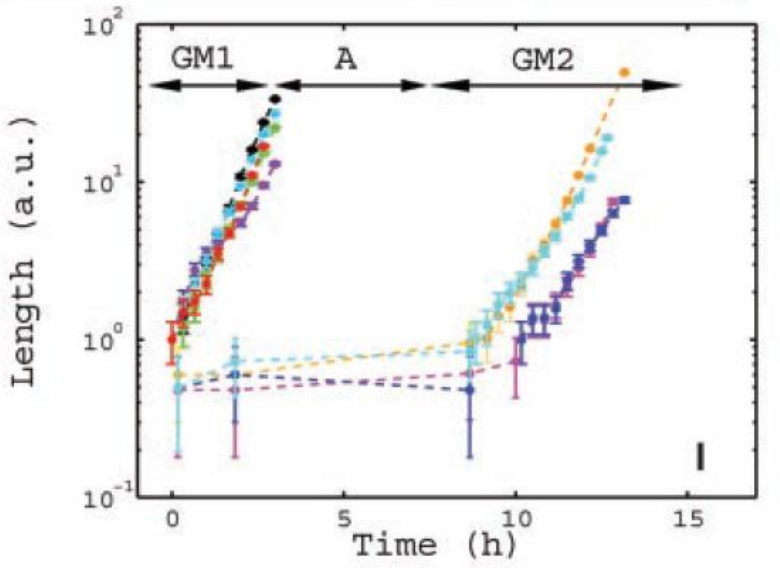


Balaban *et al.* (2004), *Science*, 305(5690):1622-5

Growth of microbial populations

- If all cells have same growth rate, segregated and nonsegregated models are identical
- But: growth rate of cells in population may be heterogeneous
  - Bacterial persistence after antibiotics treatment
  - Persister cellss have lower growth rate before antibiotics treatment

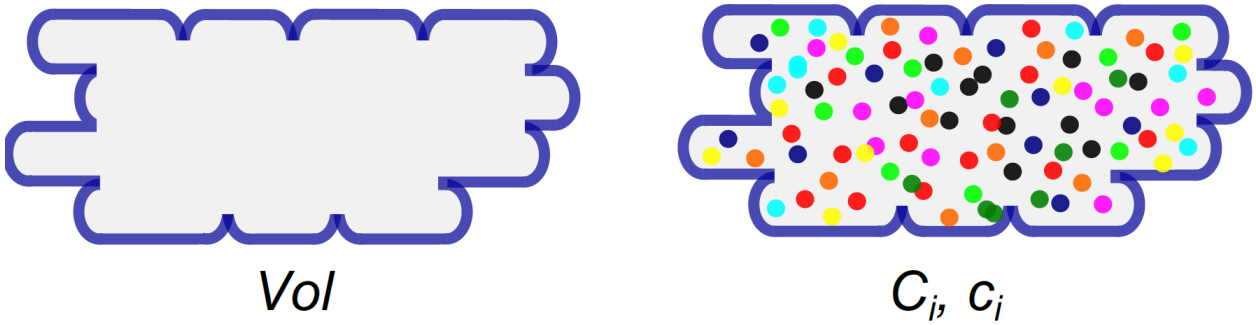


Balaban *et al.* (2004), *Science*, 305(5690):1622-5

Volume and macromolecular contents

- Growth is fueled by biochemical processes
- Models describing molecular constituents and biochemical reactions in which they are involved

  Structured *vs* unstructured models



Volume and macromolecular contents

- Basic assumption: volume proportional to biomass (total mass of molecular constituents in cells)

  Dry mass of constituent *i* C_i $[\mathrm{g}]$

  Biomass B $[\mathrm{g}]$

$$Vol \sim \sum_i C_i = B$$

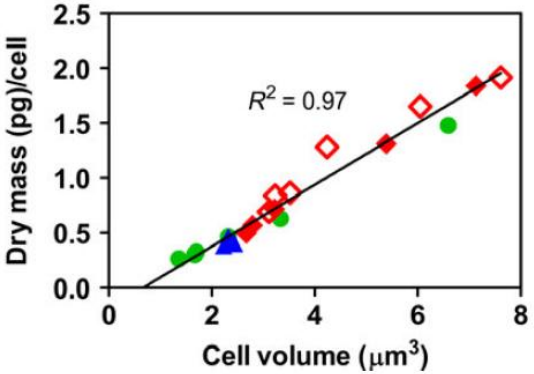
- In other words, biomass density $1/\delta$ $[\mathrm{g\,L^{-1}}]$ is constant:

$$Vol = \delta \cdot \sum_i C_i = \delta \cdot B$$

Volume and macromolecular contents

- Assumption of constant biomass density supported by experimental data

  Biomass density approximately $300\ \mathrm{g\ L^{-1}}$



Conditions	Strain	Description	Medium	Symbols
Nutrient limitation	NCM3722	Wild type	Various nutrient	●
Translation Inhibition with Cm	NCM3722	Wild type	Glucose with Cm	▲
Glucose LacZ OE	NQ1389	Titratable LacZ expression	Glucose with cTc	◆
Glucose +cAA LacZ OE	NQ1389	Titratable LacZ expression	Glucose+cAA with cTc	◇

Basan *et al.* (2015), *Mol. Syst. Biol.*, 11:836-5

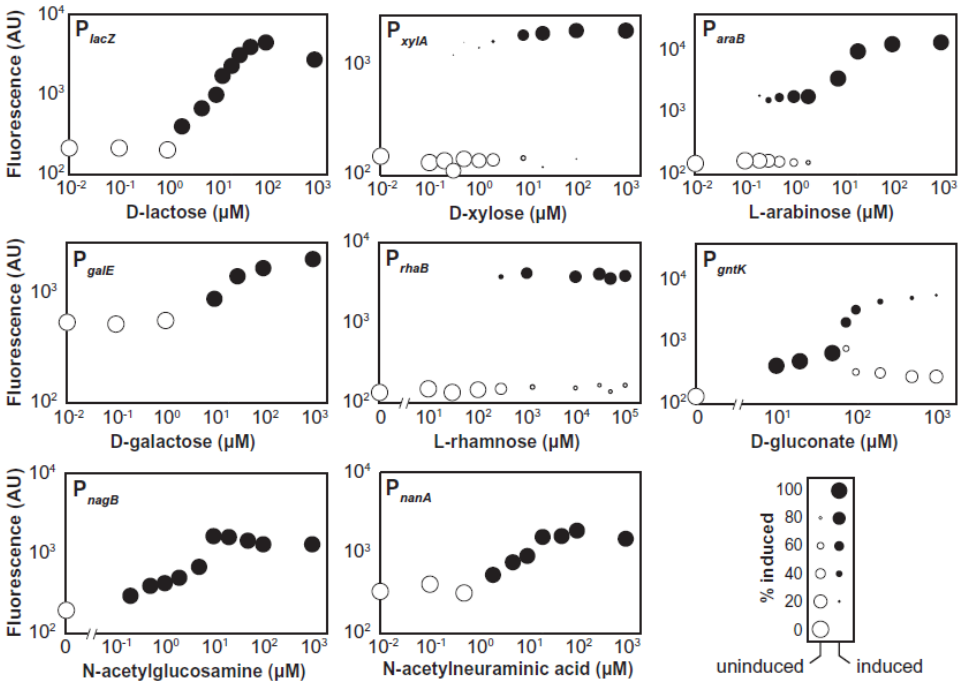
Volume and macromolecular contents

- Concentration c_i $[\mathrm{g}]$ of molecular constituent *i* in population

$$c_i = C_i / Vol$$

- If all cells have same concentration, then c_i also applies to individual cells
- But: concentrations may be heterogeneous, leading to different growth phenotypes

  Enzymes for secondary carbon sources in *E. coli*



Afroz *et al.* (2014), *Mol. Microbiol.*, 93(6):1093-1103

Volume and macromolecular contents

- Concentration c_i $[\mathrm{g}]$ of molecular constituent *i* in population

$$c_i = C_i / Vol$$

- If all cells have same concentration, then c_i also applies to individual cells
- Consequence of proportionality of mass and volume: total concentration is constant

$$\sum_i c_i = \sum_i C_i / Vol = B / Vol = 1/\delta$$

Volume and macromolecular contents

- ODE model of dynamics of molecular constituent *i* :

$$\dot{c}_i = \frac{\dot{C}_i \cdot Vol - C_i \cdot \dot{V}ol}{Vol^2} = \frac{\dot{C}_i}{Vol} - \frac{C_i}{Vol} \cdot \frac{\dot{V}ol}{Vol}$$

$$= \frac{\dot{C}_i}{Vol} - \mu \cdot c_i .$$

Appearance of term for **growth dilution** of individual constituents

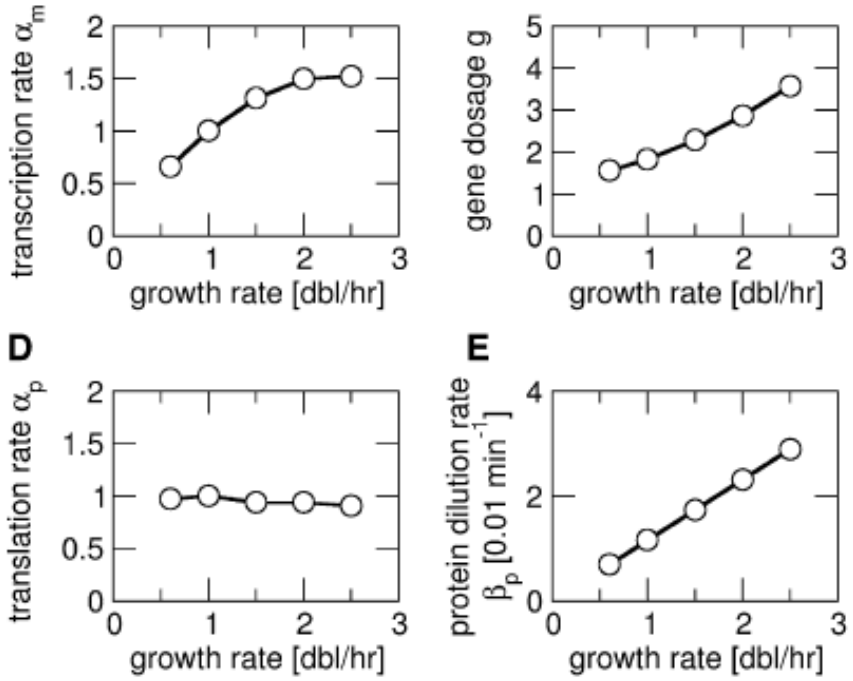
- Growth rate follows from dynamics of molecular constituents

$$\mu = \frac{\dot{V}ol}{Vol} = \delta \cdot \sum_i \frac{\dot{C}_i}{Vol} = \delta \cdot \frac{\dot{B}}{Vol}$$

No growth dilution if mass of all constituents remains constant

Volume and macromolecular contents

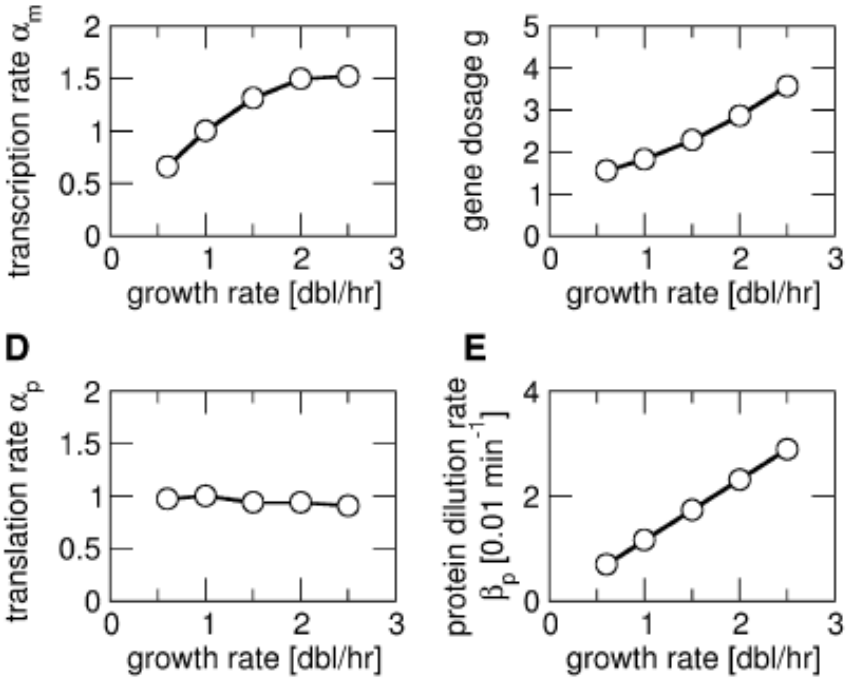
- Growth dilution may have an important effect on the concentration of cellular constituents
  - Changes in rate of protein synthesis and decay of **constitutive gene**

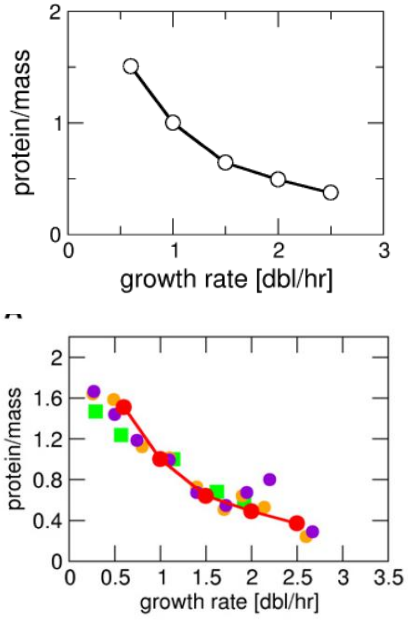


Klumpp *et al.* (2009), *Cell*, 139(7):1366-75

Volume and macromolecular contents

- Growth dilution may have an important effect on the concentration of cellular constituents
  - Changes in rate of protein synthesis and decay of **constitutive gene**
  - Concentration of gene product is growth-rate dependent





Klumpp *et al.* (2009), *Cell*, 139(7):1366-75

Biochemical reactions underlying growth

- Term $\dot{C}_i / Vol$ represents net effect of biochemical reactions on concentration of molecular constituent *i*
- Change of variables: $X_i = C_i / \alpha_i \ [\mathrm{mol}]$

  Rate of reactions based on physical encounters of molecules

$$x_i = X_i / Vol$$

- ODE model of dynamics of molecular constituent *i* :

$$\dot{x}_i = \frac{\dot{X}_i}{Vol} - \mu \cdot x_i$$

Biochemical reactions underlying growth

- Reformulation of reaction rates $\dot{X}_i / Vol$
  - Rate of reaction *j* : $v_j \quad [\mathrm{mol\ L^{-1}\ h^{-1}}]$
  - Stoichiometry of constituent *i* in reaction *j* : N_{ij}

Biochemical reactions underlying growth

- Reformulation of reaction rates $\dot{X}_i / Vol$
  - Vector of reaction rates: v
  - Row in stoichiometry matrix for constituent *i* : N_i
  - Vector of molecular constituents: x
- Reformulation of ODE model

$$\dot{x}_i = N_i \cdot v - \mu \cdot x_i$$

Biochemical reactions underlying growth

- Reformulation of reaction rates $\dot{X}_i / Vol$
  - Vector of reaction rates: v
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- Reformulation of ODE model

$$\dot{x}_i = N_i \cdot v - \mu \cdot x_i$$

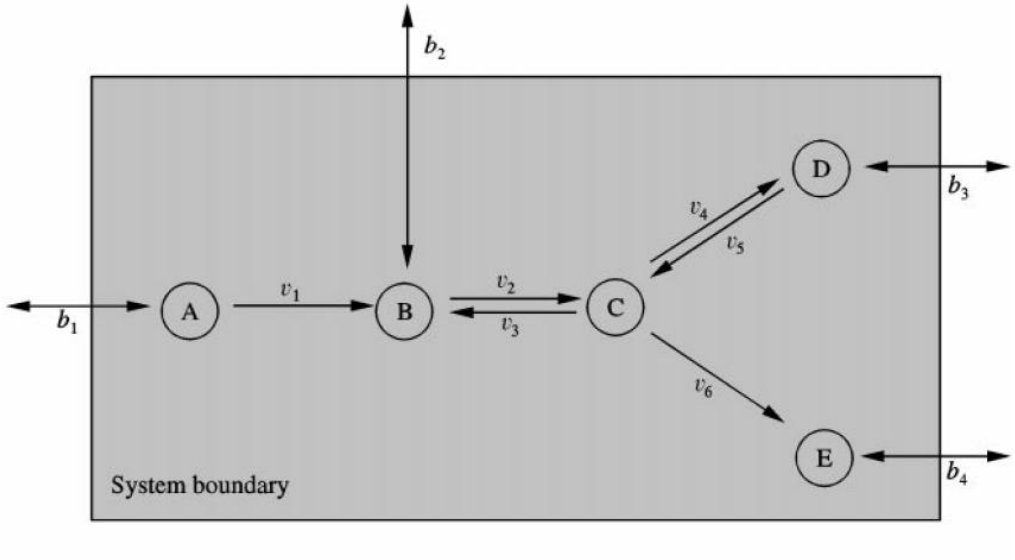
- Stoichiometry model of biochemical reactions

$$\dot{x} = N \cdot v - \mu \cdot x$$

Biochemical reactions underlying growth

- Stoichiometry matrix N describes structure of reaction network

  Internal reactions and exchange reactions, reversible and irreversible



$$N = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \end{bmatrix} \quad v = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \\ b_1 \\ b_2 \\ b_3 \\ b_4 \end{bmatrix}$$

Schilling *et al.* (2000), *J. Theor. Biol.*, 203(3):229-48

Biochemical reactions underlying growth

- Stoichiometry model of biochemical reactions

$$\dot{x} = N \cdot v - \mu \cdot x$$

- Expression of growth rate

$$\mu = \delta \cdot \sum_i \frac{\dot{C}_i}{Vol} = \delta \cdot \sum_i \alpha_i \cdot \frac{\dot{X}_i}{Vol}$$
$$= \delta \cdot \sum_i \alpha_i \cdot N_i \cdot v(x).$$

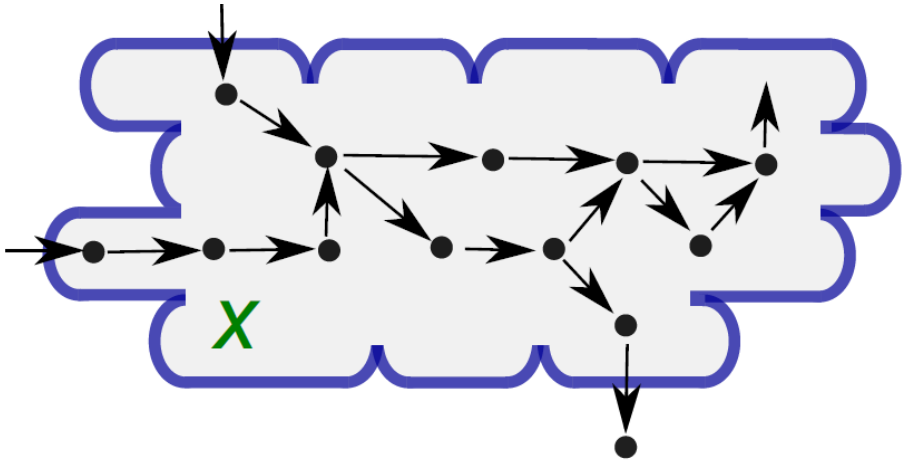
  - Rate of accumulation of (mass of) constituents (within unit volume per unit time) relative to total amount of constituents (within unit volume)
  - Not *ad-hoc* definition, but derived from basic assumptions

Biochemical reactions underlying growth

- ODE model for growth of microbial populations:

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

$$\mu = \delta \cdot \sum_i \alpha_i \cdot N_i \cdot v(x).$$



- Reaction rates depend on concentrations x of substrates, products, effectors

Biochemical reactions underlying growth

- ODE model for growth of microbial populations:

$$\dot{x} = N \cdot v(x) - \mu \cdot x,$$
$$\mu = \delta \cdot \sum_i \alpha_i \cdot N_i \cdot v(x).$$

- Reaction rates depend on concentrations x of substrates, products, effectors

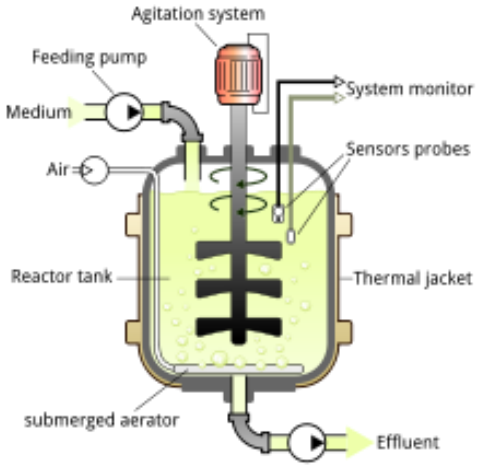
  Mass-action kinetics, Henri-Michaelis-Menten kinetics, Monod-Wyman-Changeux kinetics, …

$$v(x, e) = V_{max} \cdot x / (K_m + x)$$
$$V_{max} = k_{cat} \cdot e$$

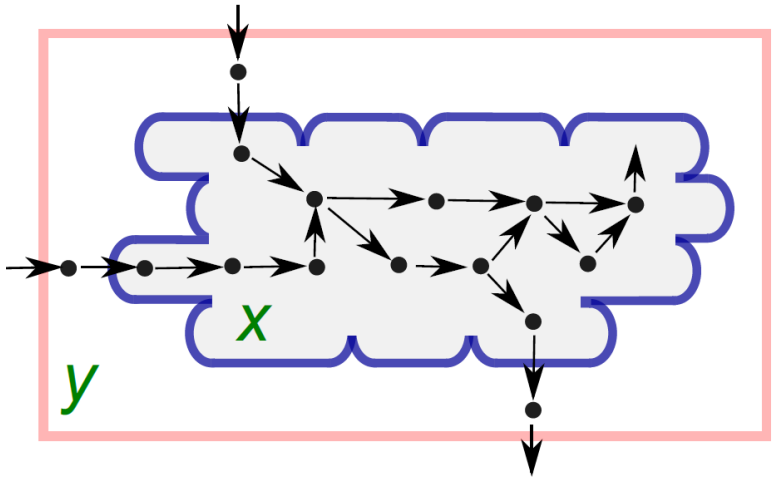
Heinrich and Schuster (1996), *The Regulation of Cellular Systems*, Chapman & Hall

Growth in a changing environment

- No explicit model of the environment
  - Some reactions in v correspond to uptake of substrates or secretion of products
- Environment modeled as bioreactor filled by liquid medium of fixed volume
  - Substrate/product concentrations in medium: $y \quad [\mathrm{g\ L^{-1}}]$
  - Volume of medium: $Vol_{medium} \quad [\mathrm{L}]$



Source: wikpedia



Growth in a changing environment

- No explicit model of the environment
  - Some reactions in v correspond to uptake of substrates or secretion of products
- Environment modeled as bioreactor filled by liquid medium of fixed volume
  - Substrate/product concentrations in medium: $y \quad [\mathrm{g\ L^{-1}}]$
  - Volume of medium: $Vol_{medium} \quad [\mathrm{L}]$
- ODE model for dynamics of substrate/product concentrations in medium

$$\dot{y} = \alpha_y \cdot E \cdot v(x, y) \cdot (\, Vol / \, Vol_{medium})$$

  - Stoichiometry matrix for exchange reactions: E
  - Diagonal matrix of molar mass coefficients: α_y

Growth in a changing environment

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  - Some reactions in v correspond to uptake of substrates or secretion of products
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  - Volume of medium: $Vol_{medium} \quad [\mathrm{L}]$
- ODE model for dynamics of substrate/product concentrations in medium

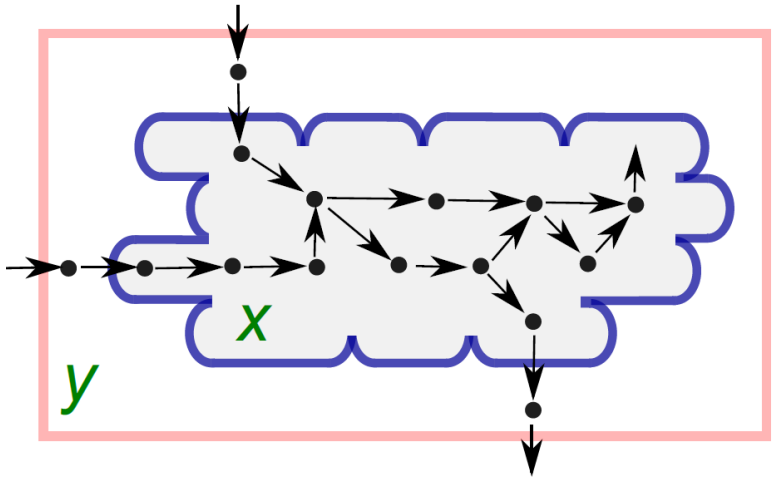
$$\frac{Vol}{Vol_{medium}} = \delta \cdot \frac{\sum_i C_i}{Vol_{medium}} = \delta \cdot b,$$

$$\dot{y} = \delta \cdot \alpha_y \cdot E \cdot v(x, y) \cdot b.$$

Growth in a changing environment

- ODE model for growth of microbial populations:

$$\dot{x} = N \cdot v(x,y) - \mu \cdot x,$$
$$\dot{y} = \delta \cdot \alpha_y \cdot E \cdot v(x,y) \cdot b,$$
$$\mu = \delta \cdot \sum_i \alpha_i \cdot N_i \cdot v(x,y),$$
$$\dot{b} = \mu \cdot b,$$



Growth in a changing environment

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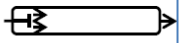
$$\dot{y} = \delta \cdot \alpha_y \cdot E \cdot v(x,y) \cdot b,$$
$$\mu = \delta \cdot \sum_i \alpha_i \cdot N_i \cdot v(x,y),$$
$$\dot{b} = \mu \cdot b,$$

- Model applies to batch cultivation, but can be easily adapted for continuous culture or fed-batch culture

Bastin and Dochin (1990), *On-Line Estimation and Adaptive Control of Bioreactors*, Elsevier, 1990

Growth in a changing environment

- Bioreactor models have been mostly used in context of biotechnological applications
- **But**: they also apply to complex natural environments, such as digestive tracts of vertebrates and insects

Organ shape and location in horse digestive tract	Example of organ names	Reactor shape	Modelized reactor	Scheme
	Stomach (human) Rumen (cow) Crop (hoazin) Saccular forestomach (kangaroo) Proctodeum P3 (termite)	Open sac-like reactor	Continuously stirred tank reactor (CSTR)	
	Caecum (rabbit)	Closed sac-like reactor	Batch reactor	
	Large intestine (human)	Large tubular reactor	CSTR in series	
	Small intestine (human) Tubiform forestomach (kangaroo)	Narrow tubular reactor	Plug-flow reactor	

Godon *et al.* (2013), *BioEnergy Res.*, 6(3):1063-81

Towards integrated models of the cell

- Integrated models of the cell are emerging, but some interesting precursors exist
  - Coarse-grained model of an *E. coli* cell

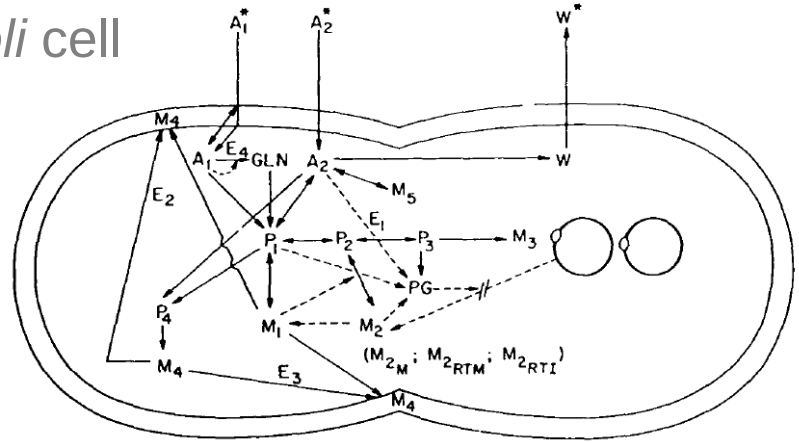


FIGURE 7 An idealized sketch of the model of *E. coli B/rA* growing in a glucose-ammonium salts medium with glucose or ammonia as the limiting nutrient. At the time shown the cell has just completed a round of DNA replication and initiated cross-wall formation and a new round of DNA replication. Solid lines indicate the flow of material, while dashed lines indicate flow of information. Reproduced with permission from Shuler and Domach, 1983.

A_1 = ammonium ion
A_2 = glucose (and associated compounds in the cell)
W = waste products (CO_2, H_2O, and acetate) formed from energy metabolism during aerobic growth
P_1 = amino acids
P_2 = ribonucleotides
P_3 = deoxyribonucleotides
P_4 = cell envelope precursors
M_1 = protein (both cytoplasmic and envelope)
$M_{2_{RTI}}$ = immature "stable" RNA
$M_{2_{RTM}}$ = mature "stable" RNA (*r*-RNA and *r*-RNA—assume 85% *r*-RNA throughout)
M_{2_M} = messenger RNA
M_3 = DNA
M_4 = non-protein part of cell envelope (assume 16.7% peptidoglycan, 47.6% lipid, and 35.7% polysaccharide)
M_5 = glycogen
PG = *ppGpp*
E_2, E_3 = molecules involved in directing cross-wall formation and cell envelope synthesis—the approach used in the prototype model was used here but more recent experimental support is available
GLN = glutamine
E_1 = glutamine synthetase
*—the material is present in the external environment.

Domach *et al.* (1984), *Biotechnol. Bioeng.*, 26(3):203-16

Towards integrated models of the cell

- Integrated models of the cell are emerging, but some interesting precursors exist

  Coarse-grained model of an *E. coli* cell

- Model has evolved into minimal, **functionally complete model** of chemoheterotrophic bacterium

Model structure	Count	Examples
Compartments	4	Cytoplasm, cell membrane, whole cell, medium
Chemical species	408	Glucose-6P, alanine, mRNAs, proteins
Reactions	570	Fructose-6P synthesis, CTP synthesis
Rate parameters	570	Mass action or Michaelis–Menten rate constants
Saturation parameters	581	Michaelis–Menten-like saturation parameters
Inhibition parameters	25	Michaelis–Menten-like inhibition parameters
Rate rules	1	Methylation state of chromosome
Algebraic rules	1	Cell width (CW)
Events	36	DNA replication initiation, cell division
Constraints	408	Each species must have mass >0
Genes	241	Protein and stable RNA coding genes
Single coding genes	102	*dnaB*, *pgi*, etc.
Gene clusters	19	*replisome*, etc.
Genes in clusters	139	Ribosomal proteins, *dnaE*, etc.

Shuler *et al.* (2012), *Methods Mol. Biol.*, 881:573-610

Towards integrated models of the cell

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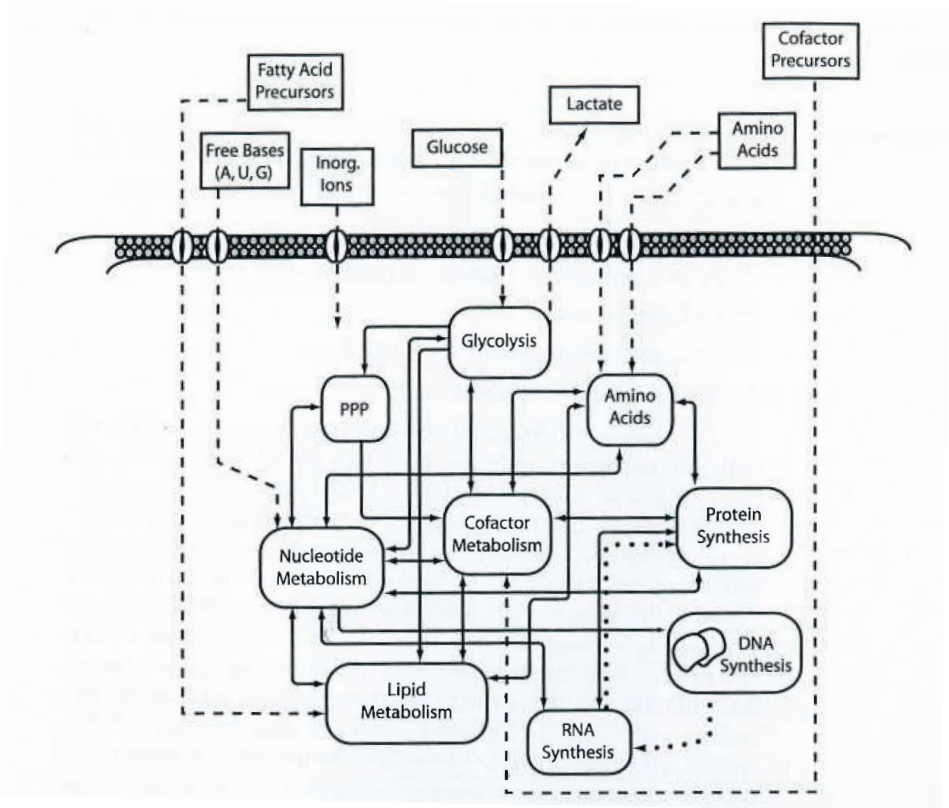
  Coarse-grained model of an *E. coli* cell

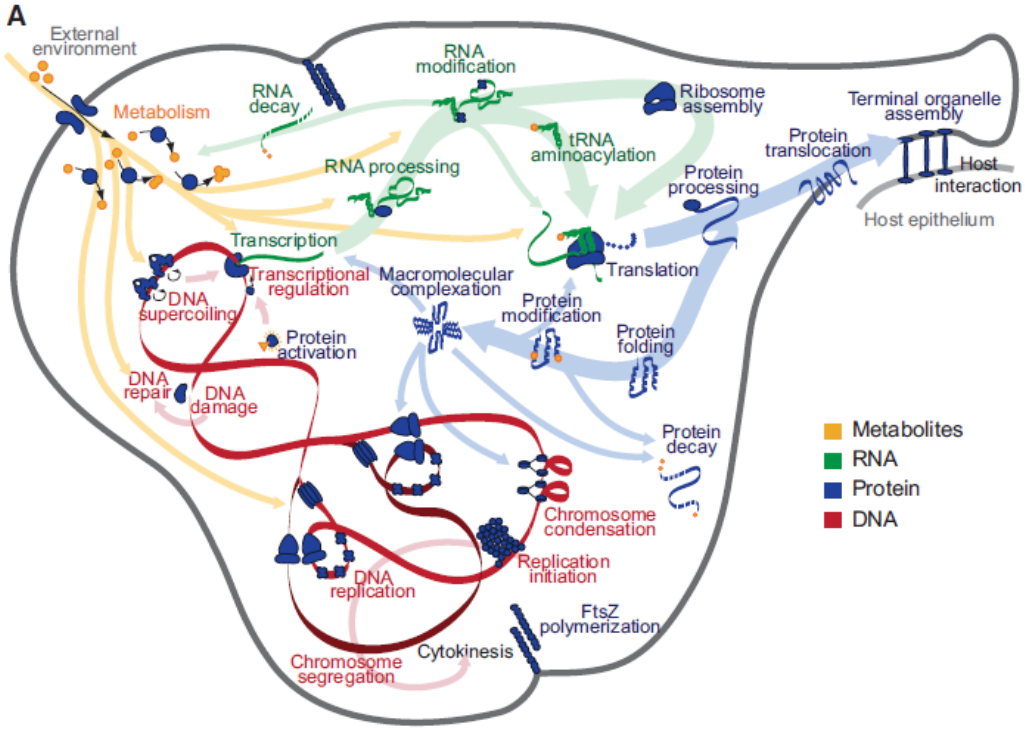
- Model has evolved into minimal, **functionally complete model** of chemoheterotrophic bacterium



Shuler *et al.* (2012), *Methods Mol. Biol.*, 881:573-610

Whole-cell model *M. genitalium*

- Metabolic networks are integrated with gene networks and signalling networks
  - Complex multi-level system with feedback across different time-scales

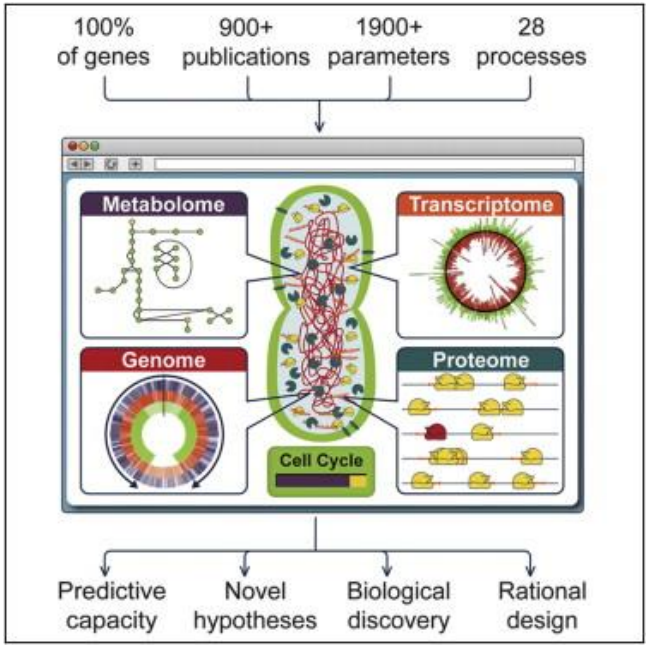


Whole-cell model of *Mycoplasma genitalium*

Karr *et al.* (2012), *Cell*, 150(2): 389-401

Whole-cell model *M. genitalium*

- Whole-cell model represents huge modelling effort:
  - Whole-genome model including **complete** known metabolic, gene, and signalling networks



Karr *et al.* (2012), *Cell*, 150(2): 389-401

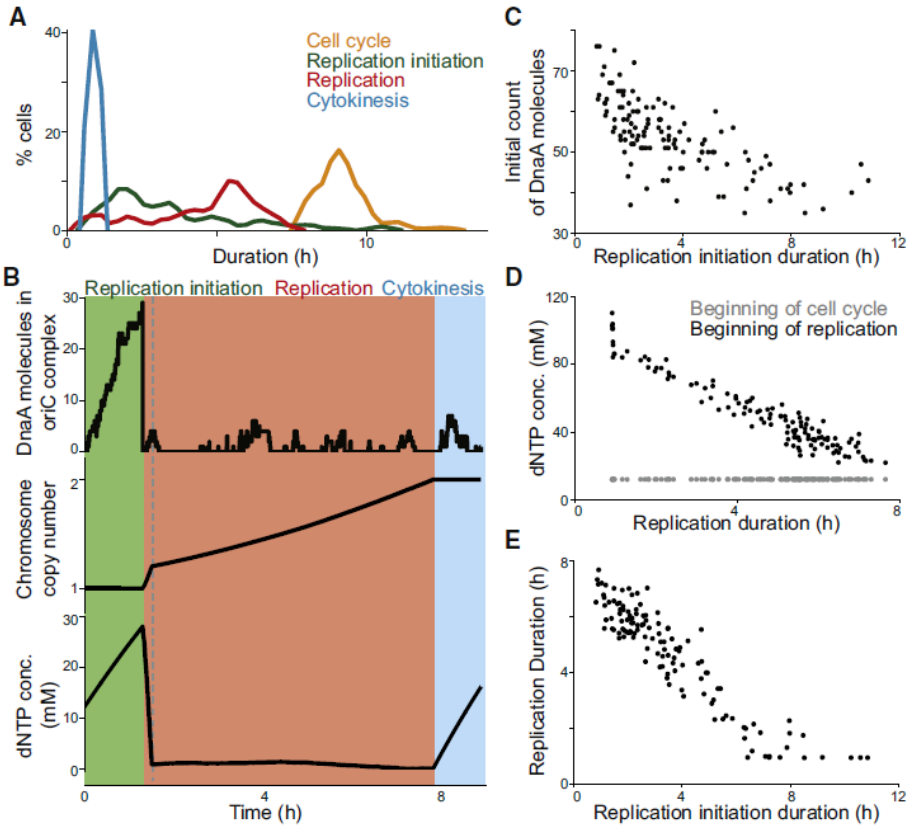
  - Variety of **formalisms** to model the 28 modules: FBA, kinetic ODE models, Boolean models, Markov chains, …
  - Cell cycle simulated for >100 cells, >30 mutants on 128-core machine

Whole-cell model *M. genitalium*

- Whole-cell simulation of *M. genitalium* cell cycle

Whole-cell model *M. genitalium*

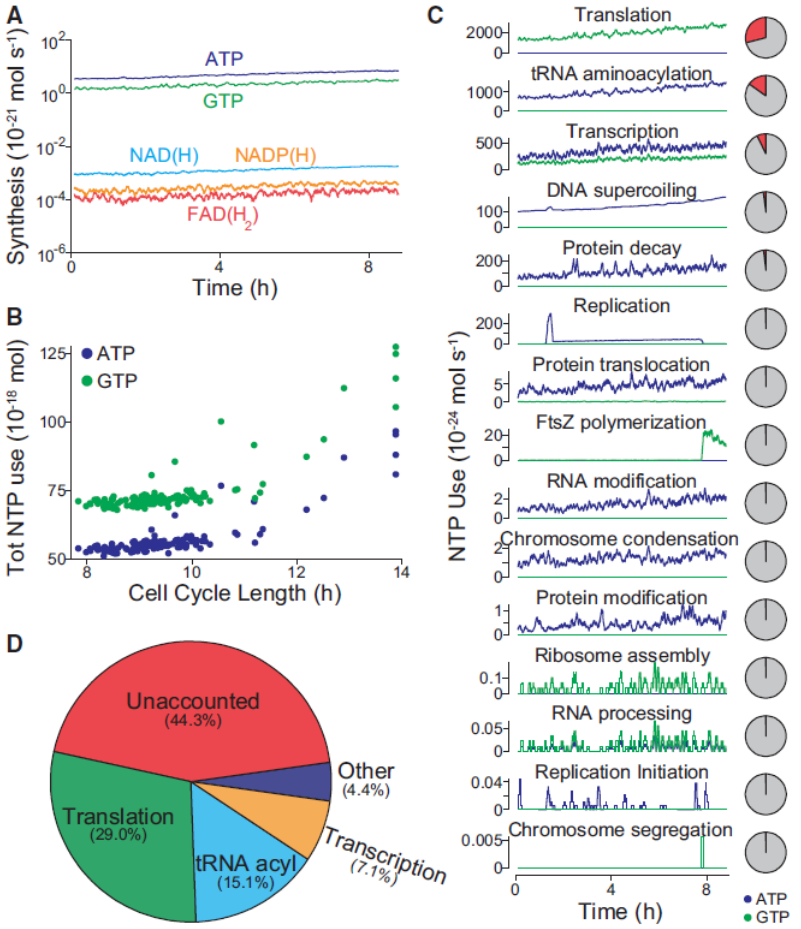
- Whole-cell simulations have provided new insights into **robustness of cell-cycle duration**
  - High variability of replication initiation buffered by dNTP-dependent duration of replication
  - This metabolic control of replication leads to decreased variability of cell-cycle length



Karr *et al.* (2012), *Cell*, 150(2): 389-401

Whole-cell model *M. genitalium*

- Whole-cell simulations have provided new insights into **global use and allocation of energy**
  - Transcription and translation most costly processes
  - Energy use largely independent of cell-cycle length
  - Usage of almost half of produced energy not accounted for!



Karr *et al.* (2012), *Cell*, 150(2): 389-401

Large-scale integrated models: conclusions

- Large-scale integrated models help analyze the dynamics of interactions between multiple functions of the cell
  - Models allow predictions to be confronted with experimental data and performance of thought experiments
- But large-scale integrated models have problems as well!
  - Models **difficult to construct**, to debug and to maintain
  - Huge **number of parameters**, many unknown: parameter estimation is a difficult problem requiring many data of high quality
  - How do we **extract fundamental insights** on cell functioning from large, mechanistic models?

Large-scale integrated models: conclusions

- Large-scale integrated models help analyze the dynamics of interactions between multiple functions of the cell
  - Models allow predictions to be confronted with experimental data and performance of thought experiments
- But large-scale integrated models have problems as well!

On Exactitude in Science

Jorge Luis Borges, *Collected Fictions*, translated by Andrew Hurley.

…In that Empire, the Art of Cartography attained such Perfection that the map of a single Province occupied the entirety of a City, and the map of the Empire, the entirety of a Province. In time, those Unconscionable Maps no longer satisfied, and the Cartographers Guilds struck a Map of the Empire whose size was that of the Empire, and which coincided point for point with it. The following Generations, who were not so fond of the Study of Cartography as their Forebears had been, saw that that vast Map was Useless, and not without some Pitilessness was it, that they delivered it up to the Inclemencies of Sun and Winters. In the Deserts of the West, still today, there are Tattered Ruins of that Map, inhabited by Animals and Beggars; in all the Land there is no other Relic of the Disciplines of Geography.

—Suarez Miranda, *Viajes de varones prudentes*, Libro IV, Cap. XLV, Lerida, 1658

Resource allocation models

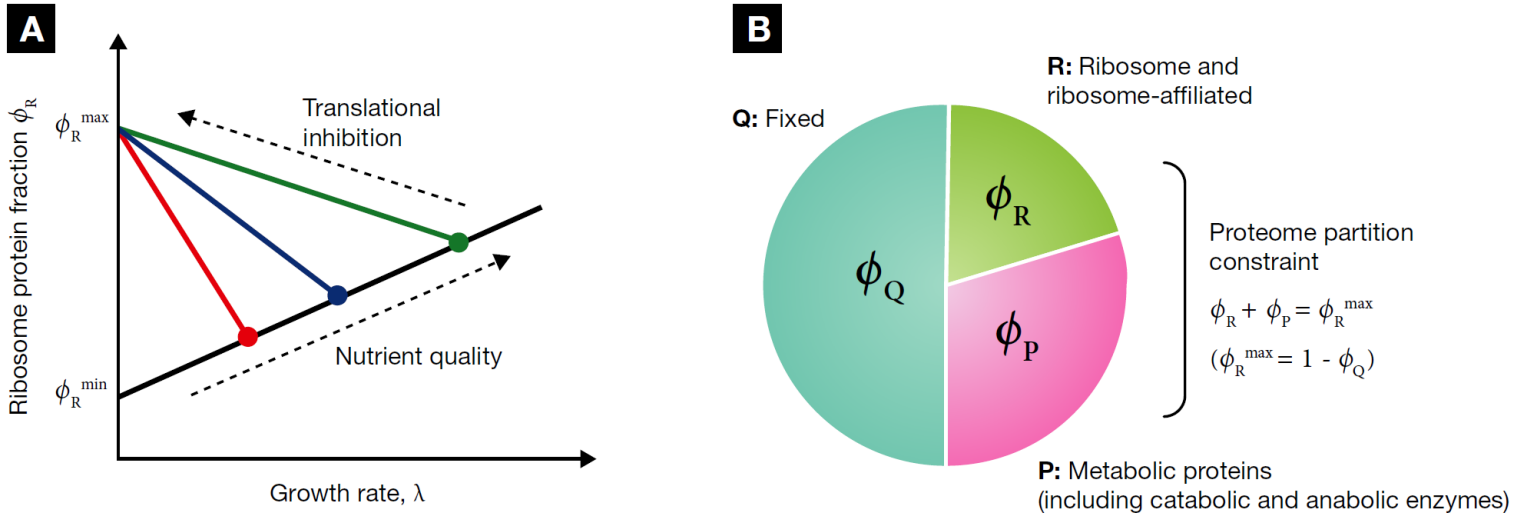
- Difficulties encountered with large-scale integrated models have motivated **simplified models**
- Example of simplified models: **resource allocation models**
- Reorganization of gene expression in response to changes in environment is **resource allocation problem**

  How does cell distribute available resources over cellular functions?

Resource allocation in bacteria

- Empirical **growth laws** quantify resource allocation in bacteria
  - Different protein categories
  - Mass fraction (at steady state) is growth-rate dependent
  - Mass fraction (at steady state) varies with translation capacity

Scott *et al.* (2014), *Mol. Syst. Biol.*, 10:747



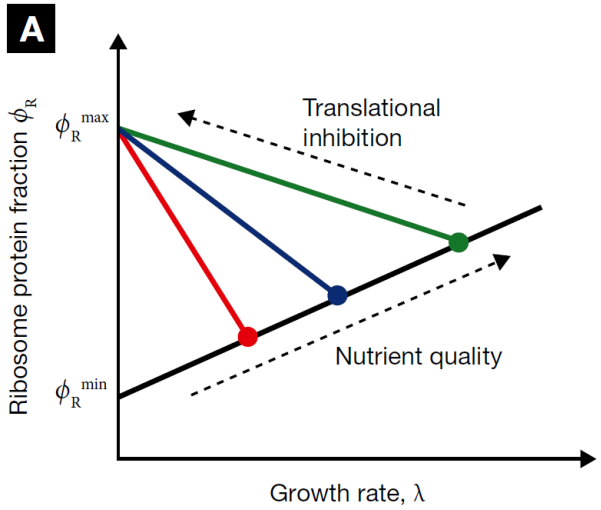
Resource allocation in bacteria

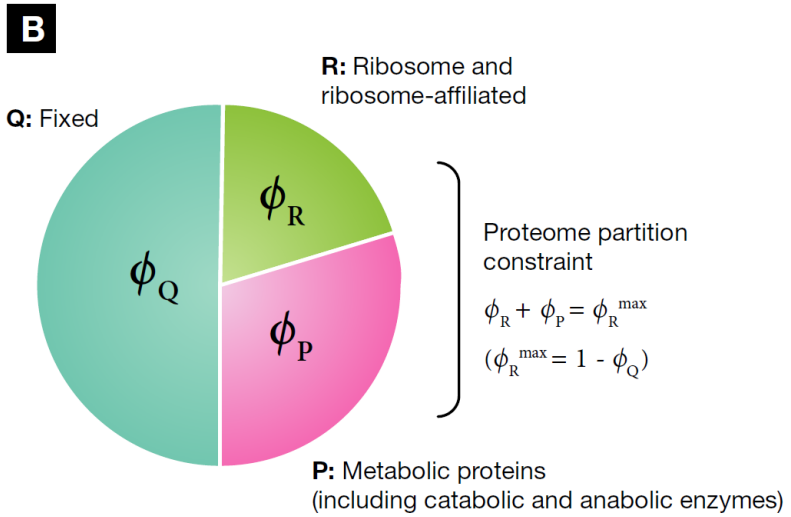
- Empirical **growth laws** quantify resource allocation in bacteria

$$\phi_R = \phi_R^{min} + \frac{\lambda}{\gamma} \qquad \phi_R = \phi_R^{max} - \frac{\lambda}{\nu}$$

  - Translation efficiency γ
  - Nutritional efficiency ν
  - Growth rate λ

Scott *et al.* (2014), *Mol. Syst. Biol.*, 10:747





Resource allocation in bacteria

- Empirical **growth laws** quantify resource allocation in bacteria

$$\phi_R = \phi_R^{min} + \frac{\lambda}{\gamma} \qquad \phi_R = \phi_R^{max} - \frac{\lambda}{\nu}$$

- Which mechanisms underlie these growth laws?
- Resource allocation and growth laws can be studied using coarse-grained **self-replicator models**

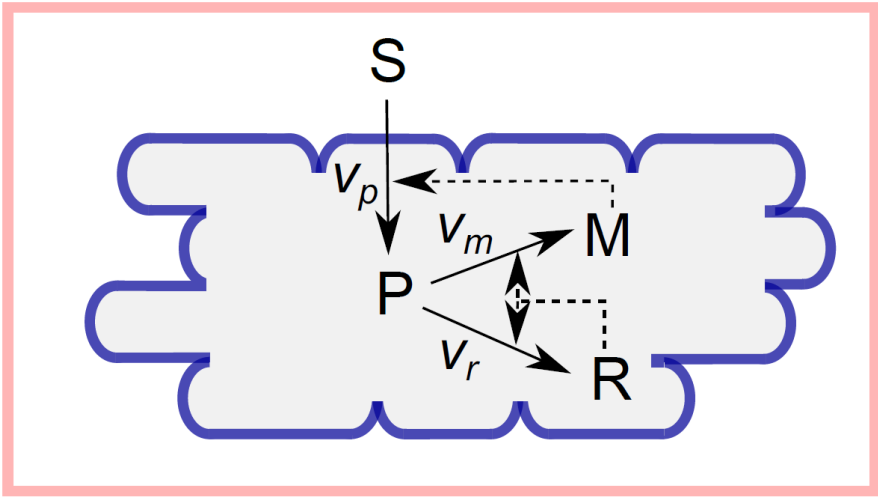
Molenaar *et al.* (2009), *Mol. Syst. Biol.*, 5:323

Hinshelwood (1952), *J. Chem. Soc. (Res.)*, 745-55

Self-replicator model of bacterial growth

- Reorganization of gene expression in response to changes in environment is **resource allocation problem**
- Resource allocation in bacteria can be studied using coarse-grained **self-replicator models**

S: substrate
P: precursor metabolites
M: metabolic machinery (enzymes)
R: gene expression machinery (ribosomes)



Giordano *et al.* (2016), *PLoS Comput. Biol.,* 12(3): e1004802

Self-replicator model of microbial growth

- Model of self-replicator falls within modeling framework developed above

$$\begin{bmatrix} \dot{p} \\ \dot{r} \\ \dot{m} \end{bmatrix} = \begin{bmatrix} n_p & -n_r & -n_m \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \cdot \begin{bmatrix} v_p(m,s) \\ v_r(r,p) \\ v_m(r,p) \end{bmatrix} - \mu \cdot \begin{bmatrix} p \\ r \\ m \end{bmatrix},$$

$$\dot{s} = -\delta \cdot \alpha_s \cdot v_p(m,s) \cdot b,$$

$$\mu = \delta \cdot \alpha_p \cdot n_p \cdot v_p(m,s),$$

$$\dot{b} = \mu \cdot b,$$

Giordano *et al.* (2016), *PLoS Comput. Biol.*, 12(3): e1004802

de Jong *et al.* (2017), *J. Roy. Soc. Interface*, 14:20170502

Self-replicator model of microbial growth

- Model of self-replicator falls within modeling framework developed above
- Rate equations
  - Definition of total protein synthesis rate $v_{ps} = n_r \cdot v_r + n_m \cdot v_m$
  - Rate equations:

$$v_{ps}(p, r) = k_r \cdot r \cdot \frac{p}{p + K_r},$$

$$v_p(s, m) = k_m \cdot m \cdot \frac{s}{s + K_m},$$

- Resource allocation parameter α

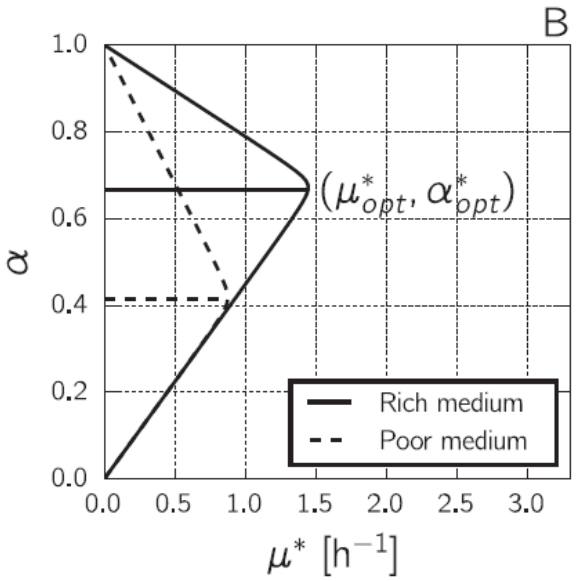
$$n_r \cdot v_r = \alpha \cdot v_{ps}$$

$$n_m \cdot v_m = (1 - \alpha) \cdot v_{ps}$$

$$0 \leq \alpha \leq 1$$

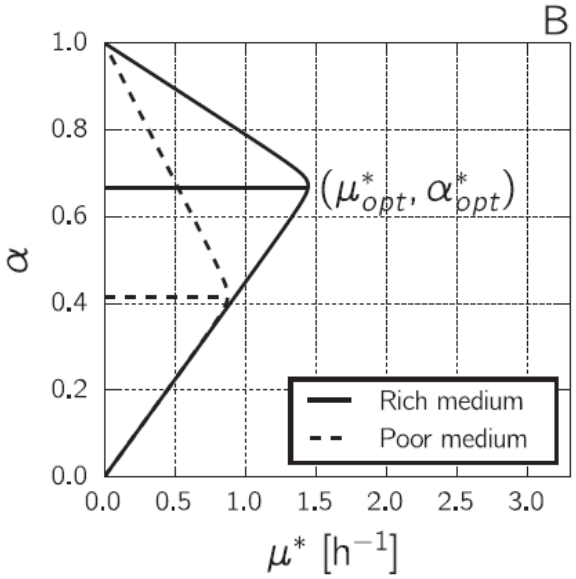
Self-replicator model and growth laws

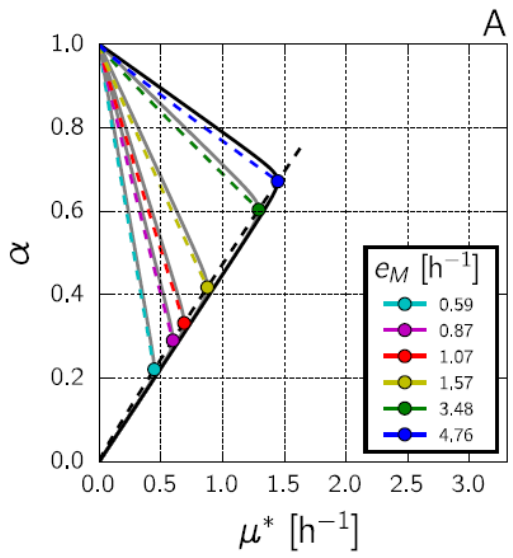
- Self-replicator model reproduces steady-state growth laws under assumption of growth-rate maximization
  - Reasonable parameter values from literature

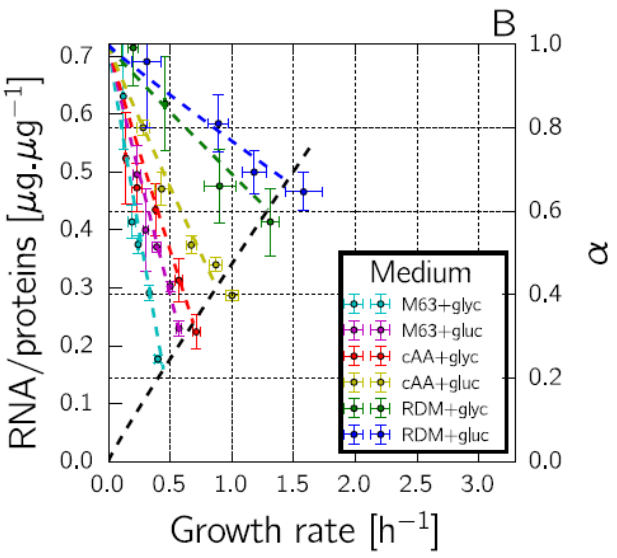


Self-replicator model and growth laws

- Self-replicator model reproduces steady-state growth laws under assumption of growth-rate maximization
  - Reasonable parameter values from literature
  - RNA/protein fraction proxy for resource allocation parameter α



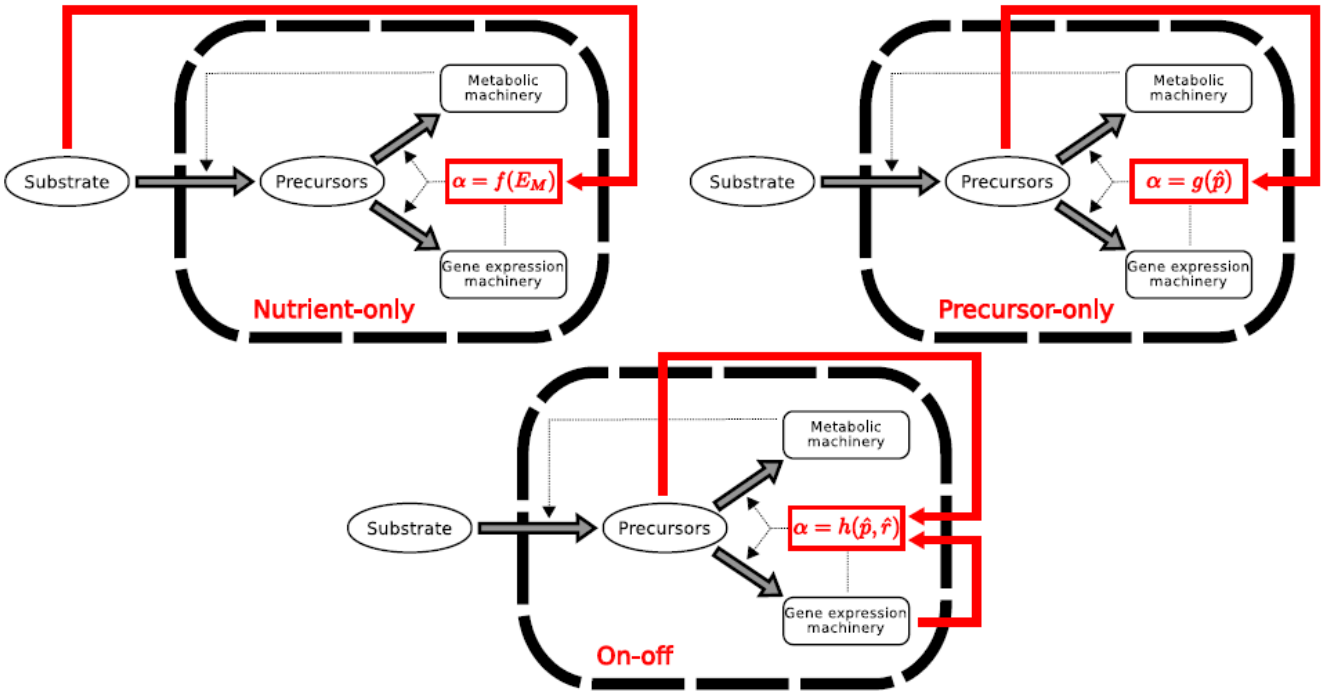




Scott *et al.* (2010), *Science,* 330(6007):1099-102

Feedback growth control strategies

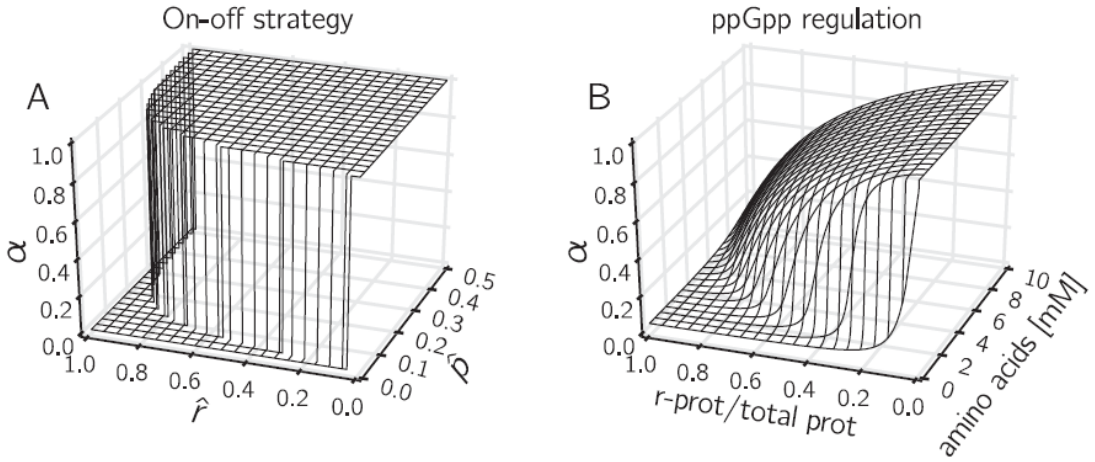
- Which mechanisms allow bacteria to adapt resource allocation over various environments?
- Different strategies can implement **feedback growth control**
  - Exploit information on system variables and/or environment



Feedback growth control strategies

- **On-off control strategy** maintains balance between precursors and gene expression machinery at all times
- On-off strategy resembles ppGpp regulation in bacteria
  - Effect of ppGpp regulation derived from kinetic model of ppGpp system

Bosdriesz *et al.* (2015), *FEBS J.*, 282:209-



Conclusions

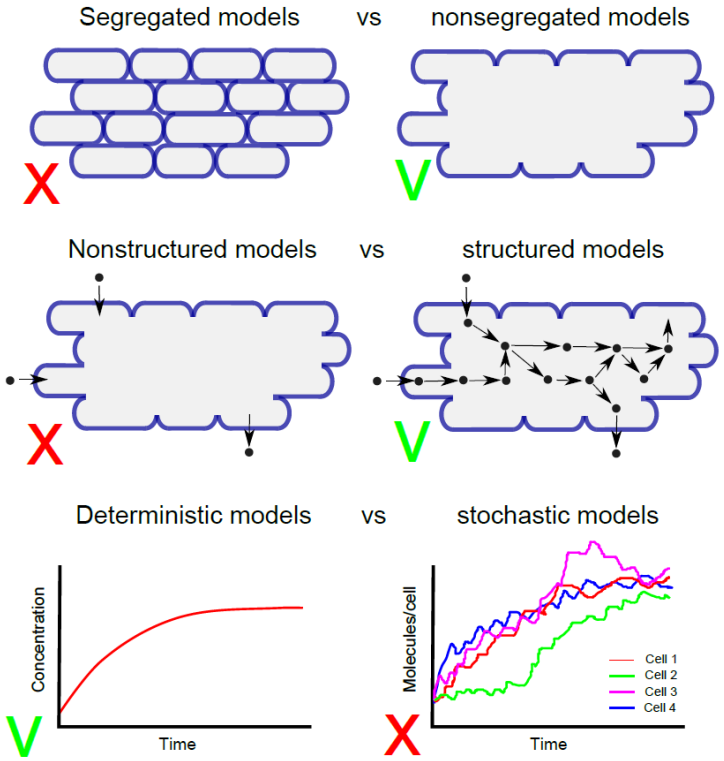
- Adaptation of bacteria to their environment involves reorganisation of cellular physiology
- Increasingly powerful methods have become available to experimentally quantify cellular adaptation
  - Transcriptomics, proteomics, fluxomics, metabolomics, …
- Adaptation process achieved by large and complex regulatory networks
  - Nonlinear dynamical systems with feedback across different time-scales
- Fundamental questions on network functioning remain unanswered and require integrated models of the cell
  - Multiple functions, multiple regulatory levels, interactions with environment and ecosystem, …

Conclusions

- Several approaches have been tried to develop and exploit integrated models of the cell
  - Flux balance models
  - Kinetic models of cellular functions: towards whole-cell models
  - Resource allocation models
- Issues for development of such models:
  - Scope
  - Granularity
  - Mathematical methods
  - …

Conclusions

- Modeling framework comes with number of fundamental assumptions



- Most importantly, models are tools for a purpose: **a different model for a different question**

Most fundamental questions are still open

- How does the multi-level **feedback structure** of the network give rise to **dynamical properties** of adaptive response?

  Can we formulate **general laws** that explain a variety of phenomena on the molecular level?

- How does repertoire of dynamical properties of the cell respond to **challenges from ecosystem**?

  Why have these properties been **evolutionary conserved** in environment?

  How do bacterial cells cooperate and evolve in **consortia of microorganisms**?

Merci !

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