Microbial growth control in changing environments

Theoretical and experimental study of resource allocation in *Escherichia coli*

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PhD Thesis Defense
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**Microbial growth**

Cell composition is a resource allocation problem

Blount, *eLife* 2015
RESOURCE ALLOCATION OBEYS GROWTH LAWS

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Why such regularities?

Scott et al, Science 2010
Growth laws are explained if microorganisms maximize their growth rate.

Growth laws result from a balance between supply and demand of precursors.

GROWTH LAWS WERE ESTABLISHED AT BALANCED GROWTH

(B) Balanced growth

- Exponential growth \( \left( \frac{dB}{dt} = \mu B \right) \)
- Steady state \( \left( \frac{dx}{dt} = 0 \right) \)
- Experimentally and theoretically convenient
Growth laws were established at balanced growth

(B) Balanced growth

- Exponential growth \( \left( \frac{dB}{dt} = \mu B \right) \)
- Steady state \( \left( \frac{dx}{dt} = 0 \right) \)
- Experimentally and theoretically convenient

Growth laws were established for laboratory conditions that are seldom encountered in nature
No growth laws for changing conditions

Steady-state conditions

Environment

Resource allocation

poor rich
No growth laws for changing conditions

Steady-state conditions
- Poor environment
- Rich environment

Dynamical conditions
- Poor environment
- Rich environment
No growth laws for changing conditions

Transitions are more difficult to study
**Problem Statement**

How do microorganisms dynamically reallocate their resources after a change in the environment?
**APPROACH**

Theoretical approach
What is the optimal way to dynamically allocate resources during a growth transition?
- What is the optimal resource allocation strategy?
- Can the strategy be linked to known molecular mechanisms?

Experimental approach
Do bacteria implement the theoretically optimal strategy of resource allocation?
- Measure resource allocation during a transition
- Compare with the optimal strategy
THEORETICAL APPROACH

Dynamical Allocation of Cellular Resources as an Optimal Control Problem: Novel Insights into Microbial Growth Strategies

Collaborators

► Francis Mairet (Inria Sophia-Antipolis Méditerranée, project-team Biocore)
► Jean-Luc Gouzé (Inria Sophia-Antipolis Méditerranée, project-team Biocore)

Published in Giordano et al, PLoS Comput Biol 2016
**SELF-REPLICATOR MODEL OF RESOURCE ALLOCATION**

Two biochemical (macro)reactions:

**Metabolism:** \( S \xrightarrow{V_M} P \)

**Macromolecule synthesis:** \( P \xrightarrow{V_R} \alpha R + (1 - \alpha)M \)
**TWO-DIMENSIONAL DYNAMICAL SYSTEM**

Precursors: \[
\frac{dP}{dt} = V_M - V_R
\]

GEM: \[
\frac{dR}{dt} = \alpha \cdot V_R
\]
Two-dimensional dynamical system

Precursors: \[ \frac{dp}{dt} = \nu_M - \nu_R - \mu \cdot p \]

GEM: \[ \frac{dr}{dt} = \alpha \cdot \nu_R - \mu \cdot r \]

Assuming...

Volume: \( V_{\text{ol}} = \beta (M + R) \) \Rightarrow Growth rate: \( \mu = \beta \frac{V_R}{V_{\text{ol}}} = \beta \nu_R \)

Michaelis-Menten kinetics \Rightarrow \( \nu_R = \frac{k_R \cdot p}{K_R + p} \cdot r \)

\( \nu_M = e_M \cdot (1/\beta - r) \)

How does the cell choose the resource allocation parameter \( \alpha \)?
MODEL PREDICTS THE STEADY-STATE GROWTH LAWS

Data from Scott et al, Science, 2010
MODEL PREDICTS THE STEADY-STATE GROWTH LAWS

Data from Scott et al, Science, 2010
MODEL PREDICTS THE STEADY-STATE GROWTH LAWS

![Graph showing the relationship between α and μ* for different values of e_M (h⁻¹). The graph includes data points for e_M = 0.59, 0.87, 1.07, 1.57, 3.48, and 4.76.]

Data from Scott et al, Science, 2010
MODEL PREDICTS THE STEADY-STATE GROWTH LAWS

Choosing the optimal $\alpha$ for each environment predicts the empirical growth laws

Data from Scott et al, Science, 2010
**GROWTH MAXIMIZATION DURING TRANSITIONS**

New objective: maximize biomass produced during an environmental transition

\[ J(\alpha) = \int_0^\tau \mu(t, \hat{p}, \hat{r}, \alpha) \, dt \]

[Diagram A: \( \hat{r} \) vs. \( \hat{p} \) with an optimal point marked.]

[Diagram B: \( t \) vs. \( E_M \) with a transition phase highlighted.]
**GROWTH MAXIMIZATION DURING TRANSITIONS**

**New objective:** maximize biomass produced during an environmental transition

\[ J(\alpha) = \int_{0}^{\tau} \mu(t, \hat{p}, \hat{r}, \alpha) \, dt \]

**Optimal solution:** bang-bang-singular strategy
**DIFFERENT CLOSED-LOOP CONTROL STRATEGIES FOR RESOURCE ALLOCATION**

All closed-loop control strategies are optimal at steady state.
Performance of control strategies during growth transition

Equivalent strategies at steady state produce different outcomes in dynamical conditions
**Which strategy is closer to the actual regulatory mechanisms?**

The ppGpp regulatory system in *E. coli* (Bosdriesz et al, 2015)...

... is a likely candidate
WHAT WE HAVE LEARNED SO FAR

- Bang-bang resource allocation maximizes the biomass produced during a nutrient upshift
- A dynamical study uncovers differences between regulatory strategies that are equivalent at steady state
- Complex regulations are beneficial during transitions
- The ppGpp system might be an efficient way for the cell to achieve quasi-optimal resource allocation
**EXPERIMENTAL APPROACH**

**Dynamics of Resource Allocation in *E. coli* During an Acetate-Glucose Upshift**

**Collaborators**

- Irina Mihalcescu (LIPhy, Université Grenoble Alpes, team BIOP)
- Eugenio Cinquemani (Inria Grenoble – Rhône-Alpes, project-team Ibis)
EXPECTED OPTIMAL BEHAVIOR

Steady-state conditions

Dynamical conditions

Resource allocation

Environment

poor rich

poor environment rich environment

Time

▶ Rapid regulatory switches
→ high temporal resolution
▶ Probably not that stiff
→ extended observation times
▶ No reason bacteria will be synchronized
→ single-cell measurements
EXPECTED OPTIMAL BEHAVIOR

- Rapid regulatory switches
  → high temporal resolution
- Probably not that stiff
  → extended observation times
- No reason bacteria will be synchronized
  → single-cell measurements
**EXPERIMENTAL SETUP**

Quantification of gene expression machinery

- Fluorescent labeling of the RpsB subunit of the ribosome

- Isolated on the chromosome

- Growth not affected

- Integrated into ribosomes

Monitoring of single-cells during growth transition

- Microscopy and microfluidics (mother machine)

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**Strain construction**

Only *rpsB* is modified.
Pilot Experiment

- Acetate: 2.5 days (preculture)
- Acetate: 20 hours
- Glucose: 20 hours

Growth rate vs. Time [min]

Microscopy

Acetate

00:00
IMAGE ANALYSIS

- 6 fields, 15 channels = 90 lineages (68 exploitable)
- Segmentation of the cells at the bottom of the wells only (present for the entire experiment)
- Manual segmentation (selection of the 2 poles on the fluorescence images)

Raw image

Segmented image
RESULTS OF THE IMAGE ANALYSIS

Raw image

Segmented image

RFU/pixel/cell [a.u.]

Time [min]

Bacteria length [pixel]

X3Y2,W2

X3Y2,W2
RECONSTRUCTION OF THE GROWTH RATE AND RESOURCE ALLOCATION

Dynamical system

\[ \dot{r}(t) = \mu(t) \cdot \frac{\alpha(t)}{\beta} - \mu(t) \cdot r(t), \quad (1) \]
\[ \dot{V}(t) = \mu(t) \cdot V(t), \quad (2) \]

with initial conditions \( r(0) = r_0, V(0) = V_0 \)

Measurement model

\[ L(t_k) = \lambda \cdot V(t_k) + \epsilon_k, \quad (3) \]
\[ F(t_k) = \gamma \cdot r(t_k) + \eta_k, \quad (4) \]

at each time-point \( t_k, 0 \leq k \leq N - 1 \)

Problem: reconstructing \( \gamma \alpha(\cdot)/\beta \) and \( \mu(\cdot) \) from measurements \( \{F(t_0), ..., F(t_{N-1})\} \) and \( \{L(t_0), ..., L(t_{N-1})\} \)
RESULTS OF THE GROWTH-RATE RECONSTRUCTION

![Graphs showing bacterial length and growth rate over time.](image-url)
RESULTS OF THE GROWTH-RATE RECONSTRUCTION

[Diagram showing bacterial growth over time with data points and a trend line]
RECONSTRUCTION OF RESOURCE ALLOCATION ON SYNTHETIC DATA
RESULTS OF THE RECONSTRUCTION OF $\alpha(\cdot)$
RESULTS OF THE RECONSTRUCTION OF $\alpha(\cdot)$
The first oscillation in the resource allocation profile is conserved in all cells.
Still a lot to do...

We showed that:

- Dynamical resource allocation can be reconstructed via ribosome tagging and live imaging
- Kalman smoothing is convenient for such a reconstruction
- Oscillatory features are visible, but need to be confirmed

Further work should focus on:

- Long steady states before and after the upshift → crucial for calibrating the reconstruction algorithm
- More cells → for statistics, but automatic image analysis needed
- Other environmental changes, cross-validation, etc.
CONCLUSION

- Simple models are valuable for understanding fundamental principles of microbial growth
- Bang-bang regulatory scheme maximize biomass in dynamical conditions
- Complex regulation is only beneficial for unbalanced growth
- Known mechanisms of ribosome synthesis regulation (ppGpp) suggest bang-bang resource allocation during transitions
- Difficult to confirm experimentally, but preliminary results are encouraging
**PERSPECTIVE**

- Is there a fundamental relationship between the dynamics of the environment and the complexity of regulations?
- Can we apply this approach to maximize industrial production yields?
Thank you
CONTROL STRATEGIES CAN BE APPROXIMATED BY BIOLOGICALLY RELEVANT FUNCTIONS

\[ f(E_M) = \frac{E_M + \sqrt{K E_M}}{E_M + 2\sqrt{K E_M} + 1} \]

\[ g(\hat{p}) = \frac{\hat{p}^2}{0.06^2 + \hat{p}^2} \]

\[ g(\hat{p}) = \frac{\hat{p}}{\hat{p} + \frac{K}{K + \hat{p}}(1 + \hat{p})} \]
THE ON-OFF STRATEGY

\[ \alpha = h(\hat{p}, \hat{r}) = \begin{cases} 
0, & \text{if } \hat{r} > g(\hat{p}), \\
1, & \text{if } \hat{r} < g(\hat{p}), \\
\alpha_{opt}, & \text{if } (\hat{p}, \hat{r}) = (\hat{p}_{opt}, \hat{r}_{opt}). 
\end{cases} \]

with \[ g(\hat{p}) = \frac{\hat{p}}{\hat{p} + \frac{K}{K+\hat{p}} (1 + \hat{p})}. \]
Model predicts the steady-state growth laws

Choosing the optimal $\alpha$ for each environment predicts the empirical growth laws

From data in Scott et al, Science, 2010
RESULTS OF THE GROWTH-RATE RECONSTRUCTION (68 CELLS)
CELL CATEGORIES IDENTIFIED IN THE ANALYSIS

- Normal cells (N=45)
  - Growth rate for X3Y2,W2
- Pausing cells (N=11)
  - Growth rate for X1Y2,W2
- Dying cells (N=12)
  - Growth rate for X1Y1,W0
ROBUST STATISTICS FOR THE CELL CATEGORIES (GROWTH RATE)

[Graphs showing growth rate distributions for Normal, Pausing, and Dying cells over time.]
NOISE ESTIMATION (1/2)
Noise estimation (2/2)
NO GROWTH DIFFERENCE BETWEEN WT AND rpsB-TAGGED STRAINS
MATURATION / DEGRADATION

Data for rpsB-gfp

Best fit

Data for rpsB-mCherry

Best fit

Time after Cm addition [min]
COMPLETE ANALYSIS CELL 1

![Graphs showing data analysis](image)

- **Length [pixels]**
- **Growth rate [min⁻¹]**
- **RFU/pixel/cell [a.u.]**
- **γα/β [a.u.]**

The graphs illustrate the analysis of cell 1 over time, showing changes in length, growth rate, RFU/pixel/cell, and γα/β values.
COMPLETE ANALYSIS CELL 2

![Graphs showing changes in Length, Growth rate, RFU/pixel/cell, and γα/β over time.](image)
COMPLETE ANALYSIS CELL 3
COMPLETE ANALYSIS CELL 4

[Graphs showing different measurements over time: Length [pixels], Growth rate [min^-1], RFU/pixel/cell [a.u.], and γα/β [a.u.]].
COMPLETE ANALYSIS CELL 5