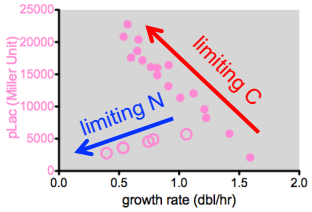
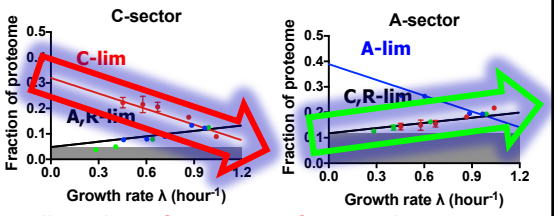
Last time: Carbon Catabolite Repression (CCR)

➔ not about carbon preferences but about carbon/nitrogen coordination

carbon catabolic enzymes

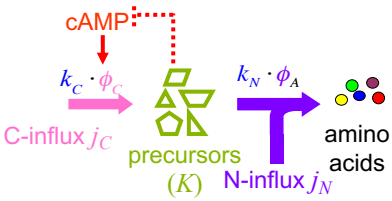


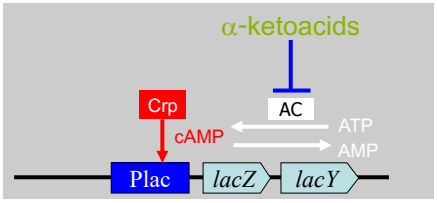
proteomics



➔ physiological function of CCR: reallocation of proteome from carbon catabolism to anabolism and protein synthesis when carbon supply is high

➔ strategy of coordination: α–ketoacids as sensor of C/N balance





➔ mechanism: not via PTS but via direct inhibition of cAMP synthesis

1

Hierarchical vs simultaneous carbon usage

Q4: If cAMP-Crp is for proteome-metabolome coordination, how is hierarchical carbon usage implemented?

Effective usage of mixed carbon sources desirable in numerous industrial applications

Absence of Diauxie during Simultaneous Utilization of Glucose and Xylose by *Sulfolobus acidocaldarius*▽†

Chijioke J. Joshua,[1,3,4] Robert Dahl,[2,3,4] Peter I. Benke,[3,4] and Jay D. Keasling[1,2,3,4,5]*

Graduate Group in Microbiology, Department of Plant and Microbial Biology, University of California, Berkeley, California 94720[1]; Department of Chemical and Biomolecular Engineering, University of California, Berkeley, California 94720[2]; Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720[3]; Joint BioEnergy Institute, 5885 Hollis Street, Emeryville, California 94608[4]; and Department of Bioengineering, University of California, Berkeley, California 94720[5]

Received 11 October 2010/Accepted 8 January 2011

A new carbon catabolite repression mutation of *Escherichia coli*, *mlc**, and its use for producing isobutanol

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Available online 4 May 2012

➔ Can we manipulate the order of carbon hierarchy?

2

Hierarchical vs simultaneous carbon usage

Q4: If cAMP-Crp is for proteome-metabolome coordination, how is hierarchical carbon usage implemented?

growth rate (1/h)		oaa	succ	pyr	glyc	glucose
	alone	0.79 ± 0.01	0.46 ± 0.01	0.61 ± 0.02	0.63 ± 0.01	0.85 ± 0.00
mannose	0.42 ± 0.00	0.87 ± 0.01	0.64 ± 0.00	0.70 ± 0.02	0.65 ± 0.01	0.84 ± 0.00
xylose	0.61 ± 0.00	0.88 ± 0.01	0.71 ± 0.01	0.80 ± 0.01	0.64 ± 0.01	0.84 ± 0.01
glycerol	0.63 ± 0.01	0.93 ± 0.00	0.72 ± 0.01	0.85 ± 0.03	--	0.84 ± 0.00
maltose	0.67 ± 0.00	0.90 ± 0.00	0.77 ± 0.00	0.85 ± 0.00	0.70 ± 0.00	0.84 ± 0.01
glucose	0.85 ± 0.00	0.94 ± 0.00	0.86 ± 0.01	0.82 ± 0.01	0.84 ± 0.00	--

$\lambda_{12} > \max\{\lambda_1, \lambda_2\}$ simultaneous utilization

$\lambda_{12} \approx \max\{\lambda_1, \lambda_2\}$ hierarchical utilization

3

Hierarchical vs simultaneous carbon usage

Q4: If cAMP-Crp is for proteome-metabolome coordination, how is hierarchical carbon usage implemented?

growth rate (1/h)		oaa	succ	pyr	glyc	glucose
	alone	0.79 ± 0.01	0.46 ± 0.01	0.61 ± 0.02	0.63 ± 0.01	0.85 ± 0.00
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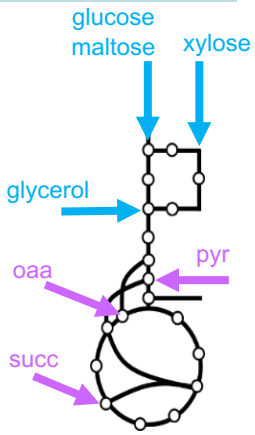
glycerol	0.63 ± 0.01	0.93 ± 0.00	0.72 ± 0.01	0.85 ± 0.03	--	0.84 ± 0.00
maltose	0.67 ± 0.00	0.90 ± 0.00	0.77 ± 0.00	0.85 ± 0.00	0.70 ± 0.00	0.84 ± 0.01
glucose	0.85 ± 0.00	0.94 ± 0.00	0.86 ± 0.01	0.82 ± 0.01	0.84 ± 0.00	--



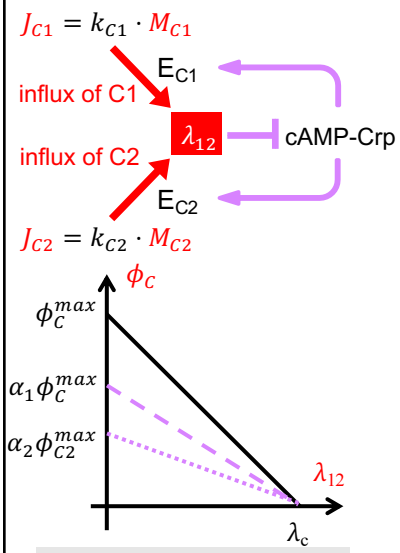
$\lambda_{12} > \max\{\lambda_1, \lambda_2\}$ simultaneous utilization

$\lambda_{12} \approx \max\{\lambda_1, \lambda_2\}$ hierarchical utilization

4

Simultaneous utilization of two C-sources in steady state

Consider two substrates C1, C2, with single-substrate growth rate λ_1, λ_2



$\phi_C^{max} = \phi^{max} - \phi_{R,0}$

$\lambda_C = \phi_C^{max} \cdot \gamma_0 \nu_A / (\gamma_0 + \nu_A)$

total protein synthesis flux on two substrates:

$\lambda_{12} \cdot M = J_{C1} \cdot Y_{C1} + J_{C2} \cdot Y_{C2}$
$= \nu_{C1} M_{C1} + \nu_{C2} M_{C2}$

Y_{Ci} yield of Ci
$\nu_{Ci} = k_{Ci} Y_{Ci}$

$\Rightarrow \lambda_{12} = \nu_{C1}\phi_{C1} + \nu_{C2}\phi_{C2}$

other constraints: $\lambda_{12} = \gamma\phi_R$, $\lambda_{12} = \nu_A\phi_A$

$\phi_R + \phi_A + \phi_C = \phi^{max}$

$\Rightarrow \phi_C = \phi_C^{max} \cdot (1 - \lambda_{12}/\lambda_C)$

$\phi_C = \phi_{C0} + \phi_{C1} + \phi_{C2}$

generic (non flux-carrying) C-proteins

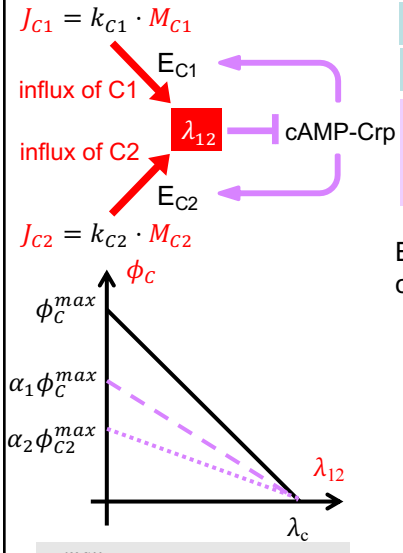
no further known constraints to fix ϕ_{C1}, ϕ_{C2}
➔ optimization: $\phi_{C2}^* = 0$ if $\nu_{C2} < \nu_{C1}$
➔ hierarchical utilization: $\lambda = \max\{\lambda_1, \lambda_2\}$

Alternative: common regulation (cAMP-Crp)
-- induced by C1: $\phi_{C1} = \alpha_1 \cdot \phi_C$
-- induced by C2: $\phi_{C2} = \alpha_2 \cdot \phi_C$

6

Simultaneous utilization of two C-sources in steady state

Consider two substrates C1, C2, with single-substrate growth rate λ_1, λ_2



$\phi_C^{max} = \phi^{max} - \phi_{R,0}$

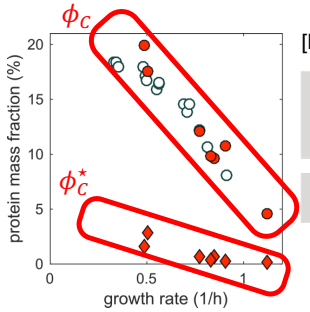
$\lambda_C = \phi_C^{max} \cdot \gamma_0 \nu_A / (\gamma_0 + \nu_A)$

$\Rightarrow \lambda_{12} = \nu_{C1}\phi_{C1} + \nu_{C2}\phi_{C2}$

$\Rightarrow \phi_C = \phi_C^{max} \cdot (1 - \lambda_{12}/\lambda_C)$

Alternative: common regulation (cAMP-Crp)
-- induced by C1: $\phi_{C1} = \alpha_1 \cdot \phi_C$
-- induced by C2: $\phi_{C2} = \alpha_2 \cdot \phi_C$

Empirical data: flux-carrying catabolic enzymes comprise a small portion of the C-sector



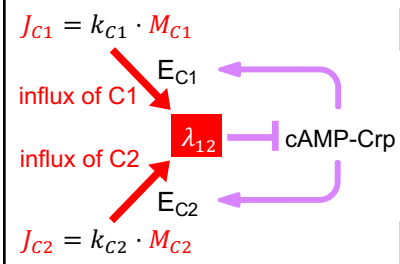
[Mori et al, MSB (2021)]

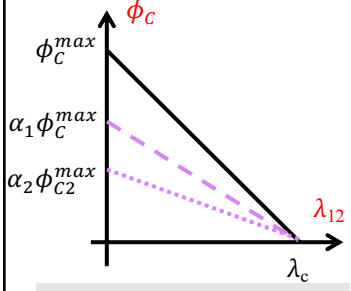
$\phi_C^\star < 5\% \cdot \phi_C$

7

Simultaneous utilization of two C-sources in steady state

Consider two substrates C1, C2, with single-substrate growth rate λ_1, λ_2





$\phi_C^{max} = \phi^{max} - \phi_{R,0}$

$\lambda_C = \phi_C^{max} \cdot \gamma_0 \nu_A/(\gamma_0 + \nu_A)$

$\Rightarrow \lambda_{12} = \nu_{C1}\phi_{C1} + \nu_{C2}\phi_{C2}$

$\Rightarrow \phi_C = \phi_C^{max} \cdot (1 - \lambda_{12}/\lambda_C)$

Alternative: common regulation (cAMP-Crp)
-- induced by C1: $\phi_{C1} = \alpha_1 \cdot \phi_C$
-- induced by C2: $\phi_{C2} = \alpha_2 \cdot \phi_C$

$\Rightarrow \lambda_{12} = (\nu_{C1}\alpha_1 + \nu_{C2}\alpha_2)\phi_C^{max}(1 - \lambda_{12}/\lambda_C)$

relate parameters to λ_1, λ_2 :

C1 alone: $\lambda_1 = \nu_{C1}\phi_{C1} = \nu_{C1}\alpha_1\phi_C^{max}(1 - \lambda_1/\lambda_C)$

C2 alone: $\lambda_2 = \nu_{C2}\phi_{C2} = \nu_{C2}\alpha_2\phi_C^{max}(1 - \lambda_2/\lambda_C)$

$$\Rightarrow \frac{\lambda_{12}}{1 - \lambda_{12}/\lambda_C} = \frac{\lambda_1}{1 - \lambda_1/\lambda_C} + \frac{\lambda_2}{1 - \lambda_2/\lambda_C}$$

$$\Rightarrow \lambda_{12} = \frac{\lambda_1 + \lambda_2 - 2\lambda_1\lambda_2/\lambda_C}{1 - \lambda_1\lambda_2/\lambda_C^2} > \max\{\lambda_1, \lambda_2\}$$

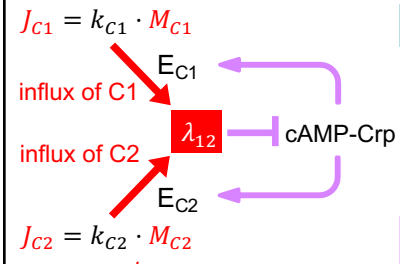
growth-rate "addition" formula!

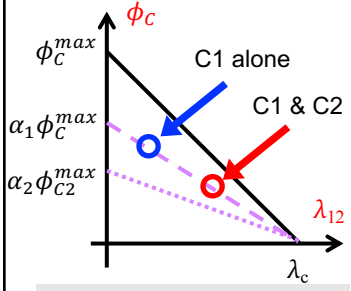
[Hermsen et al, MSB (2015)]

8

Simultaneous utilization of two C-sources in steady state

Consider two substrates C1, C2, with single-substrate growth rate λ_1, λ_2





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$$\Rightarrow \lambda_{12} = \frac{\lambda_1 + \lambda_2 - 2\lambda_1\lambda_2/\lambda_C}{1 - \lambda_1\lambda_2/\lambda_C^2} > \max\{\lambda_1, \lambda_2\}$$

growth-rate "addition" formula!

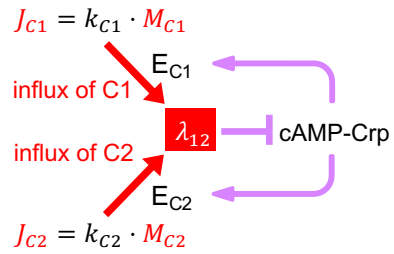
$$\lambda_{12} \approx \begin{cases} \lambda_1 + \lambda_2 & \lambda_1, \lambda_2 \ll \lambda_C \\ \lambda_C & \lambda_1 \text{ or } \lambda_2 \approx \lambda_C \end{cases}$$ speed limit

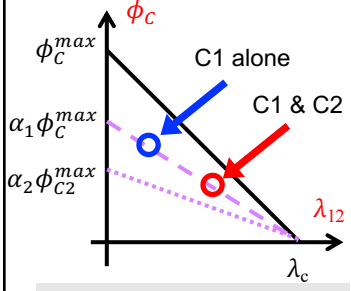
➔ key assumption:
cAMP-Crp responds to total C-flux ($\propto \lambda$),
with ϕ_{C1}, ϕ_{C2} remain on its respective C-line
when grown alone or with both C1+C2

9

Simultaneous utilization of two C-sources in steady state

Consider two substrates C1, C2, with single-substrate growth rate λ_1, λ_2





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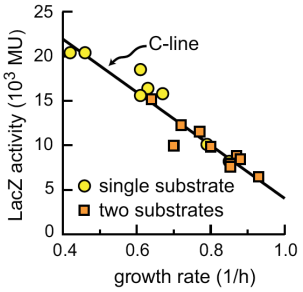
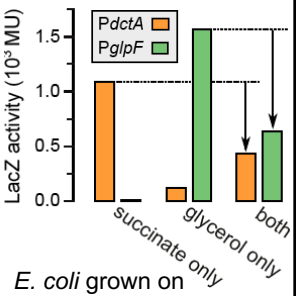


E. coli with 1mM IPTG

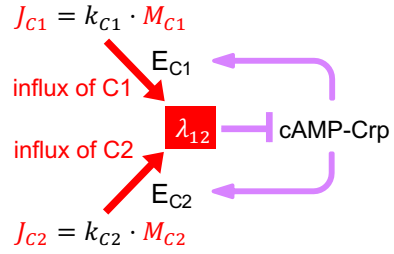


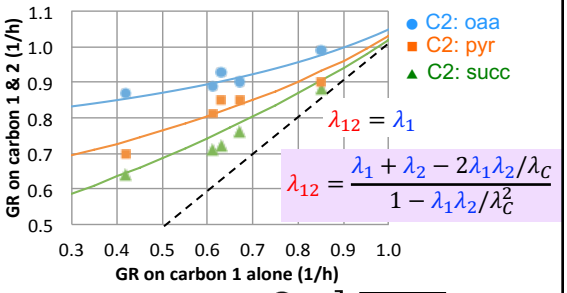
E. coli grown on glycerol/succinate

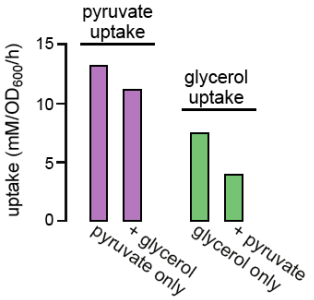
10

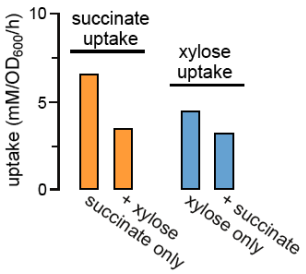
Simultaneous utilization of two C-sources in steady state

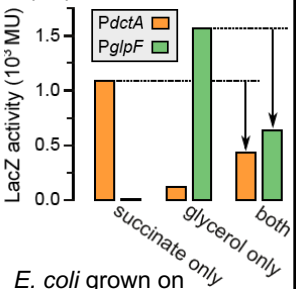
Consider two substrates C1, C2, with single-substrate growth rate λ_1, λ_2











E. coli grown on glycerol/succinate

11

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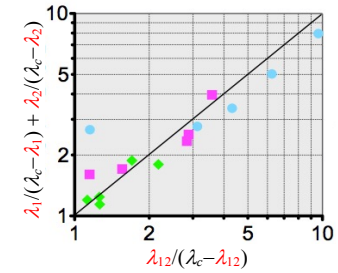

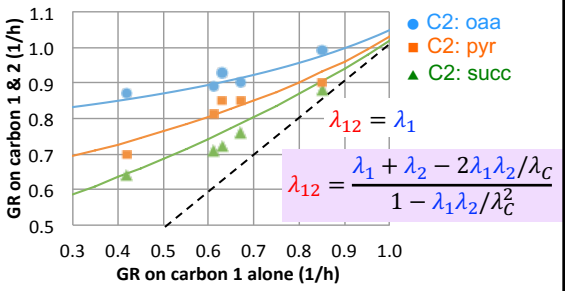

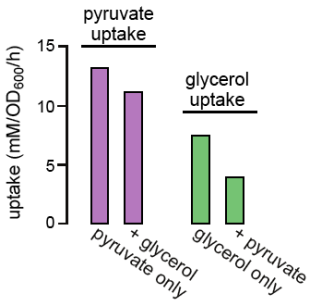

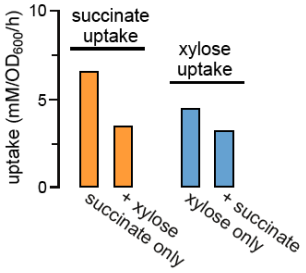

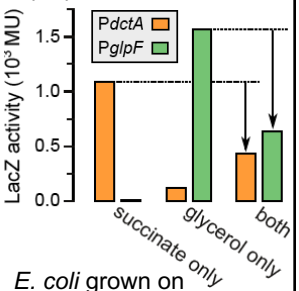

E. coli grown on glycerol/succinate

12

Hierarchical vs simultaneous carbon usage

Q4: If cAMP-Crp is for proteome-metabolome coordination, how is hierarchical carbon usage implemented?

growth rate (1/h)		oaa	succ	pyr	glyc	glucose
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mannose	0.42 ± 0.00	0.87 ± 0.01	0.64 ± 0.00	0.70 ± 0.02	0.65 ± 0.01	0.84 ± 0.00
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glycerol	0.63 ± 0.01	0.93 ± 0.00	0.72 ± 0.01	0.85 ± 0.03	--	0.84 ± 0.00
maltose	0.67 ± 0.00	0.90 ± 0.00	0.77 ± 0.00	0.85 ± 0.00	0.70 ± 0.00	0.84 ± 0.01
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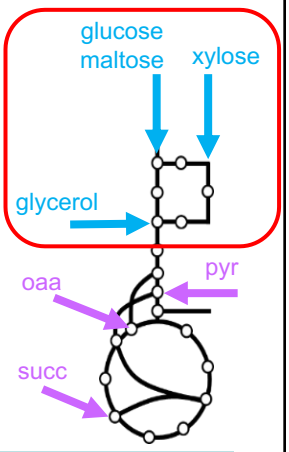


$\lambda_{12} > \max\{\lambda_1, \lambda_2\}$ simultaneous utilization

$\lambda_{12} \approx \max\{\lambda_1, \lambda_2\}$ hierarchical utilization

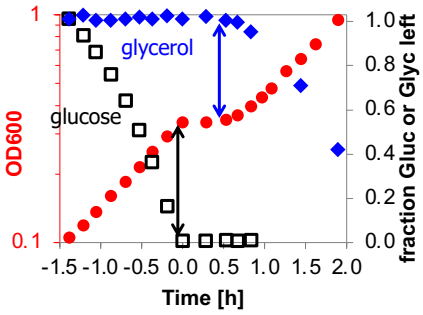
15

Hierarchical usage of glycolytic substrates

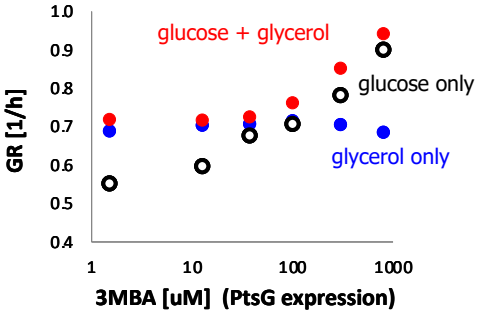
[Okano et al, Nat. Microb. (2019)]

Q4: If cAMP-Crp is for proteome-metabolome coordination, what is responsible for hierarchical carbon usage?

glucose-glycerol diauxie



steady-state growth on glucose
by titrating glucose transporter PtsG



➔ takes the faster of the two growth rates

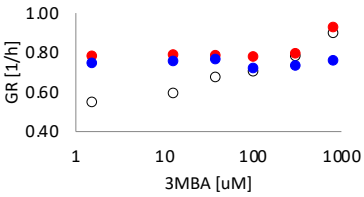
16

Hierarchical usage of glycolytic substrates

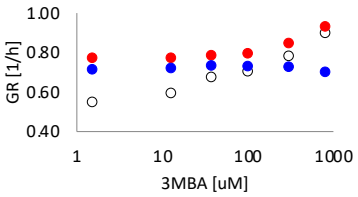
[Okano et al, Nat. Microb. (2019)]

steady-state growth on glucose using titratable PtsG

20 mM glucose / 10 mM maltose

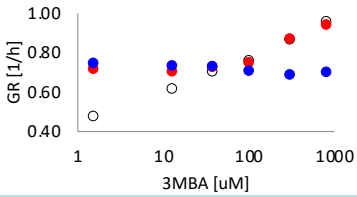


20 mM glucose / 24 mM xylose

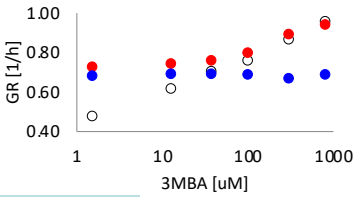


steady-state growth on lactose using titratable LacY

10 mM lactose / 10 mM maltose



10 mM lactose / 24 mM xylose



➔ takes the faster of the two growth rates

➔ not specific to PTS transport (lac uptake: proton symport)

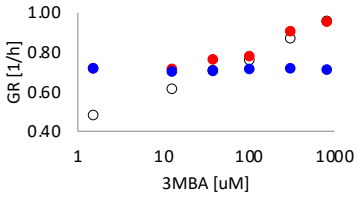
17

Hierarchical usage of glycolytic substrates

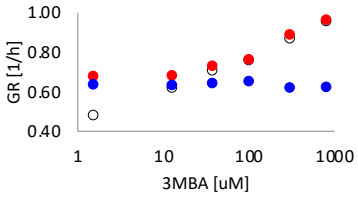
[Okano et al, Nat. Microb. (2019)]

steady-state growth on lactose using titratable LacY

10 mM lactose / 40 mM glycerol

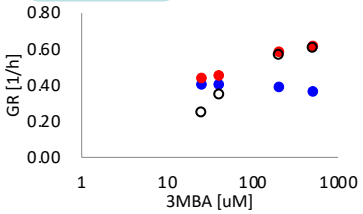


10 mM lactose / 20 mM fructose



steady-state growth on glycerol using titratable GlpFK

40 mM glycerol / 20 mM mannose



➔ hierarchical usage of glycolytic substrates is independent of the nature of the substrate

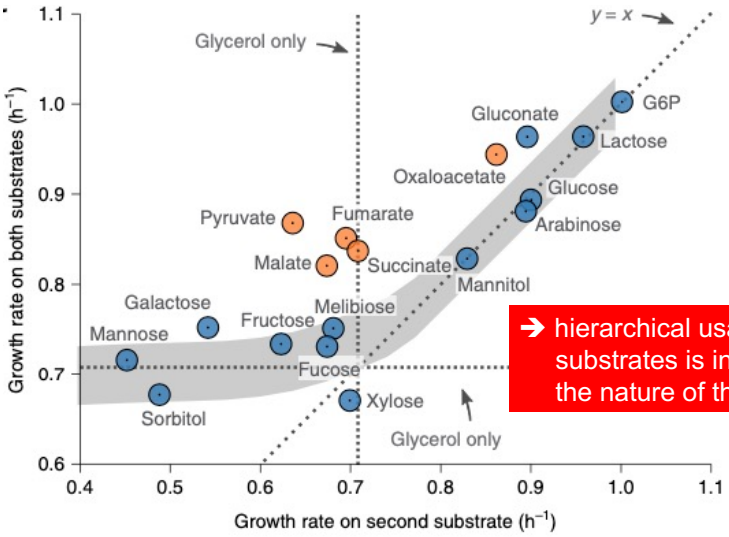
➔ takes the faster of the two growth rates

➔ not specific to PTS transport (lac uptake: proton symport)

18

Hierarchical usage of glycolytic substrates

[Okano et al, Nat. Microb. (2019)]



➔ hierarchical usage of glycolytic substrates is independent of the nature of the substrate

➔ takes the faster of the two growth rates

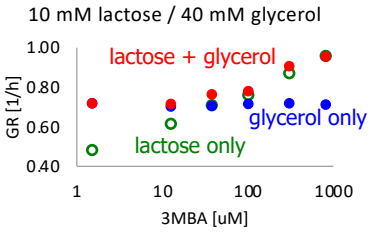
➔ not specific to PTS transport (lac uptake: proton symport)

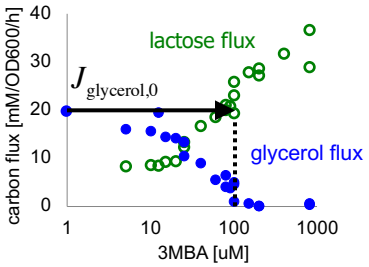
19

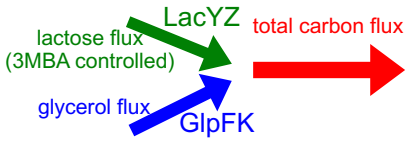
Hierarchical usage of glycolytic substrates

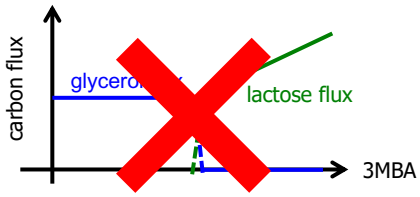
[Okano et al, Nat. Microb. (2019)]

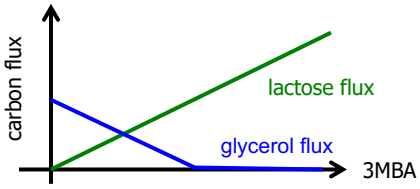
steady-state growth on lactose using titratable LacY











➔ use all available lactose

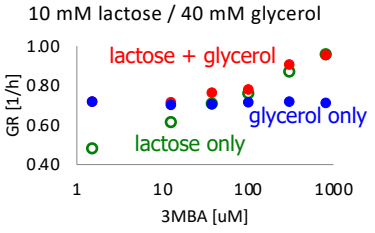
➔ glycerol uptake regulated by λ

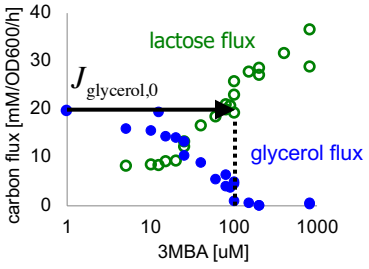
20

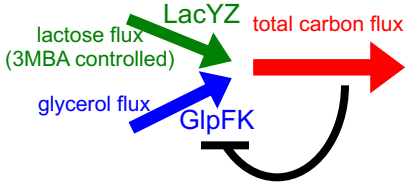
Hierarchical usage of glycolytic substrates

[Okano et al, Nat. Microb. (2019)]

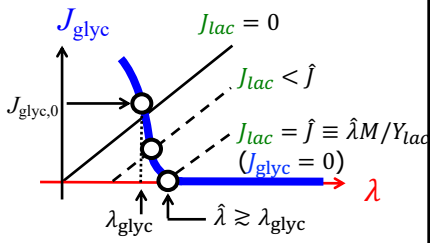
steady-state growth on lactose using titratable LacY







$$J_{lac}Y_{lac} + J_{glyc}(\lambda)Y_{glyc} = \lambda M$$



➔ use all available lactose

➔ glycerol uptake regulated by λ

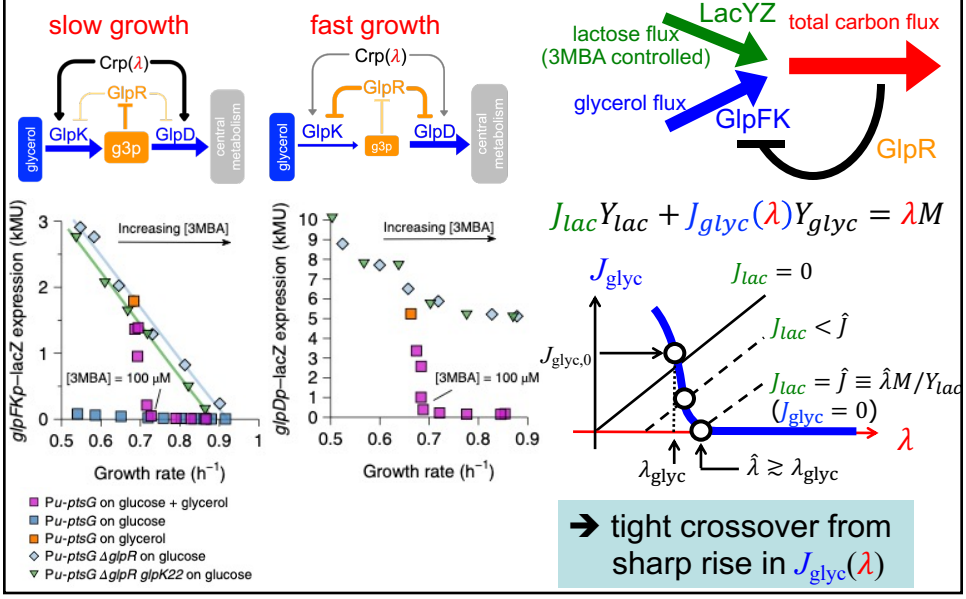
➔ tight crossover from sharp rise in $J_{glyc}(\lambda)$

21

Hierarchical usage of glycolytic substrates

[Okano et al, Nat. Microb. (2019)]

Mechanism of sharp GR-dependence



➔ tight crossover from sharp rise in $J_{glyc}(\lambda)$

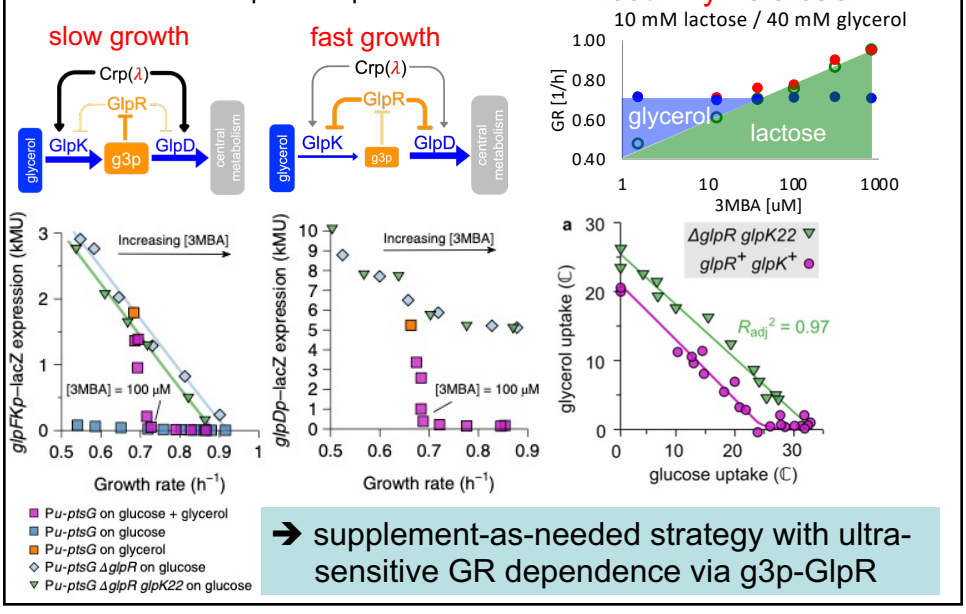
22

Hierarchical usage of glycolytic substrates

[Okano et al, Nat. Microb. (2019)]

Mechanism of sharp GR-dependence

but why hold back?



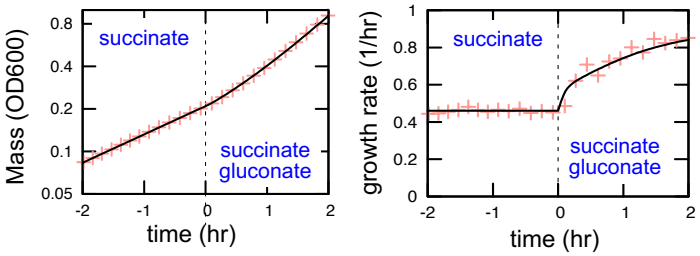
➔ supplement-as-needed strategy with ultra-sensitive GR dependence via g3p-GlpR

23

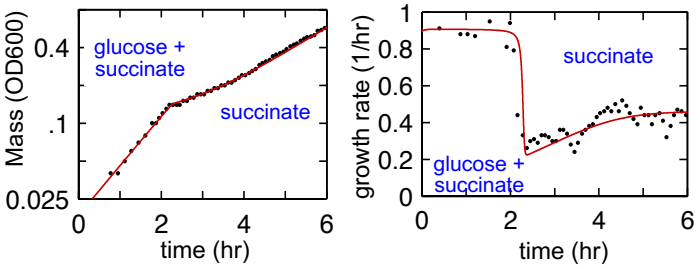
Growth transition kinetics

[Erickson, Schink, et al, Nature (2017)]

Nutrient upshift



Nutrient downshift (diauxic growth)



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Growth transition kinetics

[Erickson, Schink, et al, Nature (2017)]

Coarse-grained kinetic theory of growth involving only

-- single ordinary differential equation

-- values of the initial and final growth rates (to define C quality)

-- steady-state growth laws

➔ describes gene expression and growth curve throughout the course of the transition

➔ no need for kinetic parameters; no fitting parameter

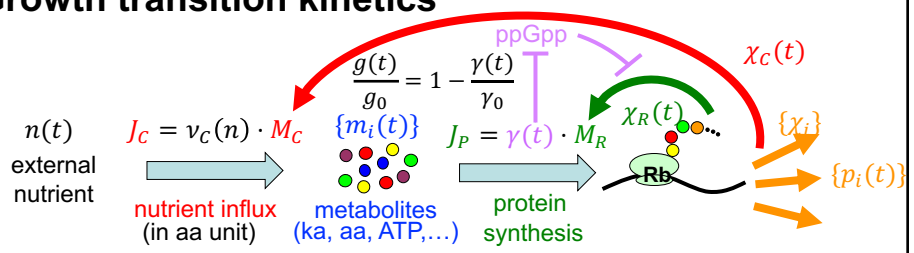
➔ works both for nutrient upshifts and downshifts

➔ same theory describes growth inhibition by antibiotics

25

Growth transition kinetics

[Erickson, Schink, et al, Nature (2017)]



Kinetics of protein synthesis:

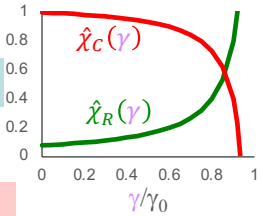
$\phi_R \equiv M_R(t)/M(t)$

$\phi_C \equiv M_C(t)/M(t)$

$\frac{d}{dt}M = J_P = \gamma(t)M_R = \nu_C M_C \xrightarrow{\lambda(t) \equiv \frac{d}{dt}\ln M} \lambda(t) = \gamma(t) \cdot \phi_R(t) = \nu_C \cdot \phi_C(t)$

$\frac{d}{dt}M_R = \chi_R(t)\, J_P \rightarrow \frac{d}{dt}\phi_R = \lambda(t) \cdot (\chi_R(t) - \phi_R(t))$

$\frac{d}{dt}M_C = \chi_C(t)\, J_P \rightarrow \frac{d}{dt}\phi_C = \lambda(t) \cdot (\chi_C(t) - \phi_C(t))$



- coupled nonlinear ODEs for $\phi_R(t)$ and $\phi_C(t)$
- requires regulatory functions $\chi_R(t)$ and $\chi_C(t)$

→ regulation of ribosome synthesis: $\chi_R(t) = \hat{\chi}_R(\gamma(t))$

→ same form as in steady state: $\hat{\chi}_R(\gamma) = \phi_R^*(\gamma) = \phi_{R,0}/(1 - \gamma/\gamma_0)$

→ repeat for regulation of catabolic enzymes: $\chi_C(t) = \hat{\chi}_C(\gamma(t)) = \phi_C^*(\gamma)$

26

Growth transition kinetics

[Erickson, Schink, et al, Nature (2017)]

"Equation of motion" for $\gamma(t) = \nu_C\phi_C(t)/\phi_R(t)$

$$\frac{d}{dt}\gamma = \gamma(t) \cdot [\nu_C\hat{\chi}_C(\gamma) - \gamma(t)\hat{\chi}_R(\gamma)]$$

➔ from soln for $\gamma(t)$, solve for $\phi_R(t)$, $\phi_C(t)$, $\lambda(t)$, $M(t)$

➔ exact solution; completely determined by λ_i, λ_f

Kinetics of protein synthesis:

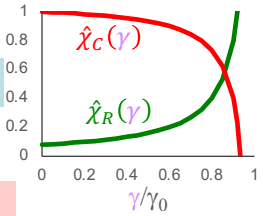
$\phi_R \equiv M_R(t)/M(t)$

$\phi_C \equiv M_C(t)/M(t)$

$\frac{d}{dt}M = J_P = \gamma(t)M_R = \nu_C M_C \xrightarrow{\lambda(t) \equiv \frac{d}{dt}\ln M} \lambda(t) = \gamma(t) \cdot \phi_R(t) = \nu_C \cdot \phi_C(t)$

$\frac{d}{dt}M_R = \chi_R(t)\, J_P \rightarrow \frac{d}{dt}\phi_R = \lambda(t) \cdot (\hat{\chi}_R(\gamma) - \phi_R(t))$

$\frac{d}{dt}M_C = \chi_C(t)\, J_P \rightarrow \frac{d}{dt}\phi_C = \lambda(t) \cdot (\hat{\chi}_C(\gamma) - \phi_C(t))$



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→ repeat for regulation of catabolic enzymes: $\chi_C(t) = \hat{\chi}_C(\gamma(t)) = \phi_C^*(\gamma)$

27

Growth transition kinetics

[Erickson, Schink, et al, Nature (2017)]

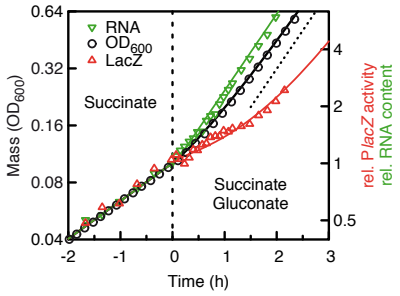
"Equation of motion" for $\gamma(t) = \nu_C \phi_C(t)/\phi_R(t)$

$$\frac{d}{dt}\gamma = \gamma(t) \cdot [\nu_C \hat{\chi}_C(\gamma) - \gamma(t)\hat{\chi}_R(\gamma)]$$

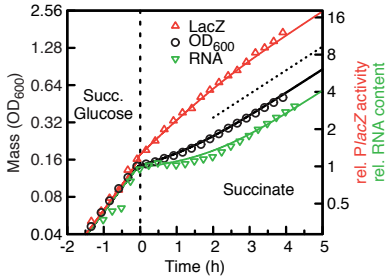
➔ from soln for $\gamma(t)$, solve for $\phi_R(t)$, $\phi_C(t)$, $\lambda(t)$, $M(t)$

➔ exact solution; completely determined by λ_i, λ_f

nutrient upshift



nutrient downshift

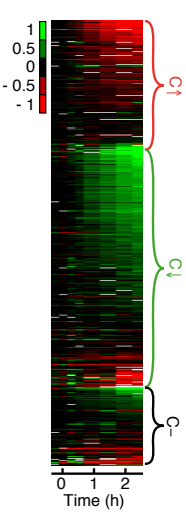


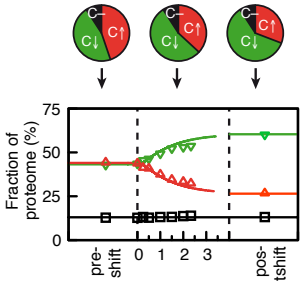
28

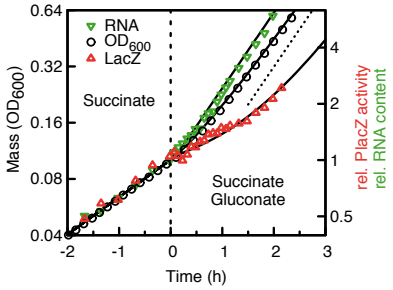
Growth transition kinetics

[Erickson, Schink, et al, Nature (2017)]

proteome-wide response to nutrient up-shift







upshift kinetics:

- rapid increase in ribosome synthesis
- rapid reduction of C-protein synthesis
- slow recovery of growth rate due to slow dilution of pre-existing C-proteins

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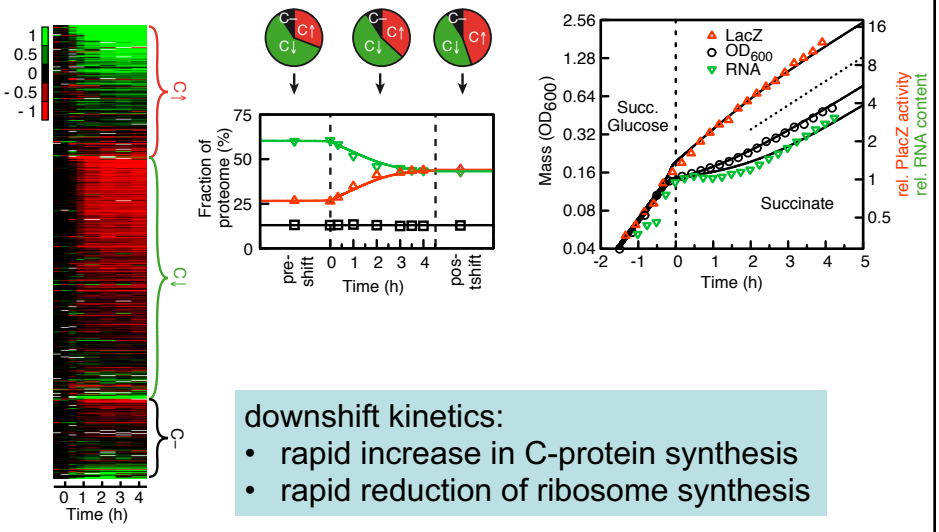
Growth transition kinetics

[Erickson, Schink, et al, Nature (2017)]

proteome-wide response to nutrient down-shift



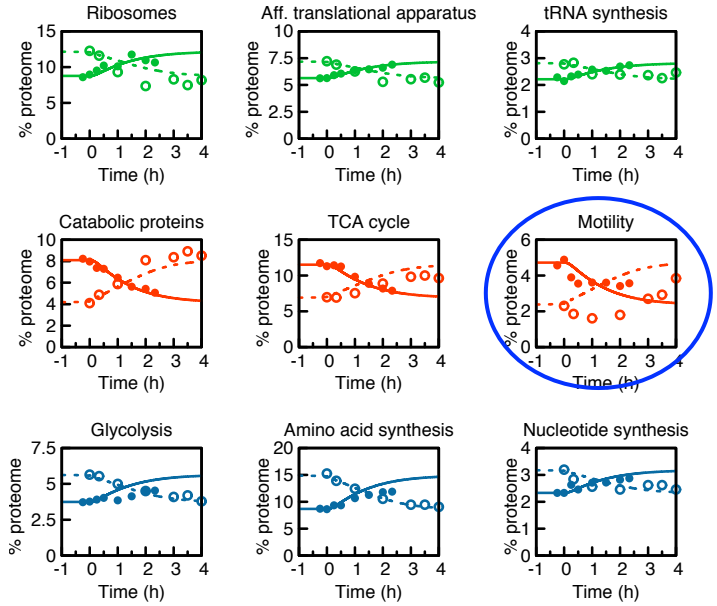
downshift kinetics:

- rapid increase in C-protein synthesis
- rapid reduction of ribosome synthesis

30

[Erickson, Schink, et al, Nature (2017)]

Kinetics of individual metabolic sectors mostly follow the predicted form



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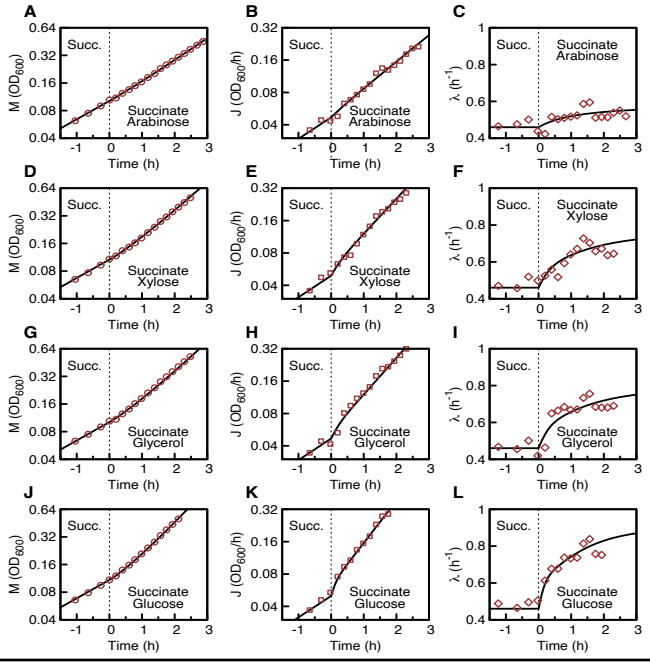
more upshifts

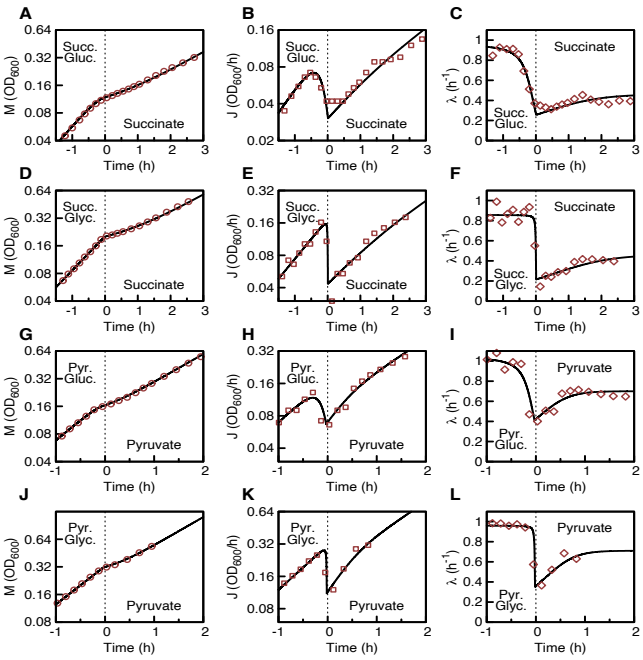
[Erickson, Schink, et al, Nature (2017)]



32

more downshifts

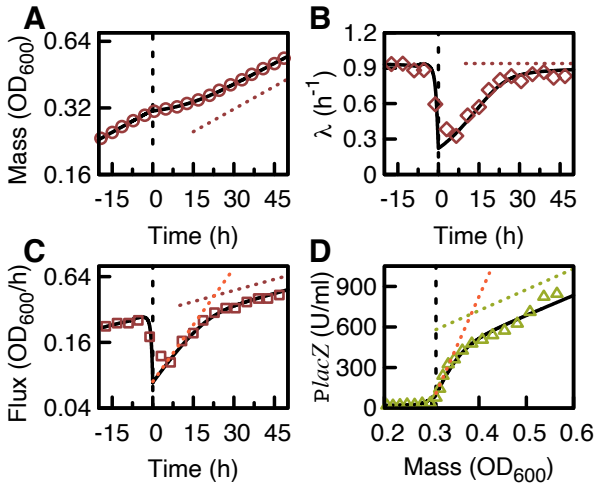
[Erickson, Schink, et al, Nature (2017)]



33

[Erickson, Schink, et al, Nature (2017)]

Glucose-lactose diauxic shift (Monod, 1947)



hierarchically utilized carbon sources; uses one fitting parameter to describe the release of inhibition of lactose uptake by glucose.

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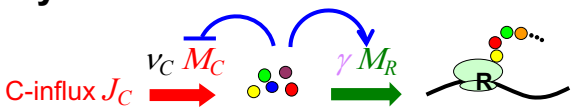
Growth inhibition by antibiotics

[Wu et al, Ph.D. thesis (2022)]

coarse-grained metabolism



Effect of translation-inhibiting drugs: inactivate ribosomes

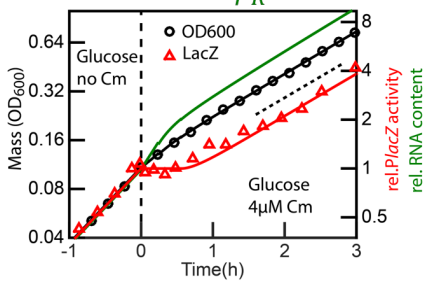
$$\phi_R^{act} = \phi_R/(1 + [\text{drug}]/K_M)$$

[Dai et al, Nat Microb. (2017)]

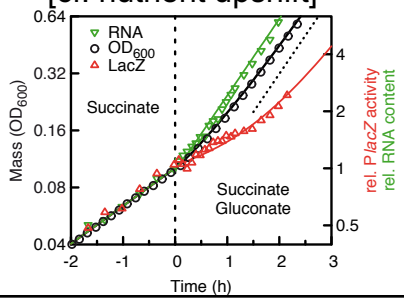
$$\frac{d}{dt}\gamma = \gamma(t) \cdot [\nu_C \hat{\chi}_C(\gamma) - \gamma(t)\hat{\chi}_R^{act}(\gamma)]$$ ➔ $\phi_R(t)$, $\phi_C(t)$, $\lambda(t)$, $M(t)$

Cm addition:

growth slows but ϕ_R increases



[cf: nutrient upshift]



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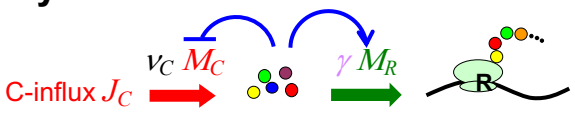
Growth inhibition by antibiotics

[Wu et al, Ph.D. thesis (2022)]

coarse-grained metabolism



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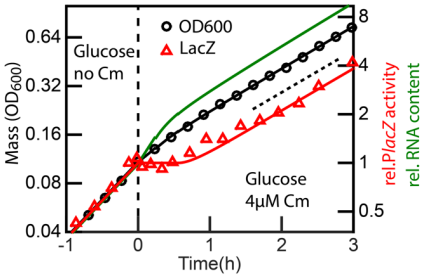
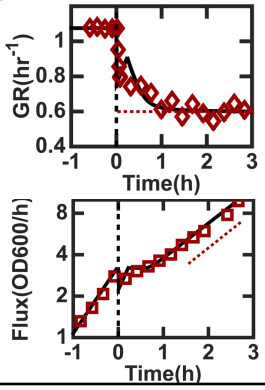
$\frac{d}{dt}\gamma = \gamma(t) \cdot [\nu_C \hat{\chi}_C(\gamma) - \gamma(t)\hat{\chi}_R^{act}(\gamma)]$ ➔ $\phi_R(t)$, $\phi_C(t)$, $\lambda(t)$, $M(t)$

Cm addition:

growth slows but ϕ_R increases





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Summary

- quantitatively predictive behaviors despite molecular complexity
- catabolite repression: not just about carbon [You et al, Nature (2013)]
  - why: proteome/metabolome coordination
  - who/how: direct inhibition of cAMP synthesis by alpha ketoacids
- simultaneous carbon usage [Hermsen et al, Mol Syst Biol (2015])]
  - increase in growth possible but cannot exceed "speed limit"
  - GR addition formula via common cAMP regulation (C-line)
- hierarchical carbon usage [Okano et al, Nature Microb. (2019)]
  - strategy: supplement-as-needed
  - mechanism: total flux sensing (cAMP) + diff regulation of uptake enzymes
  - physiological function unknown (not about optimizing resource)
- growth transition kinetics [Erickson, Schink et al, Nature (2017)]
  - strategy: flux-based regulation (translation activity via ppGpp)
  - form of regulatory function determined from steady state growth laws
  - single ODE completely captures transition kinetics; no fitting parameters
  - quantitative link to CCR still to be worked out

➔ combination of molecular vs physiological approaches

quantitative predictions & molecular mechanisms

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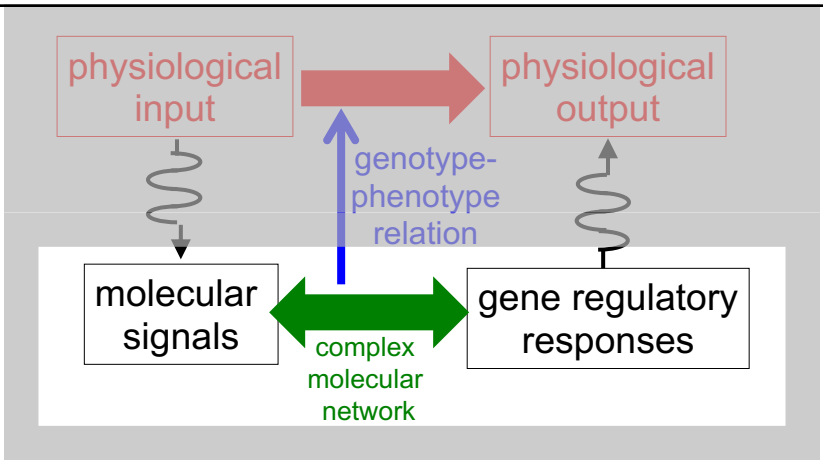

Tenet of classical molecular biology:
molecular knowledge ➔ biological function

Problem with the bottom-up approach:
quantitative, predictive understanding of the system requires many inaccessible *in vivo* parameters

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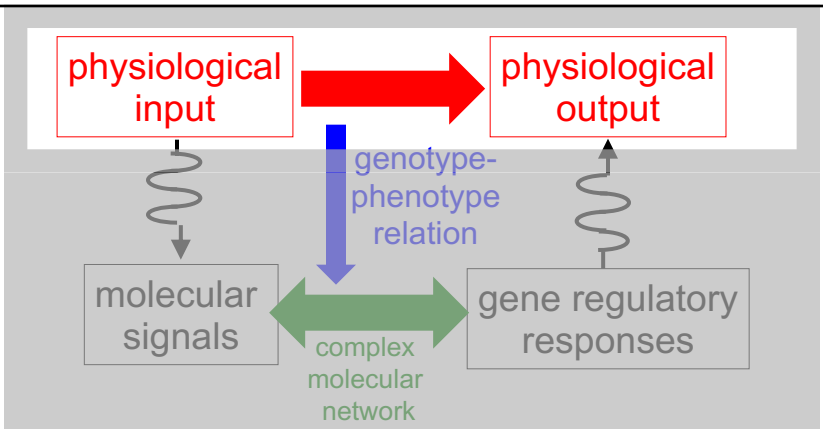

Top-down approach:
tame complexity by quantitative phenomenology ('laws')
= simple relations between physiological inputs/outputs

➔ quantitative predictions on physiological responses
➔ useful guide for synthetic biology
➔ insight on the "purpose" of regulatory mechanisms
➔ guide for regulatory strategies & molecular implementations

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