Resource allocation in microorganisms: Some control-theoretical problems

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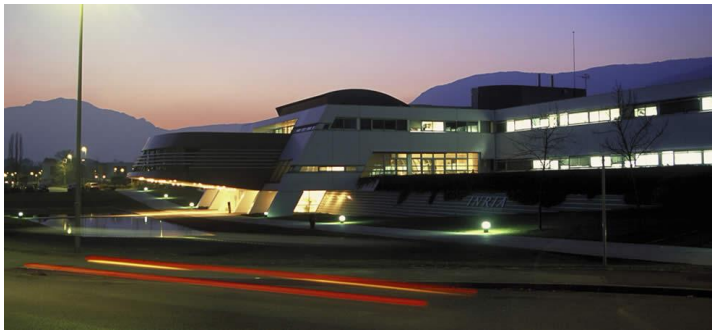
IBIS: bacterial systems biology

- **IBIS**: systems biology group at INRIA/Université Grenoble Alpes/CNRS in Grenoble

  Microbiologists, computer scientists, mathematicians, physicists, …

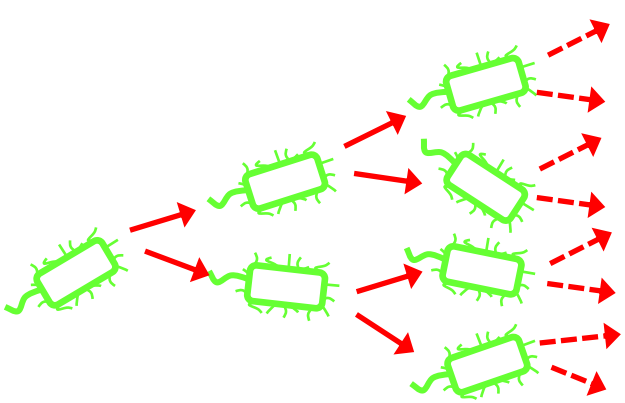
https://team.inria.fr/ibis/

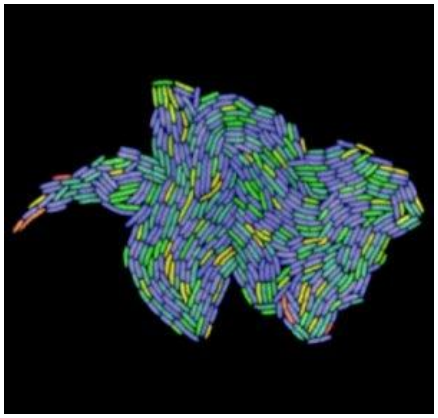


- **Objective:** understanding, predicting, and controlling the dynamics of regulatory networks in bacteria
  - Specific research problems shaped by **biological questions**
  - Problems often addressed by combination of **models and experiments**

Bacterial growth

- Bacteria are unicellular organisms geared towards **growth**
  E. coli cells have doubling times up to 20 min





Stewart *et al.* (2005), *PLoS Biol.*, 3(2): e45

- Changes in environment cause **adaptation** of growth rate, and more generally, change functioning of bacterial cell

Bacterial growth and gene expression

- Growth transitions involve changes in **gene expression**
  - Macromolecular composition of the cell

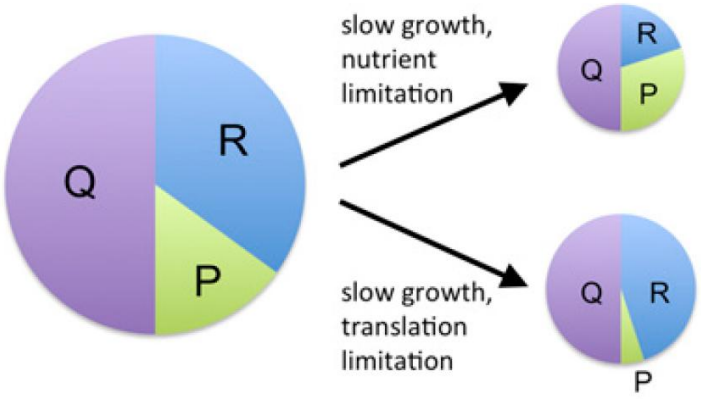
TABLE 2 Macromolecular composition of exponentially growing *E. coli* B/r as a function of growth rate at 37°C[a]

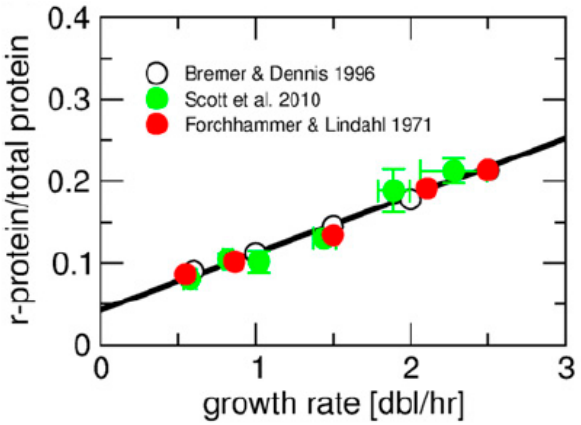
Parameter	Symbol	Units	At τ (min) and μ (doublings per h): τ, 100 μ, 0.6	τ, 60 μ, 1.0	τ, 40 μ, 1.5	τ, 30 μ, 2.0	τ, 24 μ, 2.5	Observed parameter(s)
Protein/mass	P_M	10^{17} aa/OD_{460}	6.5	5.8	5.2	5.1	5.0	*P, M*
RNA/mass	R_M	10^{16} nucl./OD_{460}	4.3	4.9	5.7	6.6	7.8	*R, M*
DNA/mass	G_M	10^{8} genomes/OD_{460}	18.3	12.4	9.3	8.0	7.6	*G, M*
Cell no./mass	C_M	10^{9} cells/OD_{460}	11.7	8.7	4.0	2.7	2.0	Cells/OD_{460}
$(P + R + G)/M$	PRD_M	μg/OD_{460}	149	137	129	131	136	
Protein/genome	P_G	10^{8} aa residues	3.5	4.7	5.6	6.3	6.6	P_M, G_M
RNA/genome	R_G	10^{7} nucl. residues	2.3	4.0	6.1	8.2	10.3	R_M, G_M
Origins/genome	O_G	Dimensionless	1.25	1.32	1.44	1.58	1.73	*C*
Protein/origin	P_O	10^{8} aa residues	2.8	3.6	3.9	4.0	3.8	P_G, O_G
Protein/cell	P_C	10^{8} aa residues	5.6	8.7	13.0	18.9	25.0	P_M, C_M
	P_C (μg)	μg/10^{9} cells	100	156	234	340	450	
RNA/cell	R_C	10^{7} nucl. residues	3.7	7.3	14.3	24.4	39.0	R_M, C_M
	R_C (μg)	μg/10^{9} cells	20	39	77	132	211	
DNA/cell	G_C	genome equiv./cell	1.6	1.8	2.3	3.0	3.8	*C, D*
	G_C (μg)	μg/10^{9} cells	7.6	9.0	11.3	14.4	18.3	
Mass/cell	M_C	OD_{460} units/10^{9} cells	0.85	1.49	2.5	3.7	5.0	C_M
	M_C (μg)	μg dry weight/10^{9} cells	148	258	433	641	865	μg/OD_{460}
Sum $P + R + G$	PRD_C	μg/10^{9} cells	127	204	322	486	679	P_G, R_G, G_C (in μg)
Origins/cell	O_C	no./cell	1.96	2.43	3.36	4.70	6.54	*C, D*
Termini/cell	T_C	no./cell	1.23	1.37	1.54	1.74	1.94	*D*
Replication forks/cell	F_C	no./cell	1.46	2.14	3.64	5.92	9.19	*C, D*

Bremer and Dennis (1996), *Escherichia Coli and Salmonella*, ASM Press, 1553-69

Bacterial growth and gene expression

- Growth transitions involve changes in **gene expression**
  - Macromolecular composition of the cell
  - Distribution of proteins over different categories

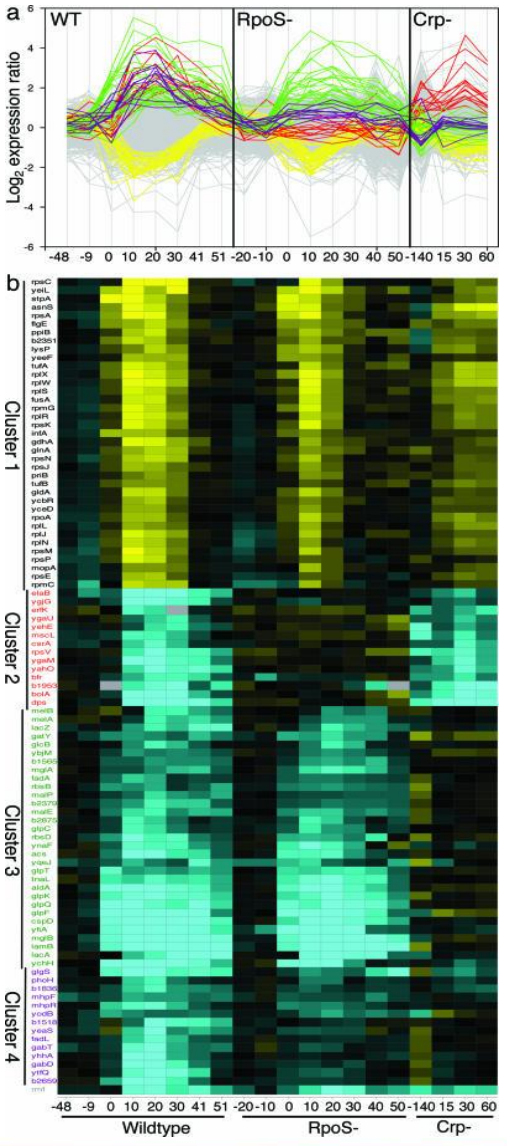




Klump *et al.* (2013), *Proc. Natl Acad. Sci. USA*, 110(42):16754-9

Bacterial growth and gene expression

- Growth transitions involve changes in **gene expression**
  - Macromolecular composition of the cell
  - Distribution of proteins over different categories
  - Expression of genes required for specific cellular functions



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

Overview

- **Adaptation of gene expression and growth as a dynamical resource allocation problem**

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

- Control of growth rate by reengineering of transcriptional control of RNA polymerase

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

Self-replicator model of bacterial growth

- Reorganization of gene expression in response to changes in environment is **resource allocation problem**
  - How does cell optimally distribute available resources over cellular functions?
  - For microorganisms, “optimal” often interpreted as “enabling maximum growth”
- Resource allocation in bacteria can be studied using simplified **self-replicator models**

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

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

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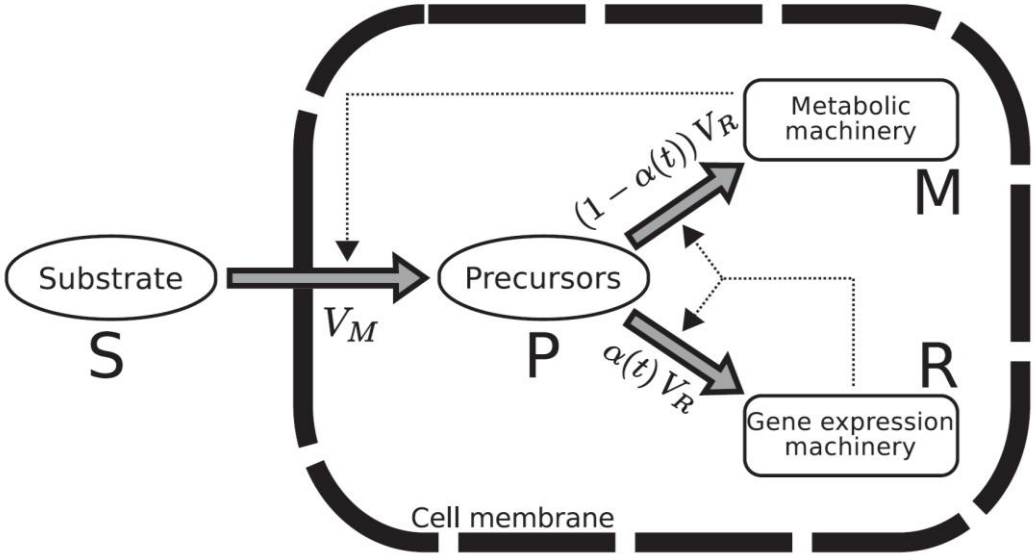

$$S \xrightarrow{V_M} P,$$

$$P \xrightarrow{V_R} \alpha R + (1-\alpha)M.$$

$$\mathrm{Vol} = \beta\,(M+R),$$

$$\mu = \frac{1}{\mathrm{Vol}}\frac{d\mathrm{Vol}}{dt}$$

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

Self-replicator model of bacterial growth

- Model of self-replicator

$$\frac{dp}{dt} = v_M(s,r) - v_R(p,r)\,(1+\beta\,p),$$

$$\frac{dr}{dt} = v_R(p,r)\,(\alpha(t) - \beta\,r),$$

$$r + m = 1/\beta$$

with

$$v_M(s,r) = k_M\,m\,\frac{s}{K_M+s} = k_M\,(1/\beta - r)\,\frac{s}{K_M+s},$$

$$v_R(p,r) = k_R\,r\,\frac{p}{K_R+p},$$

$$\mu = \frac{1}{\mathrm{Vol}}\frac{d\mathrm{Vol}}{dt} = \frac{1}{M+R}\frac{d(M+R)}{dt} = \beta\,v_R(p,r).$$

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

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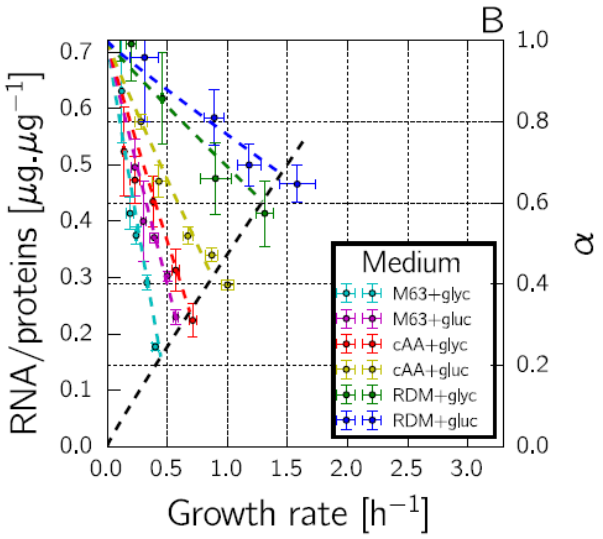
Self-replicator model and growth laws

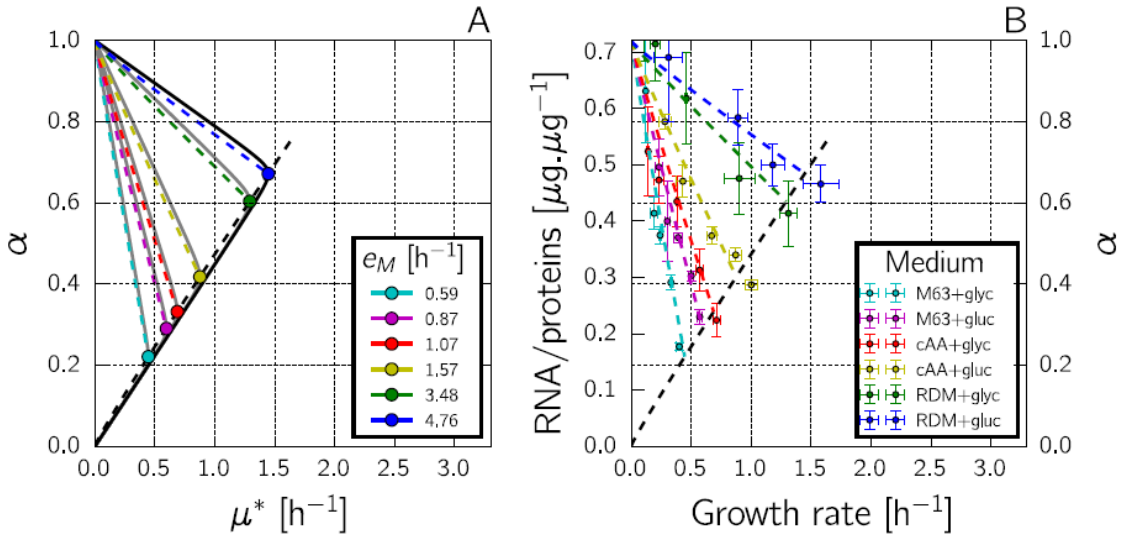
- Empirical **growth laws** show linear relation between growth rate and RNA/protein fraction at steady state
  - RNA/protein fraction proxy for resource allocation parameter α



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

Self-replicator model and growth laws

- Empirical **growth laws** show linear relation between growth rate and RNA/protein fraction at steady state
  - RNA/protein fraction proxy for resource allocation parameter α
- Self-replicator model reproduces steady-state growth laws under assumption of growth-rate maximization
  - Reasonable parameter values from literature



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

Dynamical adaptation of bacterial growth

- Bacteria rarely in steady state (constant environment) outside laboratory
- **Question**: what would be optimal resource allocation scheme in changing environment?

  Prototypical change in environment: nutrient upshift or downshift
- In framework of self-replicator, question can be formulated as **optimal control problem**

$$J(\alpha) = \int_0^\tau \mu(t)\, dt = \int_0^\tau \beta\, v_R(p, r)\, dt,$$

where α is a time-dependent function

Find $\alpha_{opt} = \arg\max_{\alpha \in \mathcal{U}} J(\alpha).$

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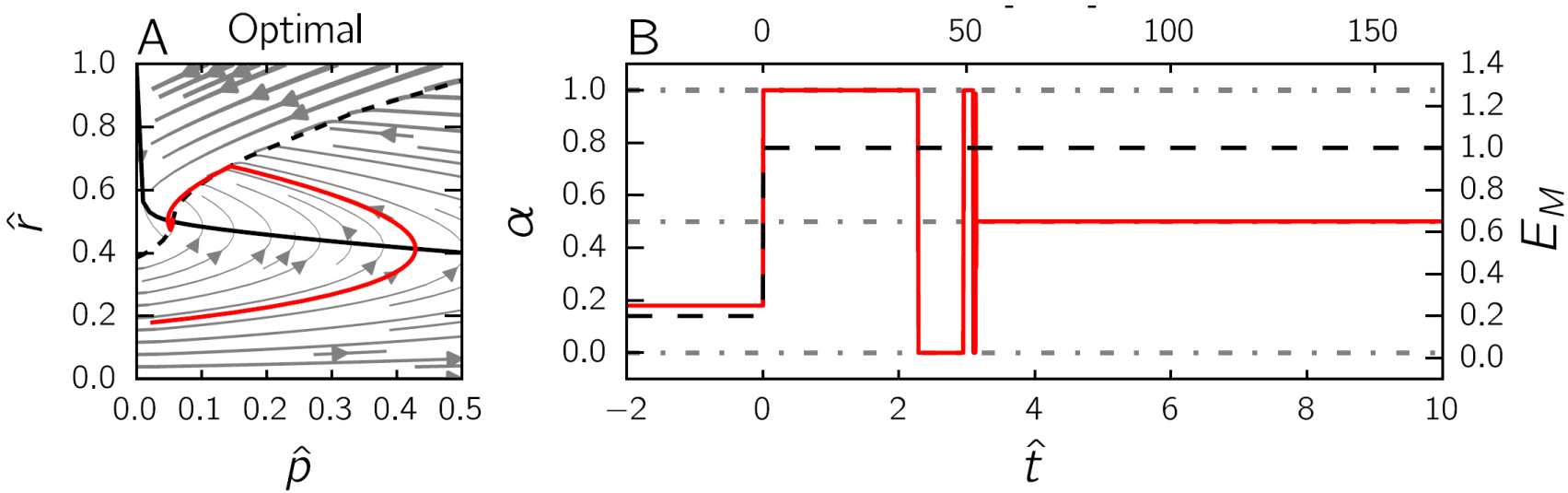
  - Prototypical change in environment: nutrient upshift or downshift
- In framework of self-replicator, question can be formulated as **optimal control problem**
- Optimal control problem can be solved using version of Pontryagin Maximum Principle
  - Nondimensionalized system, Infinite Horizon Maximum Principle, Kelley condition, chattering arc, switching curve, turnpike property

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

Dynamical adaptation of bacterial growth

- Optimal resource allocation scheme is **bang-bang singular**
  - Sequence of switches between $\alpha = 1$ (maximal synthesis of gene expression machinery) and $\alpha = 0$ (maximal synthesis of metabolic machinery)
  - α is then set to α^*_{opt}, value leading to maximal growth rate in new medium
  - Numerical solution using BOCOP



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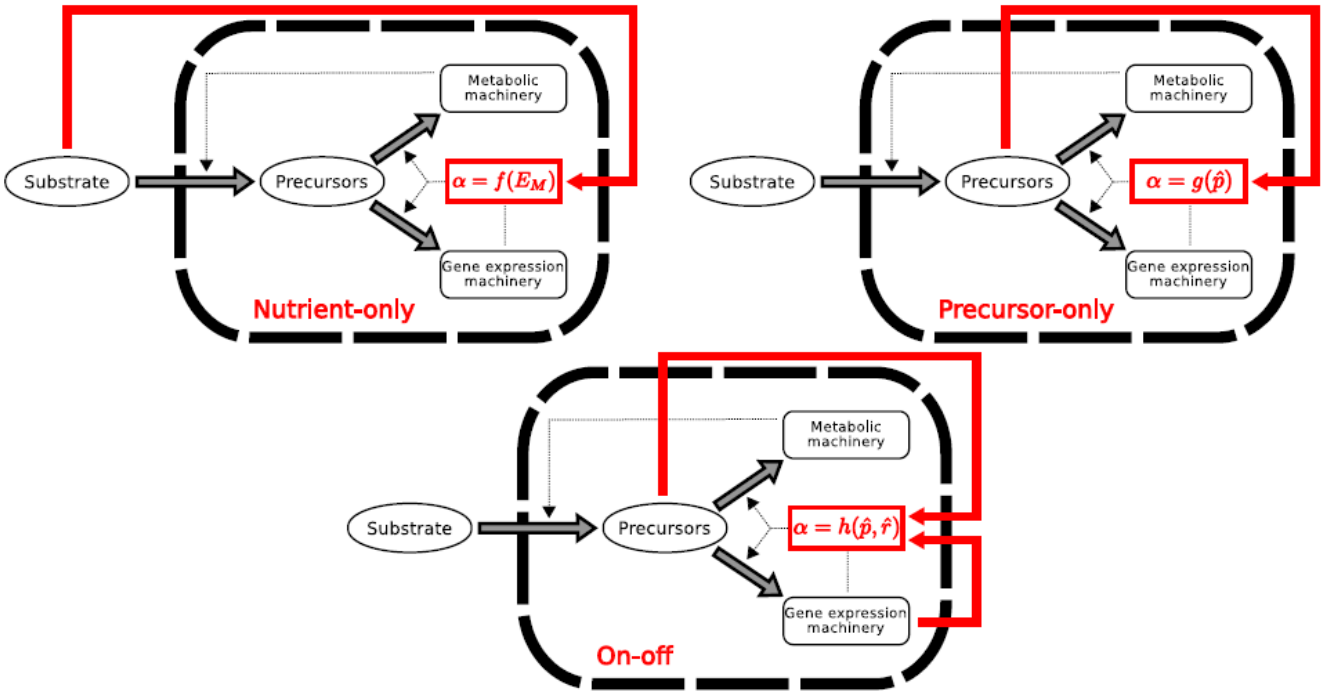
  - α is then set to α^*_{opt}, value leading to maximal growth rate in new medium
  - Numerical solution using BOCOP
- Optimal resource allocation scheme provides gold standard against which actual strategies can be compared

Feedback control strategies for growth-rate adaptation

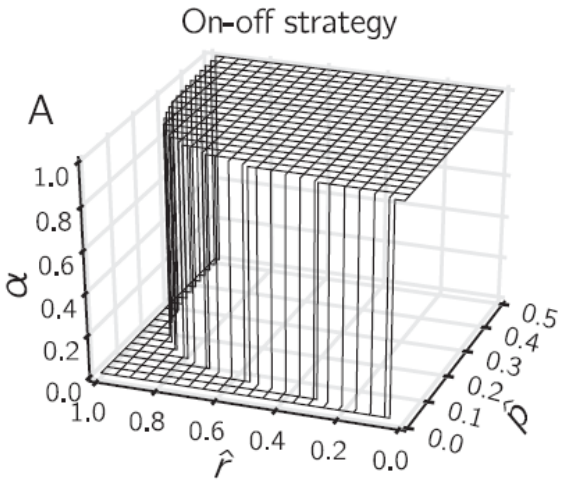
- Different strategies can implement **feedback growth control** for adapting resource allocation in response to changes in environment
  - Exploit information on system variables and/or environment

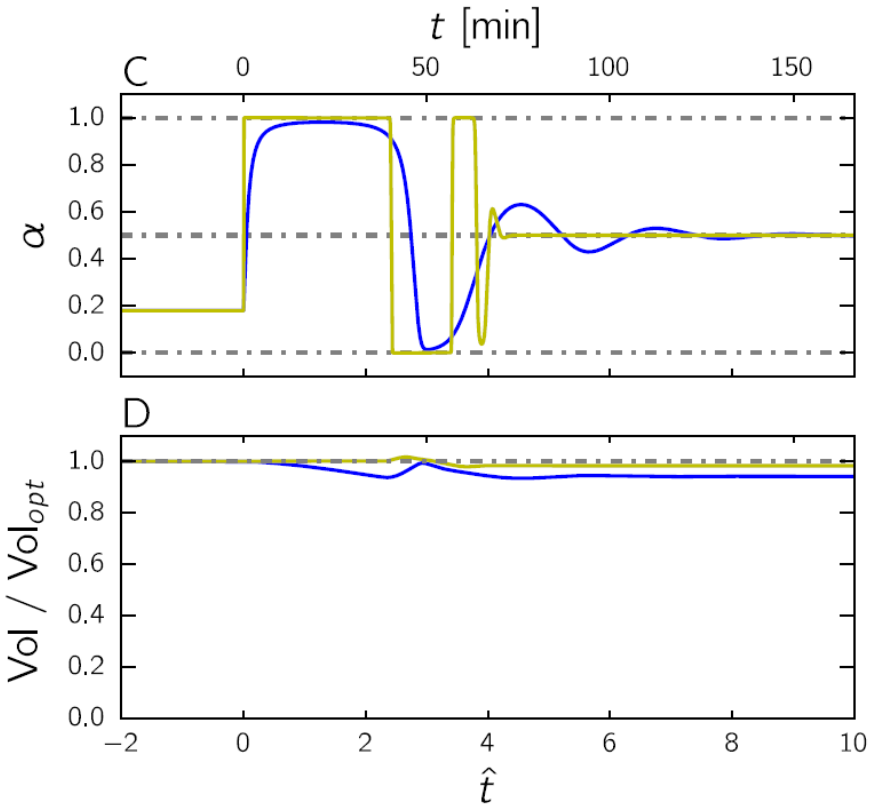


Feedback control strategies for growth-rate adaptation

- **On-off control strategy** leads to near-optimal behavior

  Drive self-replicator to optimal balance between precursors and gene expression machinery at all times

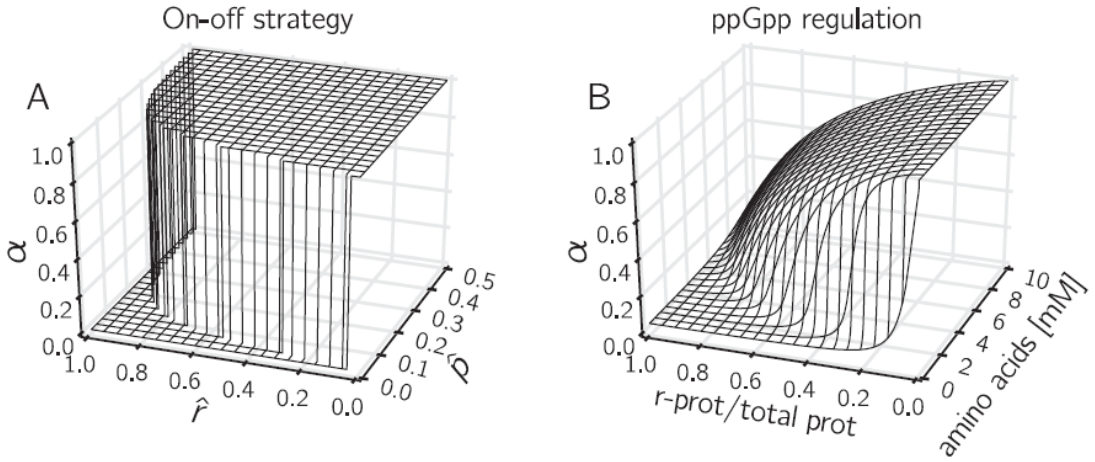




Feedback control strategies for growth-rate adaptation

- **On-off control strategy** leads to near-optimal behavior
  - Drive self-replicator to optimal balance between precursors and gene expression machinery at all times
- On-off strategy resembles effect of ppGpp regulation in bacteria
  - Effect of ppGpp regulation derived from kinetic model of ppGpp system

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



Experimental test of growth-rate adaptation

- Bang-bang schemes have been identified before in biology
  - Development of intestinal crypts: minimize time to attain mature crypt

    Itzkovitz *et al.* (2012), *Cell,* 148(3):608-19
- Some old data available in the literature consistent with bang-bang profiles after nutrient upshift in bacteria
  - Oscillatory patterns in ppGpp concentrations and ribosome synthesis rates
- However, low resolution and population-level measurements

Conclusions

- Bacterial growth can be profitably modeled by means of **self-replicators**
- Dynamic growth-rate adaptation in bacteria can be framed as **optimal control problem**
  - Predicted optimal scheme for growth-rate adaptation has bang-bang singular profile
- Ubiquitous ppGpp system has structural similarities with feedback control strategy approaching theoretical maximum
  - Sensing discrepancy between precursor/ribosome concentrations, adjust ribosome synthesis in on-off fashion
- Is predicted optimal resource allocation strategy observed experimentally?
  - Extension of self-replicators: cost of regulation, energy metabolism, …

Overview

- Adaptation of gene expression and growth as a dynamical resource allocation problem

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- **Control of growth rate by reengineering of transcriptional control of RNA polymerase**

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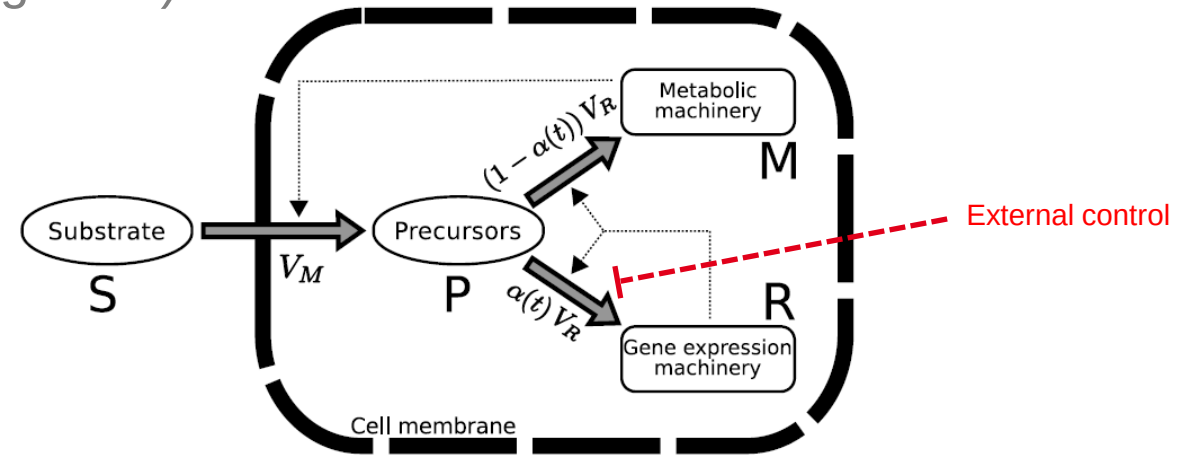
Control of gene expression machinery

- For biotechnological applications, one would like to **change** the natural resource allocation strategies of the cell

  de Jong *et al.* (2017), *Trends Microbiol.*, 25(6):480-93

- Can **control of gene expression machinery** be harnessed for this purpose?
  - Global reallocation of resources through arrest of gene expression machinery (and thus growth)



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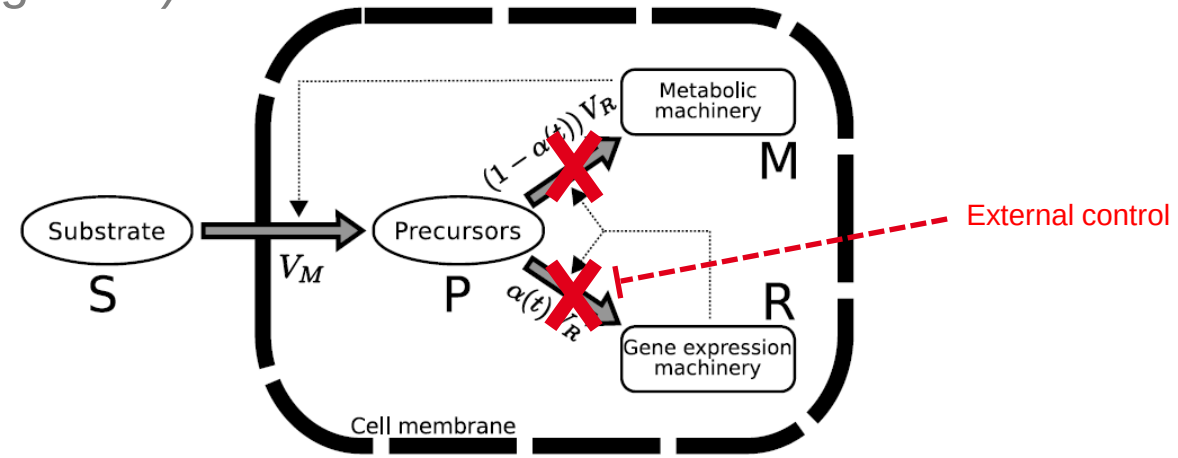

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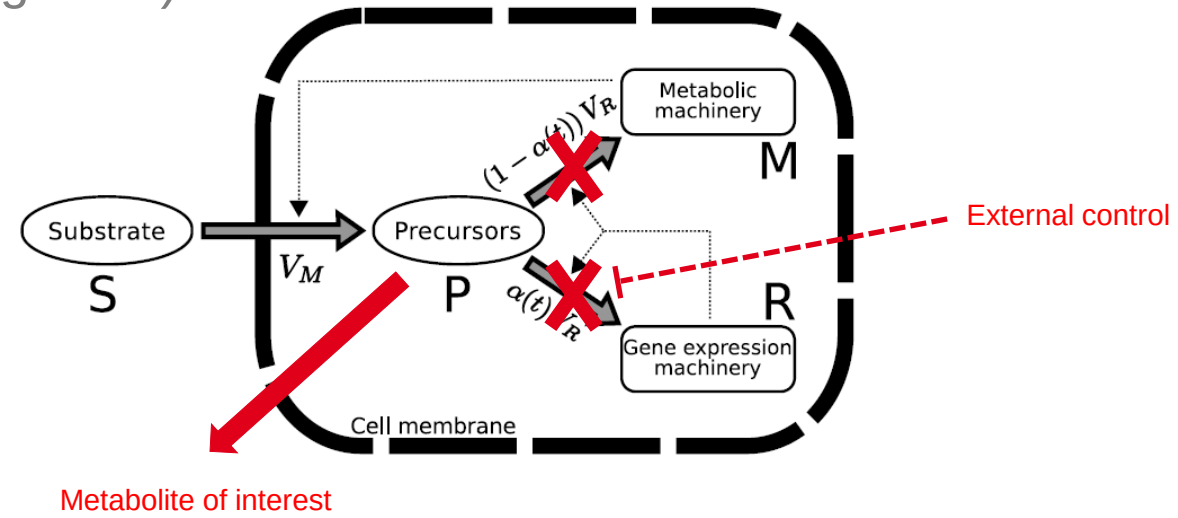

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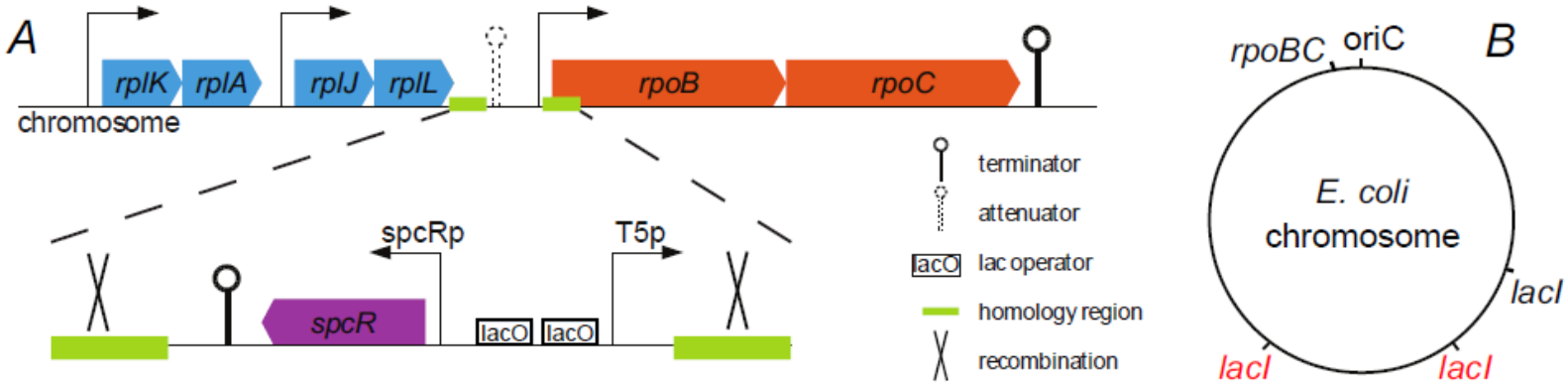
- Can **control of gene expression machinery** be harnessed for this purpose?
  - Global reallocation of resources through arrest of gene expression machinery (and thus growth)
  - Restart gene expression machinery when enzymes have been degraded

- Does this approach work?

Reengineering of gene expression machinery

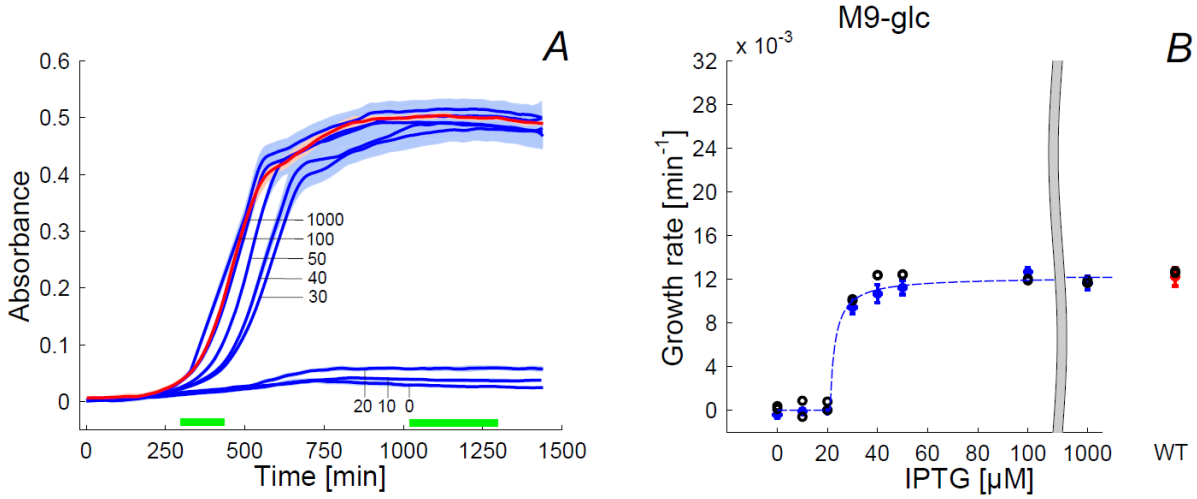
- External control of expression of **RNA polymerase**
  - Transcription of *rpoBC* operon (encoding ββ' subunits) controlled by IPTG-inducible promoter



  - Several copies of *lacI* on chromosome

Synthetic growth switch

- Reengineering of GEM results in **growth switch**



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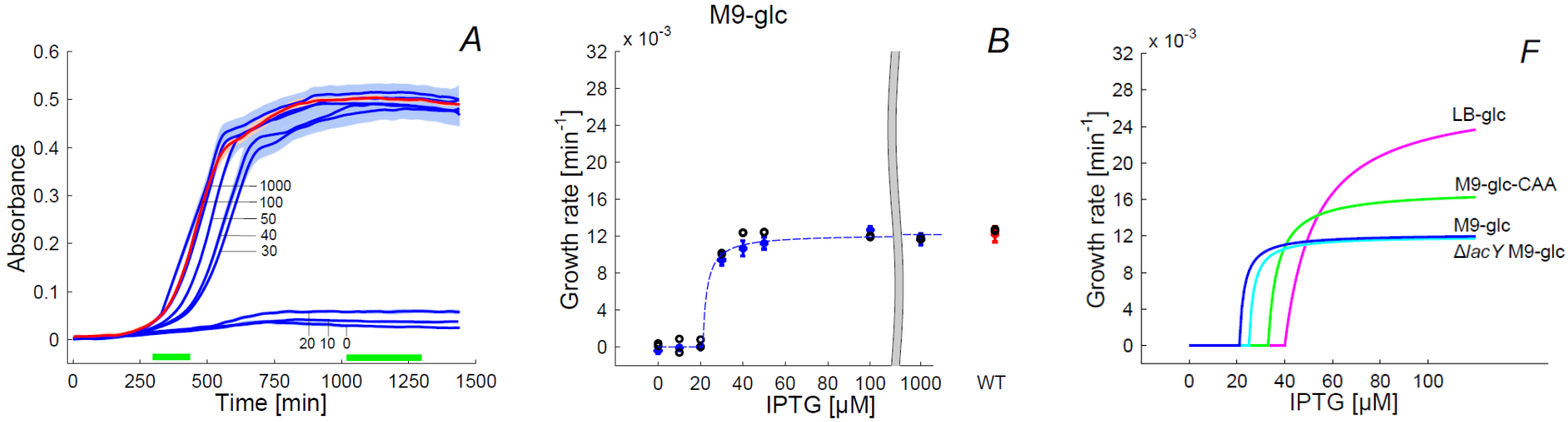
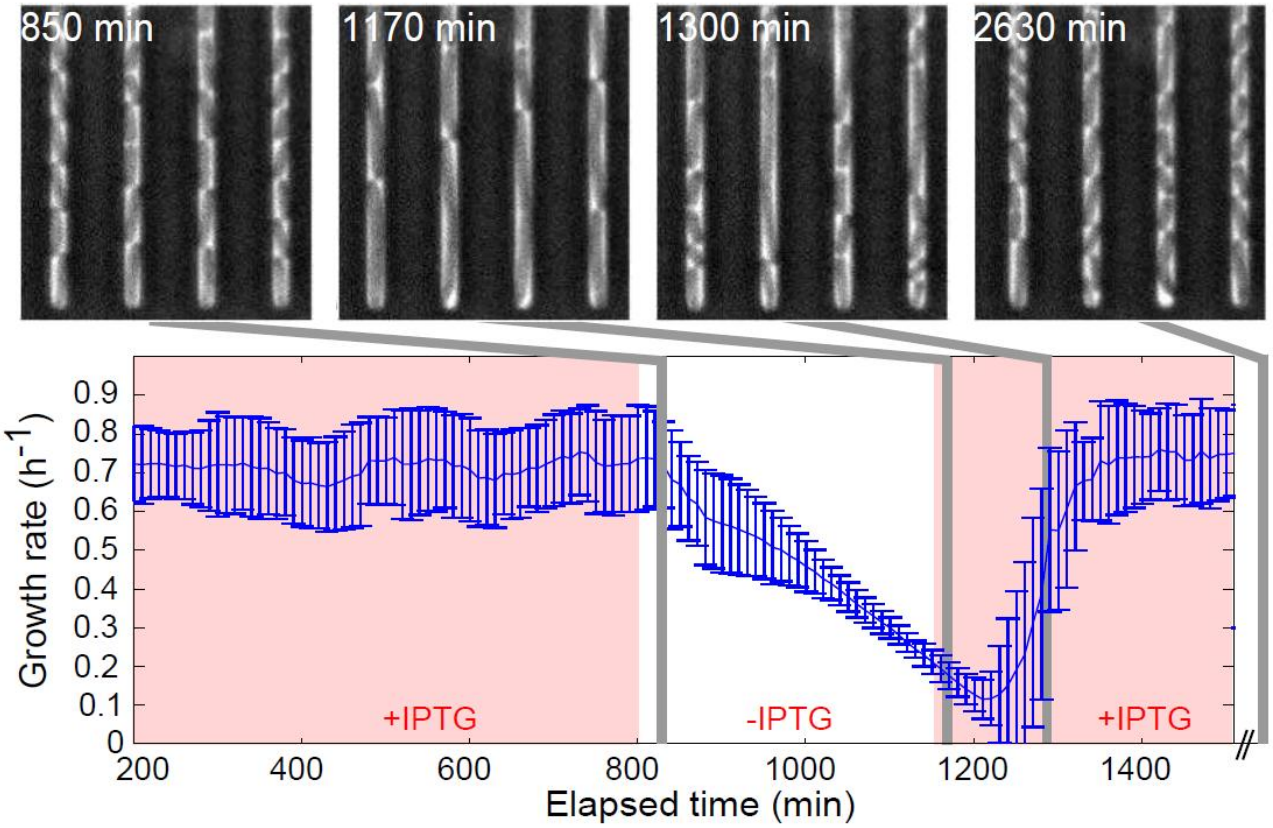


- Growth switch is **medium-independent**: works in different media supporting different maximum growth rates

Synthetic growth switch

- Growth switch is **reversible**

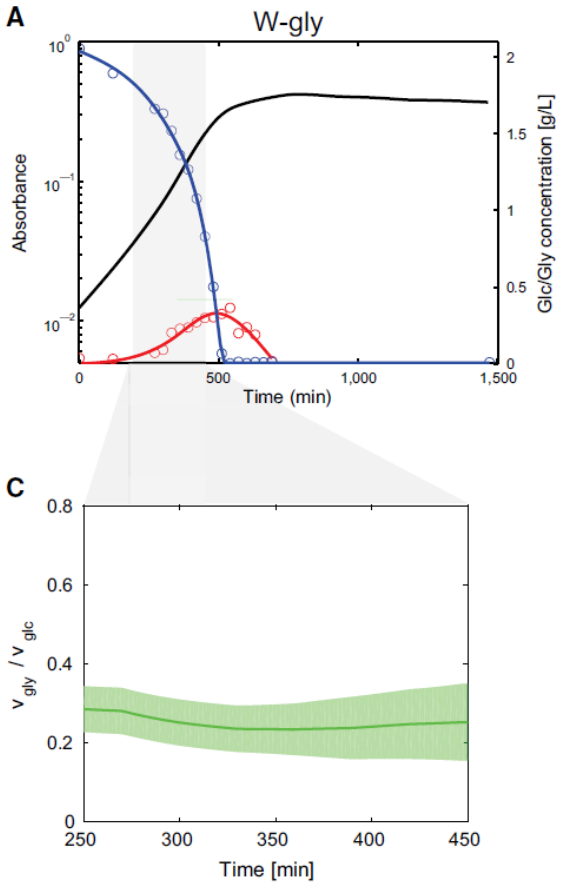
  Microfluidics/time-lapse microscopy experiment, followed by quantification of growth rate of individual cells



Growth switch improves product yields

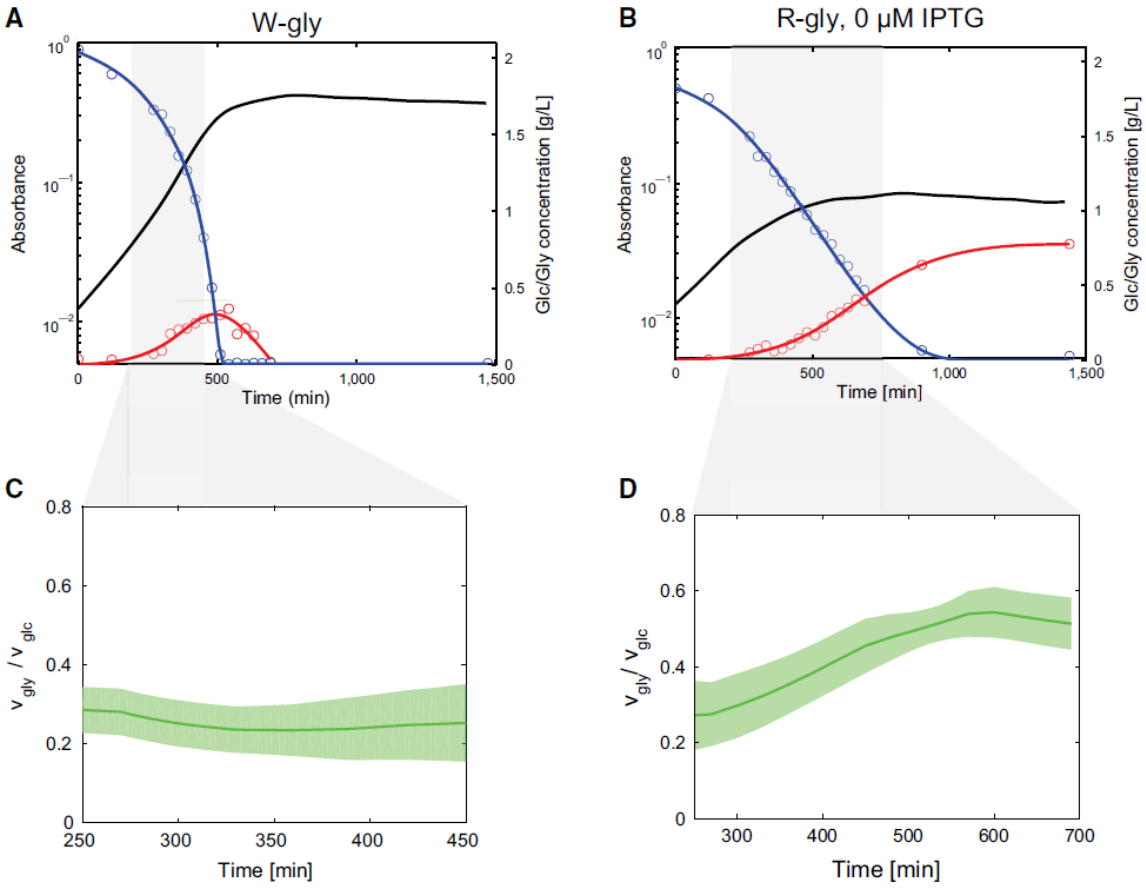
- Production of glycerol from glucose by adding plasmid carrying genes that code for glycerol pathway in yeast

Liang *et al.* (2011), *Appl. Microbial. Biotechnol.*, 89:57-62



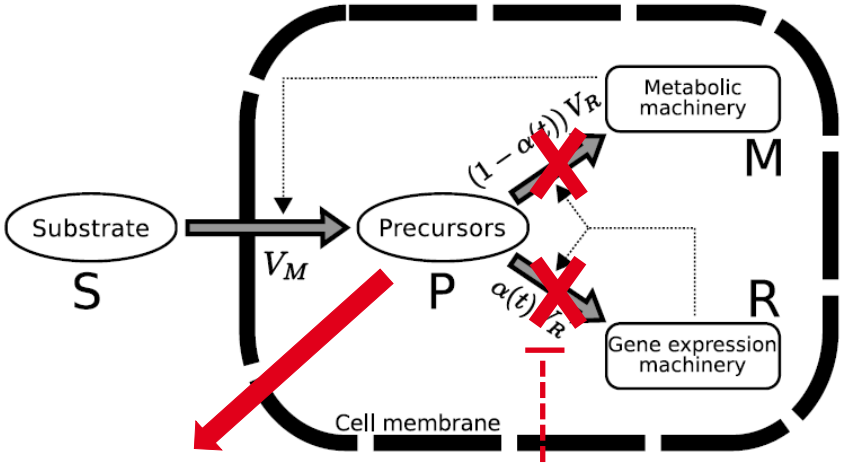
Growth switch improves product yields

- Production of glycerol from glucose by adding plasmid carrying genes that code for glycerol pathway in yeast
- External reduction of growth rate **increases production yield**
  - Yield close to predicted theoretical maximum



Optimization of growth switch

- Model of self-replicators with metabolite production and growth switch



$$\frac{ds(t)}{dt} = v_S(t) - v_M(t)\,\frac{\mathcal{V}(t)}{\mathcal{V}_{\text{ext}}},$$
$$\frac{dp(t)}{dt} = v_M(t) - v_R(t) - v_X(t) - \mu(t)\,p(t),$$
$$\frac{dr(t)}{dt} = u(t)\,v_R(t) - \mu(t)\,r(t),$$
$$\frac{dx(t)}{dt} = v_X(t) - \mu(t)\,x(t),$$
$$\frac{d\mathcal{V}(t)}{dt} = \mu(t)\,\mathcal{V}(t),$$
$$m(t) = \frac{1}{\beta} - r(t), \quad X(t) = x(t)\,\mathcal{V}(t),$$

Yegorov *et al.* (2017), in preparation

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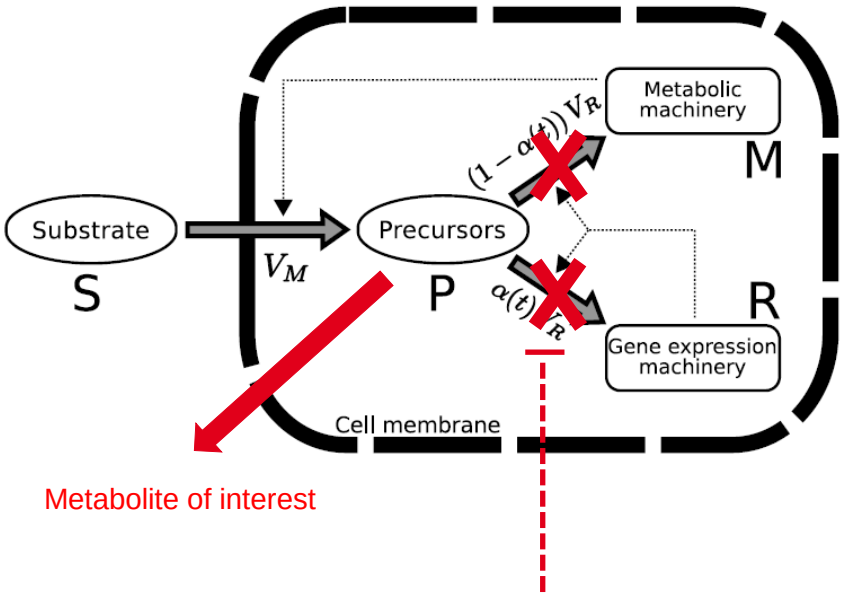

$$\frac{d\hat{s}\left(\hat{t}\right)}{d\hat{t}} = \hat{v}_S\left(\hat{t}\right) - k_2 \frac{\hat{s}\left(\hat{t}\right)\left(1-\hat{r}\left(\hat{t}\right)\right)}{K_2+\hat{s}\left(\hat{t}\right)} \frac{\mathcal{V}\left(\hat{t}\right)}{\mathcal{V}_{\text{ext}}},$$

$$\frac{d\hat{p}\left(\hat{t}\right)}{d\hat{t}} = k_2 \frac{\hat{s}\left(\hat{t}\right)\left(1-\hat{r}\left(\hat{t}\right)\right)}{K_2+\hat{s}\left(\hat{t}\right)} - \left(1+\hat{p}\left(\hat{t}\right)\right) \frac{\hat{p}\left(\hat{t}\right)\hat{r}\left(\hat{t}\right)}{K+\hat{p}\left(\hat{t}\right)} - k_1 \frac{\hat{p}\left(\hat{t}\right)\left(1-\hat{r}\left(\hat{t}\right)\right)}{K_1+\hat{p}\left(\hat{t}\right)},$$

$$\frac{d\hat{r}\left(\hat{t}\right)}{d\hat{t}} = \left(u\left(\hat{t}\right)-\hat{r}\left(\hat{t}\right)\right) \frac{\hat{p}\left(\hat{t}\right)\hat{r}\left(\hat{t}\right)}{K+\hat{p}\left(\hat{t}\right)},$$

$$\frac{d\hat{x}\left(\hat{t}\right)}{d\hat{t}} = k_1 \frac{\hat{p}\left(\hat{t}\right)\left(1-\hat{r}\left(\hat{t}\right)\right)}{K_1+\hat{p}\left(\hat{t}\right)} - \frac{\hat{p}\left(\hat{t}\right)\hat{r}\left(\hat{t}\right)}{K+\hat{p}\left(\hat{t}\right)} \hat{x}\left(\hat{t}\right),$$

$$\frac{d\mathcal{V}\left(\hat{t}\right)}{d\hat{t}} = \frac{\hat{p}\left(\hat{t}\right)\hat{r}\left(\hat{t}\right)}{K+\hat{p}\left(\hat{t}\right)} \mathcal{V}\left(\hat{t}\right),$$

$$\hat{m}\left(\hat{t}\right) = 1-\hat{r}\left(\hat{t}\right), \quad \hat{X}\left(\hat{t}\right) = \hat{x}\left(\hat{t}\right)\mathcal{V}\left(\hat{t}\right),$$

$$\hat{t} \in \left[0, \hat{T}\right].$$

Yegorov *et al.* (2017), in preparation

Optimization of growth switch

- Model of self-replicators with metabolite production and growth switch
- Optimization of metabolite production

$$\hat{X}\left(\hat{T}\right) \longrightarrow \max_{u(\cdot) \in \mathcal{U}}$$

- Preliminary results of optimal control analysis: growth phase followed by production phase

  In agreement with dynamical control schemes in biotechnology

Yegorov *et al.* (2017), in preparation

Conclusions

- Reengineering gene expression machinery leads to **reversible and medium-independent growth switch**
- Growth-arrested cells capable of **reorienting nutrient fluxes** towards increased production of metabolite of interest
  - Test on other heterologous pathways in pre-industrial setting
- Proof-of-principle shows that growth switch may be useful **extension of toolbox of biotechnological engineers**
  - But: cofactor imbalances or toxic intermediates may occur in other applications
- How can production of metabolites of interest be **dynamically optimized**?
  - Timing of arrest and restart of gene expression machinery

Contributors

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Action d'envergure Colage

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- **Open PhD position!**

ANR Maximic

Merci !

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