Last time: Carbon Catabolite Repression (CCR)

- not about carbon preferences but about carbon/nitrogen coordination
  carbon catabolic enzymes

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

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 Diauxic during Simultaneous Utilization of Glucose and Xylose by Sulfolobus acidocaldarius

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Received 11 October 2010 Accepted 8 January 2011

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

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Received 15 January 2010; accepted 30 February 2011

Can we manipulate the order of carbon hierarchy?
Hierarchical vs simultaneous carbon usage

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

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\[ \lambda_{12} > \max(\lambda_1, \lambda_2) \quad \text{simultaneous utilization} \]

\[ \lambda_{12} \approx \max(\lambda_1, \lambda_2) \quad \text{hierarchical utilization} \]
Simultaneous utilization of two C-sources in steady state
Consider two substrates C1, C2, with single-substrate growth rate $\lambda_1, \lambda_2$

$$ J_{C1} = k_{c1} \cdot M_{C1} \quad \text{influx of C1} $$

$$ J_{C2} = k_{c2} \cdot M_{C2} \quad \text{influx of C2} $$

$$ \phi_{C_{\text{max}}}^\text{C} = \phi_{C_{\text{max}}}^\text{R} - \phi_{C_{\text{R,0}}} $$

$$ \lambda_c = \phi_{C_{\text{max}}}^\text{C} \cdot \gamma_0 v_A / (\gamma_0 + v_A) $$

**total protein synthesis flux on two substrates:**

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

$Y_{C1}$ yield of C1

$Y_{C1} = \frac{v_{C1} M_{C1} + v_{C2} M_{C2}}{v_{C1}}$

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

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

$$ \phi_R + \phi_A + \phi_C = \phi_{C_{\text{max}}} $$

$$ \Rightarrow \phi_C = \phi_{C_{\text{max}}}^\text{C} \cdot (1 - \lambda_{12} / \lambda_c) $$

$$ \phi_C = \phi_{C_{01}} + \phi_{C_1} + \phi_{C_2} $$

**generic (non flux-carrying) C-proteins**

- no further known constraints to fix $\phi_{C1}, \phi_{C2}$
- optimization: $\phi_{C2} = 0$ if $v_{C2} < v_{C1}$
- 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: $\phi_{C2} = \alpha_2 \cdot \phi_C$

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

$\phi_C^* < 5\% \cdot \phi_C$
Simultaneous utilization of two C-sources in steady state

Consider two substrates C1, C2, with single-substrate growth rate $\lambda_1, \lambda_2$

\[ J_{C1} = k_{c1} \cdot M_{C1} \]

influx of C1

\[ J_{C2} = k_{c2} \cdot M_{C2} \]

influx of C2

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

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

$\lambda_{12} = \frac{\lambda_1 + \lambda_2 - 2\lambda_1\lambda_2 / \lambda_c}{1 - \lambda_1\lambda_2 / \lambda_c^2}$

growth-rate “addition” formula!

[Hermsen et al, MSB (2015)]

Alternative: common regulation (cAMP-Crp)

-- induced by C1: $\phi_{C1} = \alpha_1 \cdot \phi_c$

-- induced by C2: $\phi_{C2} = \alpha_2 \cdot \phi_c$

$\Rightarrow \lambda_{12} = (\nu_c \alpha_1 + \nu_c \alpha_2) \phi_c^{max} (1 - \lambda_{12} / \lambda_c)$

relate parameters to $\lambda_1, \lambda_2$

C1 alone: $\lambda_1 = \nu_c \phi_{C1} = \nu_c \alpha_1 \phi_c^{max} (1 - \lambda_1 / \lambda_c)$

C2 alone: $\lambda_2 = \nu_c \phi_{C2} = \nu_c \alpha_2 \phi_c^{max} (1 - \lambda_2 / \lambda_c)$

key assumption:

cAMP-Crp responds to total C-flux ($\propto \lambda$),

with $\phi_{C1}, \phi_{C2}$ remain on its respective C-line

when grown alone or with both C1+C2

\[ \lambda_{12} \approx \begin{cases} 
\lambda_1 + \lambda_2 & \lambda_1, \lambda_2 \ll \lambda_c \\
\lambda_c & \text{or} \lambda_1 \approx \lambda_2 \approx \lambda_c
\end{cases} \]

speed limit
Simultaneous utilization of two C-sources in steady state
Consider two substrates C1, C2, with single-substrate growth rate $\lambda_1, \lambda_2$

$J_{C1} = k_{C1} \cdot M_{C1}$

* influx of C1

$J_{C2} = k_{C2} \cdot M_{C2}$

* influx of C2

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

$\alpha_c = \phi^{\text{max}} \cdot \gamma_0 v_A / (\gamma_0 + v_A)$

$\lambda_1 = \frac{\lambda_1 + \lambda_2 - 2\lambda_1 \lambda_2 / \lambda_c}{1 - \lambda_1 \lambda_2 / \lambda_c}$

E. coli with 1mM IPTG

E. coli grown on glycerol/succinate

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* influx of C1

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* influx of C2

pyruvate uptake

sucinate uptake

xylose uptake

E. coli grown on glycerol/succinate
Simultaneous utilization of two C-sources in steady state

Consider two substrates C1, C2, with single-substrate growth rate $\lambda_1, \lambda_2$

$$\lambda_{12} = \frac{\lambda_1 + \lambda_2 - 2\lambda_1\lambda_2/\lambda_C}{1 - \lambda_1\lambda_2/\lambda_C}$$

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$\lambda_{12} \approx \max\{\lambda_1, \lambda_2\}$ \quad \Rightarrow \quad \text{hierarchical utilization}
Q4: If cAMP-Crp is for proteome-metabolome coordination, what is responsible for hierarchical carbon usage?

Hierarchical usage of glycolytic substrates

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

Hierarchical usage of glycolytic substrates

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

steady-state growth on glucose using \textit{titratable PtsG}

\begin{itemize}
    \item \textit{takes the faster of the two growth rates}
    \item \textit{not specific to PTS transport (lac uptake: proton symport)}
\end{itemize}
Hierarchical usage of glycolytic substrates

- steady-state growth on lactose using *titratable LacY*
- steady-state growth on glycerol using *titratable GipFK*

- Takes the faster of the two growth rates
- Not specific to PTS transport (lac uptake: proton symport)

Hierarchical usage of glycolytic substrates

- 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)
Hierarchical usage of glycolytic substrates

steady-state growth on lactose using titratable LacY

use all available lactose

glycerol uptake regulated by $\lambda$

Hierarchical usage of glycolytic substrates

steady-state growth on lactose using titratable LacY

use all available lactose

glycerol uptake regulated by $\lambda$

tight crossover from sharp rise in $J_{\text{glyc}}(\lambda)$
Hierarchical usage of glycolytic substrates

Mechanism of sharp GR-dependence

slow growth

fast growth

Hierarchical usage of glycolytic substrates

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

Hierarchical usage of glycolytic substrates

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

but why hold back?

10 mM lactose / 40 mM glycerol

supplement-as-needed strategy with ultra-sensitive GR dependence via g3p-GlpR
**Growth transition kinetics** [Erickson, Schink, et al, Nature (2017)]

**Nutrient upshift**

![Graph showing mass (OD600) vs time (hr) for succinate and gluconate](image)

**Nutrient downshift (diauxic growth)**

![Graph showing glucose + succinate and succinate mass (OD600) vs time (hr)](image)

![Graph showing succinate growth rate (1/hr) vs time (hr)](image)

---

**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
Equation of motion for Growth transition kinetics:

\[
\frac{d}{dt} \gamma(t) = \nu_C(\gamma(t)) - \gamma(t) \frac{\nu_C}{\nu_R}(t)
\]

- from soln for \(\gamma(t)\), solve for \(\phi_R(t), \phi_C(t), \lambda(t), M(t)\)
- exact solution; completely determined by \(\lambda, \lambda_f\)

Kinetics of protein synthesis:

\[
\begin{align*}
\frac{d}{dt} M &= J_R = \gamma(t) M_R = \nu_C M_C \\
\frac{d}{dt} M_R &= \chi_R(t) J_R \\
\frac{d}{dt} M_C &= \chi_C(t) J_R
\end{align*}
\]

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

\(\Rightarrow\) regulation of ribosome synthesis: \(\chi_R(t) = \tilde{\chi}_R(\gamma(t))\)

\(\Rightarrow\) same form as in steady state: \(\tilde{\chi}_R(\gamma) = \phi_{R,0}/(1 - \gamma/\gamma_0)\)

\(\Rightarrow\) repeat for regulation of catabolic enzymes: \(\chi_C(t) = \tilde{\chi}_C(\gamma(t)) = \phi_{C,0}(\gamma(t))\)
Growth transition kinetics

“Equation of motion” for $\gamma(t) = \nu C(t) / \phi_R(t)$

$$\frac{d}{dt} \gamma = \gamma(t) \cdot [\nu C \dot{\lambda}_C(t) - \gamma(t) \dot{\lambda}_R(t)]$$

- from soln for $\gamma(t)$, solve for $\phi_R(t)$, $\phi_C(t)$, $\lambda(t)$, $M(t)$
- exact solution; completely determined by $\lambda_i$, $\lambda_f$

nutrient upshift

nutrient downshift

Growth transition kinetics

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
Growth transition kinetics
proteome-wide response to nutrient down-shift

downshift kinetics:
• rapid increase in C-protein synthesis
• rapid reduction of ribosome synthesis

Kinetics of individual metabolic sectors mostly follow the predicted form
more upshifts

more downshifts
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.

Growth inhibition by antibiotics

coarse-grained metabolism

C-influx $J_C$

Effect of translation-inhibiting drugs: inactivate ribosomes

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

$\frac{d}{dt} \gamma = \gamma(t) \cdot [\nu C \hat{\lambda}_C(t) - \gamma(t) \hat{\lambda}_R^{act}(t)]$

Cm addition:

growth slows but $\phi_R$ increases

[cf: nutrient upshift]
Growth inhibition by antibiotics [Wu et al, Ph.D. thesis (2022)]

Effect of translation-inhibiting drugs: inactivate ribosomes

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

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

Cm addition:
growth slows but \( \phi_R \) increases

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)

  – 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
Tenet of classical molecular biology:

molecular knowledge $\Rightarrow$ biological function

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

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