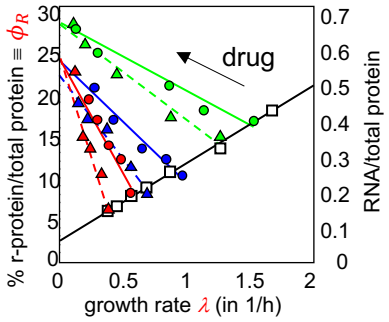
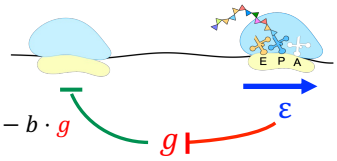
Laws of ribosome allocation



ppGpp-mediated model of flux sensing and growth control

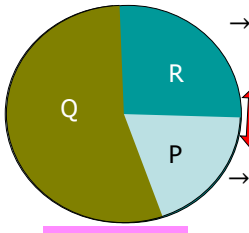
$\lambda = \varepsilon(g) \cdot \phi_R^{act}(g)$



$\phi_R^{act}(g) = \frac{a}{g} - b \cdot g$

➔ **What about control of ϕ_P ?**

Phenomenological theory of proteome allocation



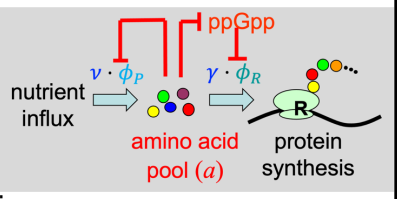
→ protein synthesis:
$\lambda(\gamma, \nu) = \gamma \cdot \phi_R$

R/P allocation according to γ, ν (state variables!)

→ nutrient uptake/synthesis:
$\lambda(\gamma, \nu) = \nu \cdot \phi_P$

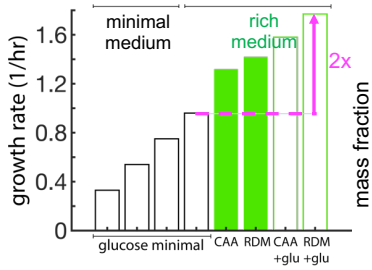
constraint: $\phi_P + \phi_R = \phi_R^{max}$

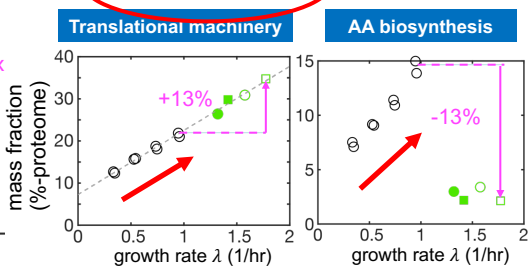
Qualitative mechanism:



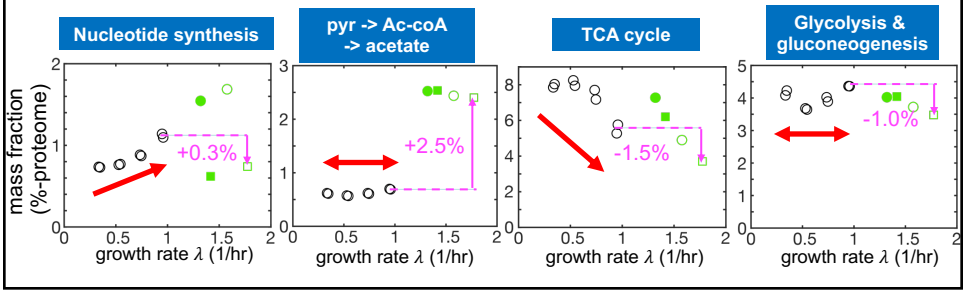
1

Proteome of *E. coli* in rich and minimal media





- increase in growth requires increased allocation to protein synthesis
- gained almost completely from proteome for AA biosynthesis
- changes in other functional groups small



2

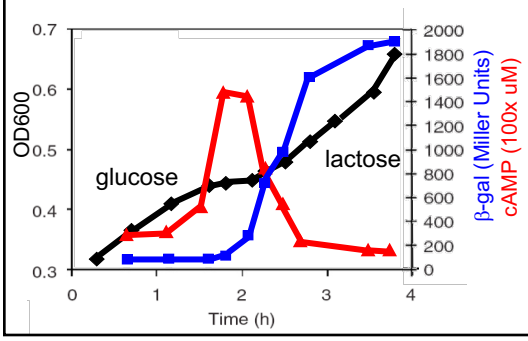
Topic 7: Catabolite repression & carbon hierarchy

• glucose effect: ability of glucose to inhibit the synthesis of certain enzymes
• all glucose-sensitive enzymes can convert their substrates to metabolites which can also be obtained more readily by the metabolism of glucose

➔ "catabolites" formed rapidly from glucose would accumulate and repress the formation of enzymes whose activity would augment the already large intracellular pools of these compounds [B. Magasanik, 1961]

carbon hierarchy (e.g., glucose-lactose diauxie)

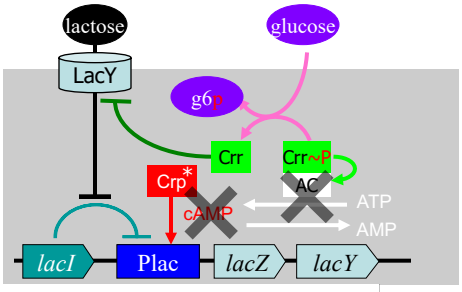
cAMP as a messenger ("inverse" of "catabolites")



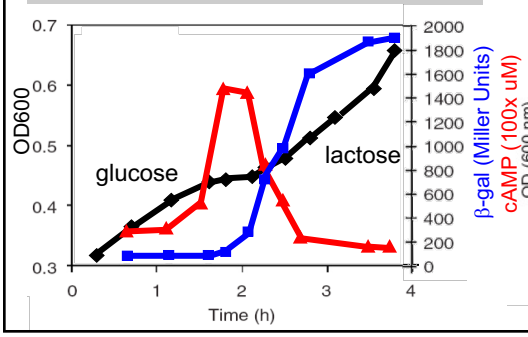
3

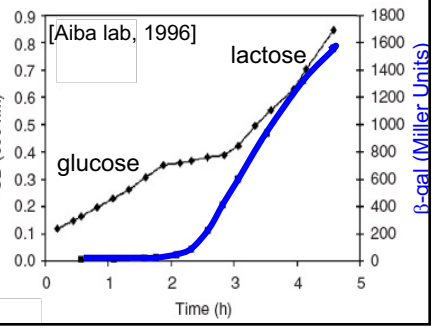
Catabolite repression & carbon hierarchy

Standard model



Problems with the standard model
• diauxie does not require cAMP
(mediated by "inducer exclusion"; later)

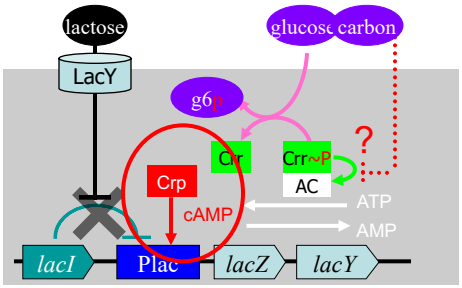




4

Catabolite repression

Standard model



[J. Mandelstam, 1962]

Carbon source	β-Galactosidase (units/mg. dry wt.)	Doubling time (min.)
Lactose	136	45
Glucose	200	48
Gluconate	260	50
Galactose	432	54
Glycerol	499	60
Succinate	600	65
Fructose	613	67
Lactate	816	65

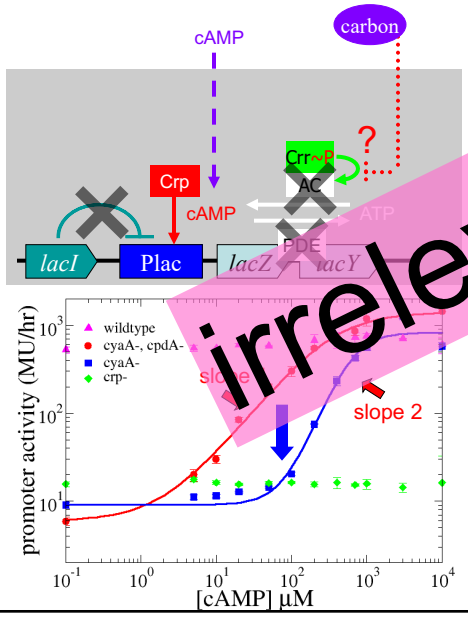
Problems with the standard model
- diauxie does not require cAMP
  (mediated by "inducer exclusion"; later)
- effect not specific to glucose

Q1: how is cAMP level controlled?
Q2: what are the "catabolites"?
Q3: physiological function?
Q4: what about carbon hierarchy?

5

Catabolite repression

Standard model



Bottom-up approach:

[Kuhlman et al, PNAS 2007]

- delete **AC** (*cyaA*) and vary ext cAMP
- Hill coeff ≈ 2
  incompatible w/ biochemistry and thermodynamic model of tsx control
  -- single Crr-binding site
  -- Crr activated by a single cAMP

irrelevant

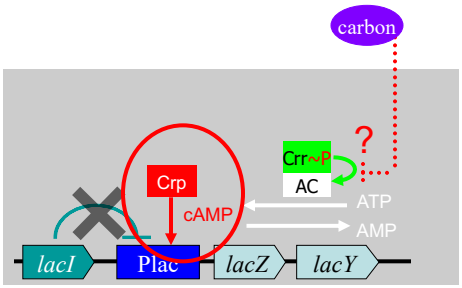
- cAMP degraded by **PDE** (*cpdA*)
- delete *cpdA*
- Hill coeff ≈ 1, agrees with tsx model

➔ role of PDE?
➔ mechanism of cooperativity?

6

Catabolite repression

Standard model



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[J. Mandelstam, 1962]

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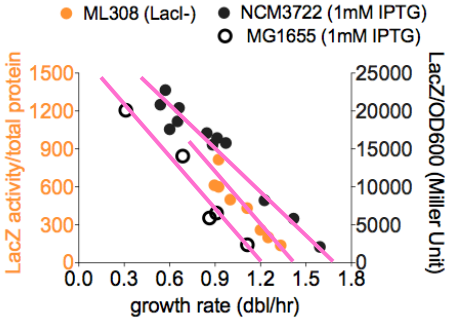


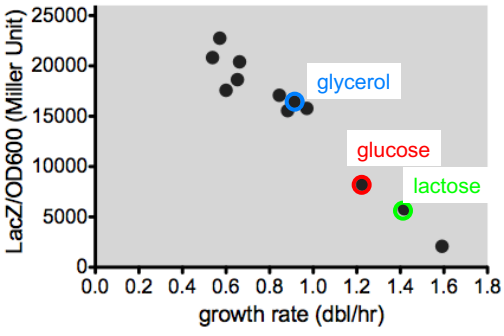
7

Physiological study of catabolite repression

E. coli K-12 NCM3722
(1mM IPTG)

C-source	dbl rate (dbl/h)	β-gal (Miller)
glucose6p +gluconate	1.59	2085
lactose	1.42	5803
glucose	1.22	8225
maltose	0.97	15789
glycerol	0.91	16431
pyruvate	0.88	15557
fructose	0.84	17080
succinate	0.66	20406
sorbitol	0.65	18636
mannose	0.60	17586
arabinose	0.57	22757
acetate	0.54	20834

[You et al, Nature 2013]

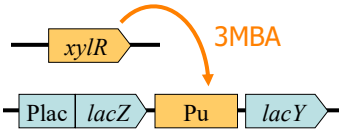


8

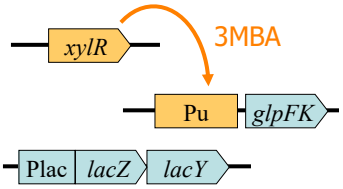
Physiological study of catabolite repression

E. coli K-12 NCM3722
(1mM IPTG)

titratable LacY expression



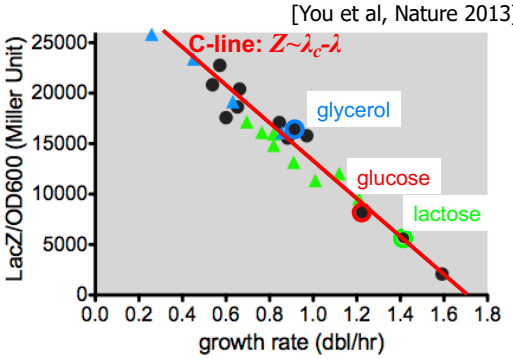
titratable GlpFK expression



➔ Up-regulation in response to reduced C-flux

Q: what about other types of growth limitation?
no effect according to known regulation

[You et al, Nature 2013]

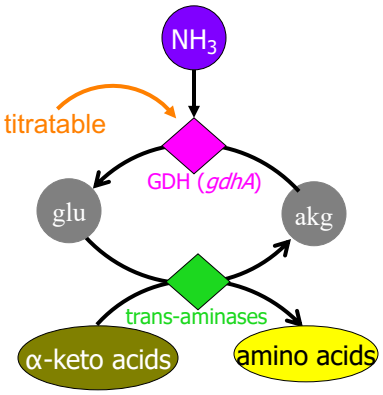


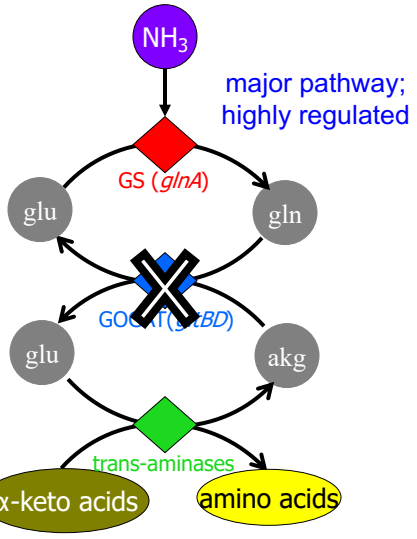
▲ lactose with titratable LacY expression
▲ glycerol with titratable GlpFK expression

9

Anabolic limitation (without affecting carbon)

two pathways of ammonium assimilation

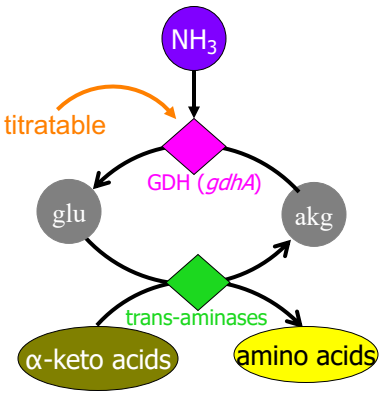




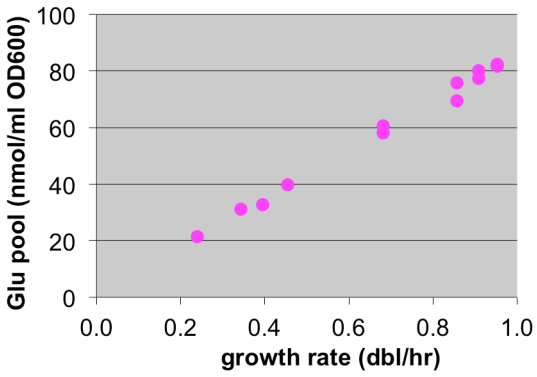
10

Anabolic limitation (without affecting carbon)

two pathways of ammonium assimilation



ΔGOGAT with titratable GDH: Glu limitation



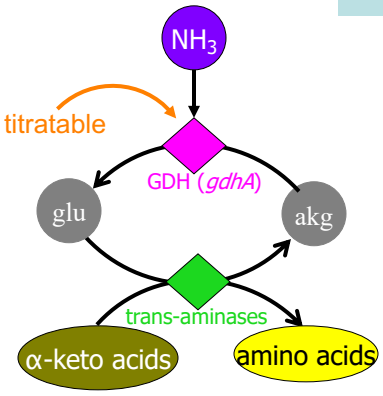
11

Physiological study of catabolite repression

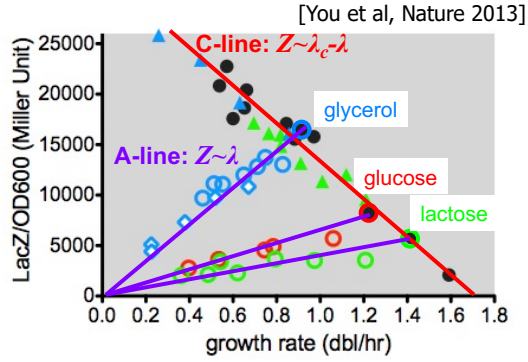
E. coli K-12 NCM3722
(1mM IPTG)

➔ Up-regulation in response to reduced C-flux

➔ Down-regulation of catabolism
upon other nutrient limitations (A-lines)



[You et al, Nature 2013]



▲ lactose with titratable LacY expression

▲ glycerol with titratable GlpFK expression

○○○ ΔgltD; titratable GDH; various C-sources

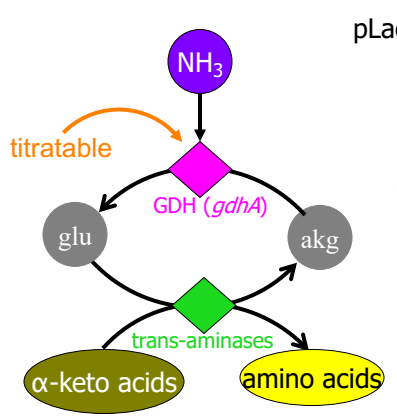
◇ S-limited chemostat; glycerol

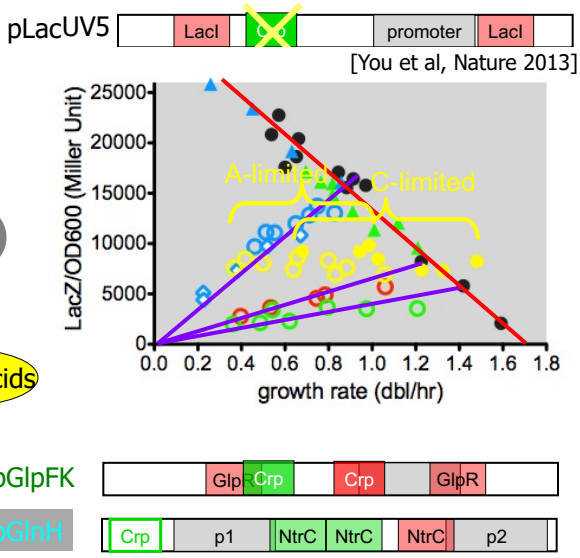
12

Physiological study of catabolite repression

E. coli K-12 NCM3722
(1mM IPTG)

Dependence on Crp-cAMP?
➔ both C- and A- lines require Crp-cAMP





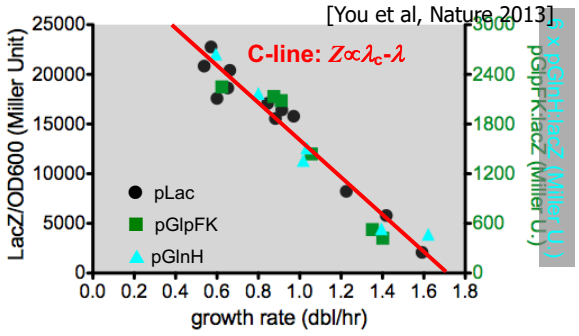
13

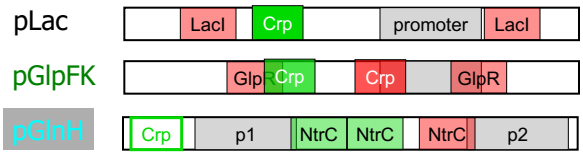
Physiological study of catabolite repression

Strain: NCM3722
(1mM IPTG)

C-source	dbl rate (dbl/h)	β-gal (Miller)
glucose6p +gluconate	1.59	2085
lactose	1.42	5803
glucose	1.22	8225
maltose	0.97	15789
glycerol	0.91	16431
pyruvate	0.88	15557
fructose	0.84	17080
succinate	0.66	20406
sorbitol	0.65	18636
mannose	0.60	17586
arabinose	0.57	22757
acetate	0.54	20834

Dependence on Crp-cAMP?
➔ both C- and A- lines require Crp-cAMP
➔ same C-line (λ_c) from different promoters

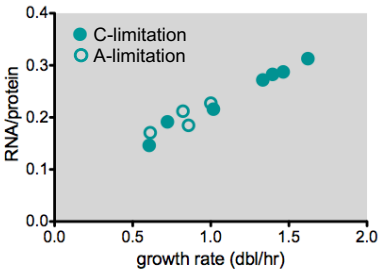


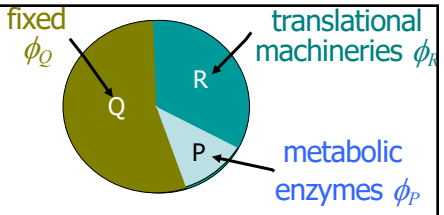


14

Model of proteome partition

[Scott et al, Science (2010)]

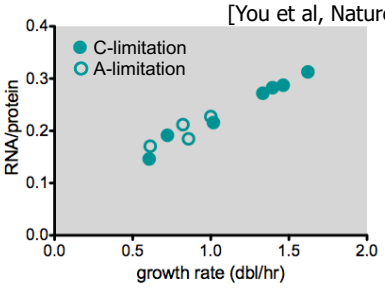


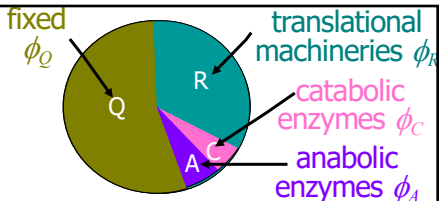


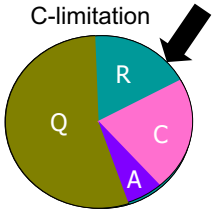
15

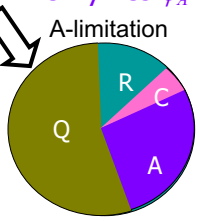
Model of proteome partition

[Scott et al, Science (2010)]
[You et al, Nature (2013)]

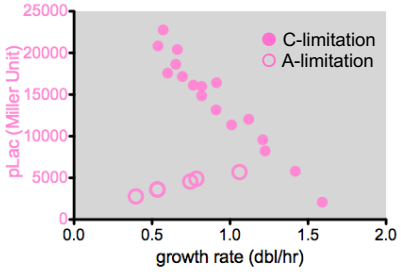




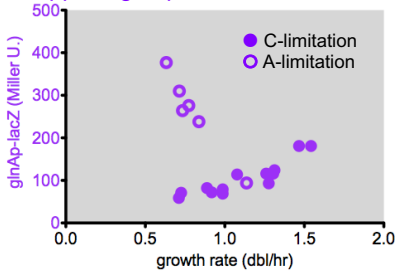




catabolic sector



➔ opposing dependence for the A-sector

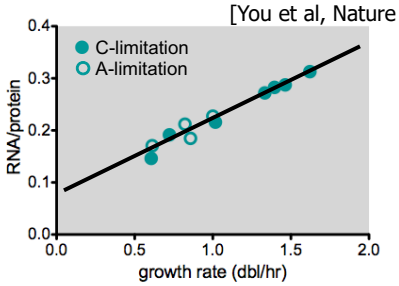


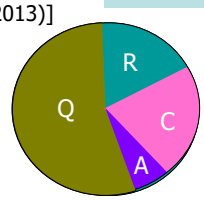
16

Model of proteome partition

Quantitative description

[Scott et al, Science (2010)]
[You et al, Nature (2013)]





constraint: $\phi_R + \phi_C + \phi_A = \phi_{max}$

protein synthesis:

$\lambda = \gamma \cdot \phi_R$

carbon influx:

$j_C = c_0 \lambda = k_C \cdot \phi_C$

nitrogen influx:

$j_N = n_0 \lambda = k_N \cdot \phi_A$

efficiency of C-uptake

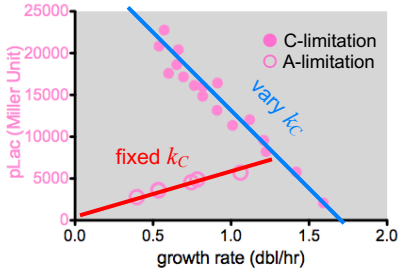
enzyme-limited

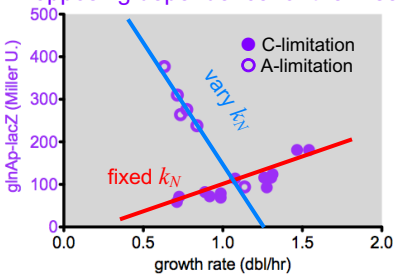
vary k_C: $\phi_C \propto \lambda_C - \lambda$; $\lambda_C \approx k_N \cdot \phi_{max}$

vary k_N: $\phi_A \propto \lambda_N - \lambda$; $\lambda_N \approx k_C \cdot \phi_{max}$

➔ opposing dependence for the A-sector

catabolic sector





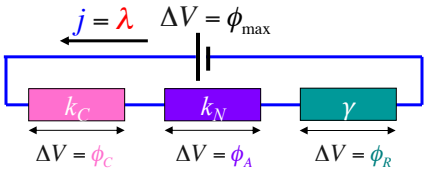
17

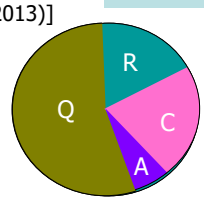
Model of proteome partition

Quantitative description

[Scott et al, Science (2010)]
[You et al, Nature (2013)]

Electrical circuit analogy:





constraint: $\phi_R + \phi_C + \phi_A = \phi_{max}$

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carbon influx:

$j_C = c_0 \lambda = k_C \cdot \phi_C$

nitrogen influx:

$j_N = n_0 \lambda = k_N \cdot \phi_A$

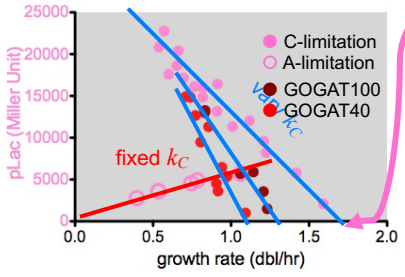
enzyme-limited

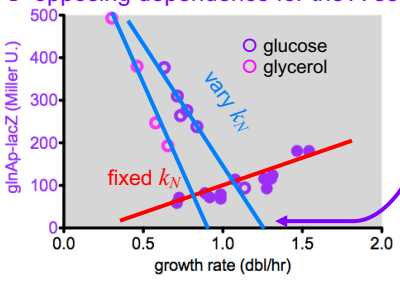
vary k_C: $\phi_C \propto \lambda_C - \lambda$; $\lambda_C \approx k_N \cdot \phi_{max}$

vary k_N: $\phi_A \propto \lambda_N - \lambda$; $\lambda_N \approx k_C \cdot \phi_{max}$

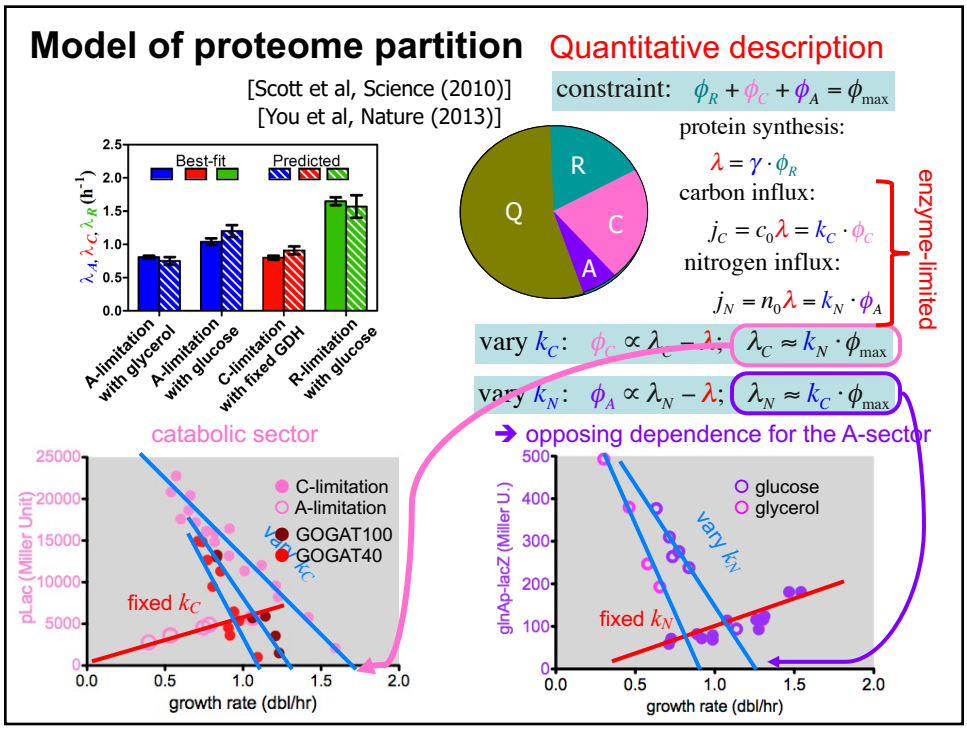
➔ opposing dependence for the A-sector

catabolic sector

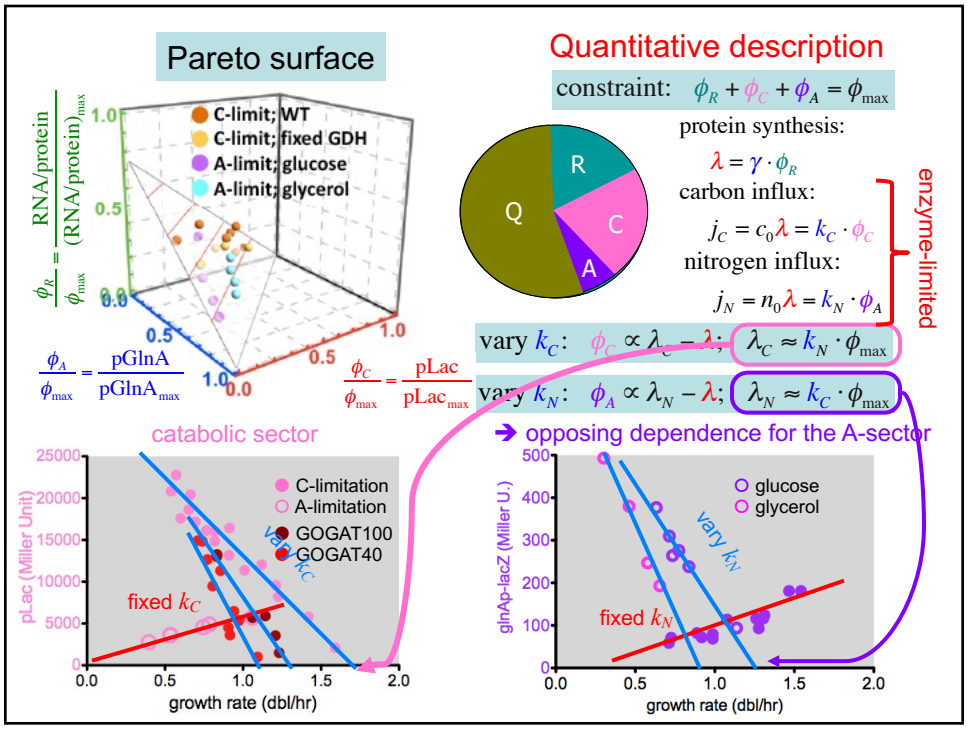




18


Model of proteome partition
Quantitative description
[Scott et al, Science (2010)]
[You et al, Nature (2013)]
Best-fit
Predicted
A-limitation with glycerol
A-limitation with glucose
C-limitation with fixed GDH
R-limitation with glucose
constraint: $\phi_R + \phi_C + \phi_A = \phi_{max}$
protein synthesis:
$\lambda = \gamma \cdot \phi_R$
carbon influx:
$j_C = c_0\lambda = k_C \cdot \phi_C$
nitrogen influx:
$j_N = n_0\lambda = k_N \cdot \phi_A$
enzyme-limited
R
C
A
Q
vary k_C: $\phi_C \propto \lambda_C - \lambda$; $\lambda_C \approx k_N \cdot \phi_{max}$
vary k_N: $\phi_A \propto \lambda_N - \lambda$; $\lambda_N \approx k_C \cdot \phi_{max}$
catabolic sector
opposing dependence for the A-sector
pLac (Miller Unit)
C-limitation
A-limitation
GOGAT100
GOGAT40
vary k_C
fixed k_C
growth rate (dbl/hr)
glnAp-lacZ (Miller U.)
glucose
glycerol
vary k_N
fixed k_N
growth rate (dbl/hr)

19


Pareto surface
Quantitative description
$\frac{\phi_R}{\phi_{max}} = \frac{RNA/protein}{(RNA/protein)_{max}}$
C-limit; WT
C-limit; fixed GDH
A-limit; glucose
A-limit; glycerol
$\frac{\phi_A}{\phi_{max}} = \frac{pGlnA}{pGlnA_{max}}$
$\frac{\phi_C}{\phi_{max}} = \frac{pLac}{pLac_{max}}$
constraint: $\phi_R + \phi_C + \phi_A = \phi_{max}$
protein synthesis:
$\lambda = \gamma \cdot \phi_R$
carbon influx:
$j_C = c_0\lambda = k_C \cdot \phi_C$
nitrogen influx:
$j_N = n_0\lambda = k_N \cdot \phi_A$
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C
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Q
vary k_C: $\phi_C \propto \lambda_C - \lambda$; $\lambda_C \approx k_N \cdot \phi_{max}$
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catabolic sector
opposing dependence for the A-sector
pLac (Miller Unit)
C-limitation
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GOGAT100
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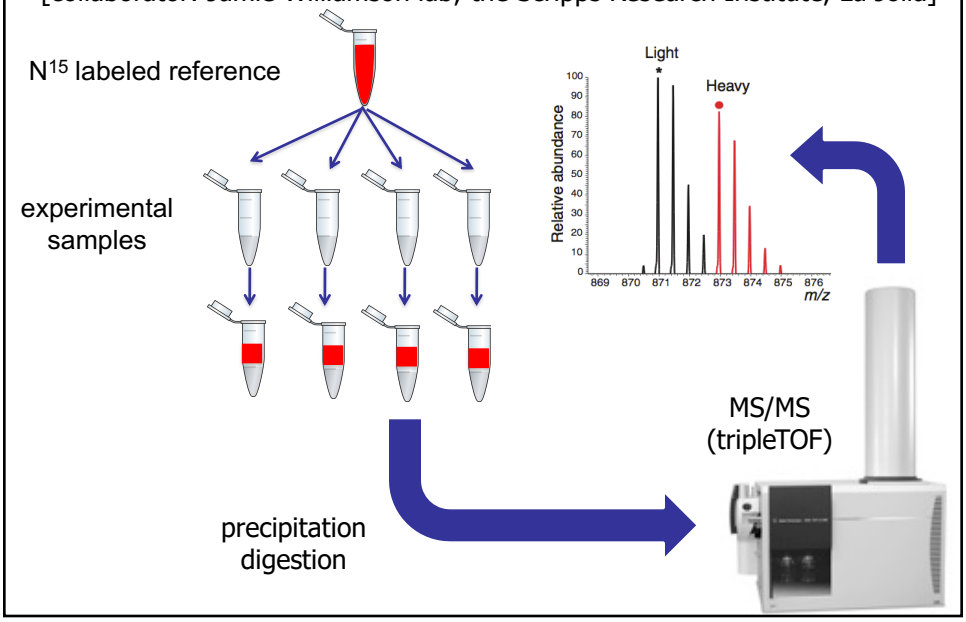
fixed k_C
growth rate (dbl/hr)
glnAp-lacZ (Miller U.)
glucose
glycerol
vary k_N
fixed k_N
growth rate (dbl/hr)

20

Proteome-wide survey by quantitative MS

[collaborator: Jamie Williamson lab; the Scripps Research Institute, La Jolla]

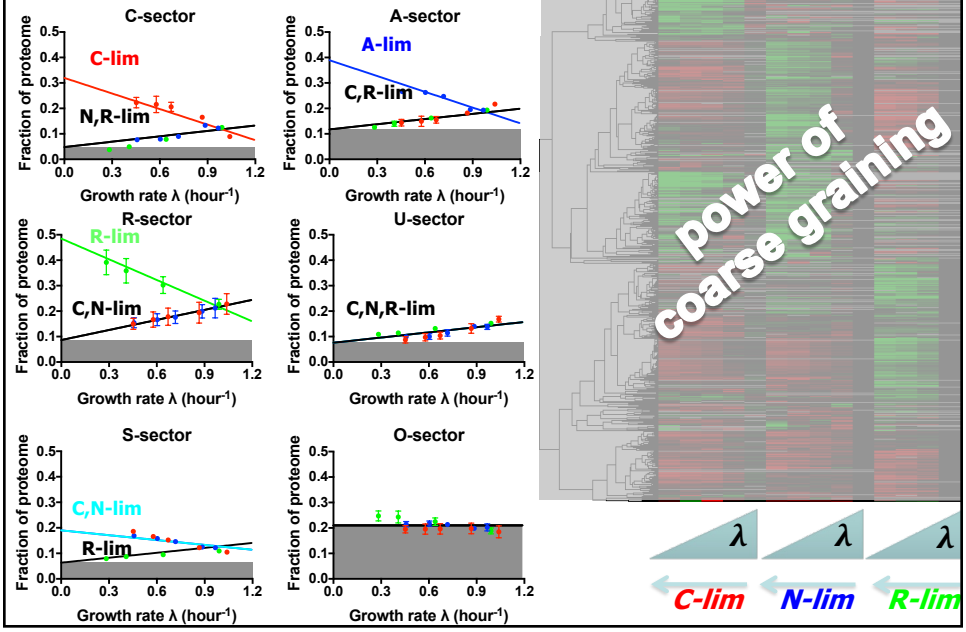


21

Quantitative Proteomics

[Hui et al, Mol Sys Biol. (2015)]

in collaboration with Jamie Williamson lab (TSRI)



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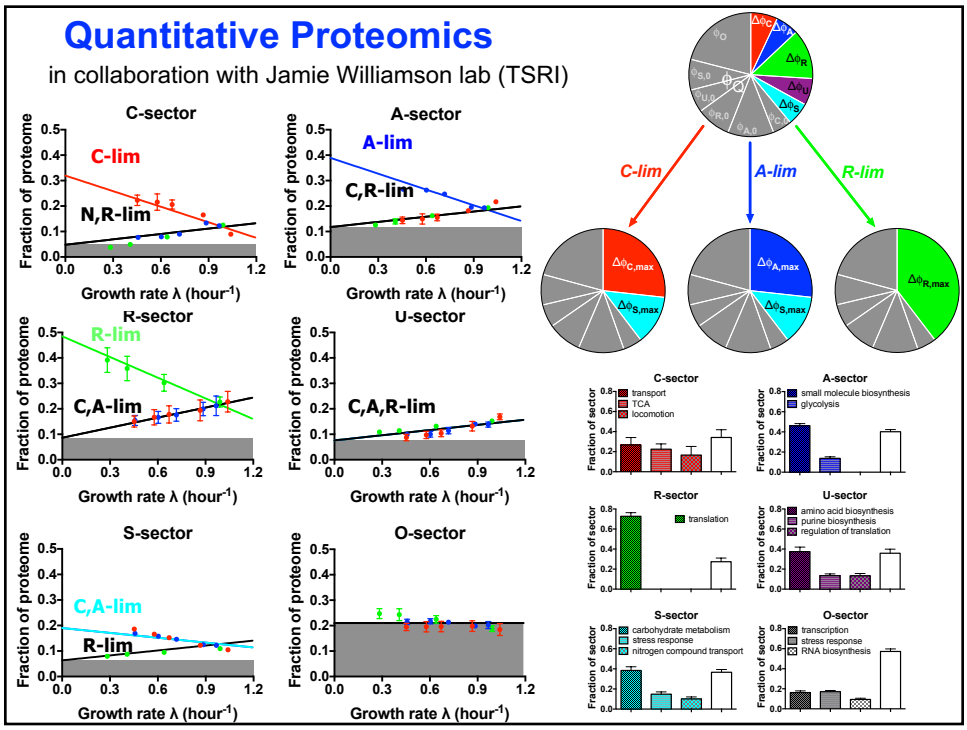

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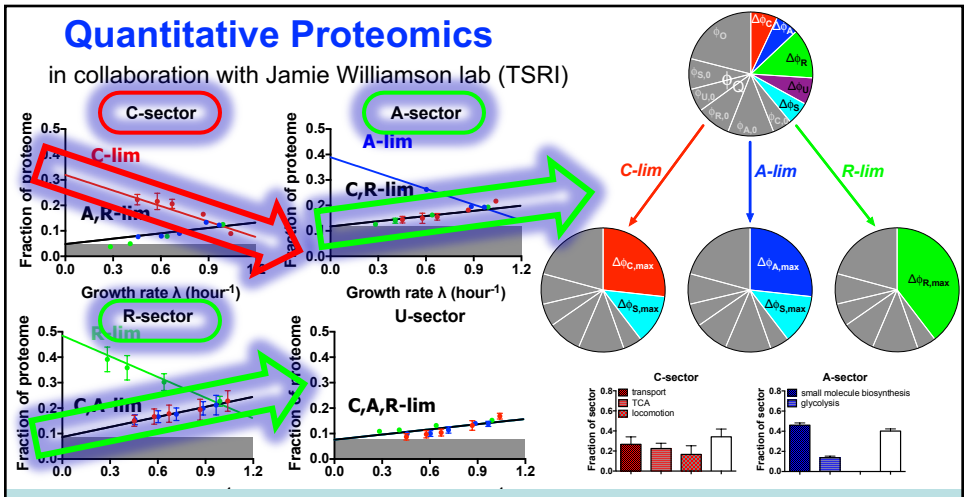


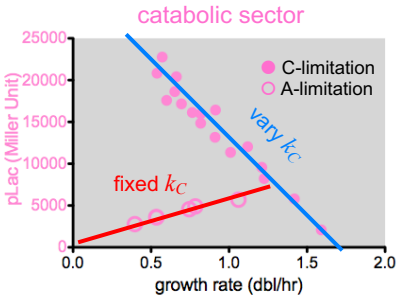
Q3: Physiological function of catabolite repression

A: proteome/metabolic coordination

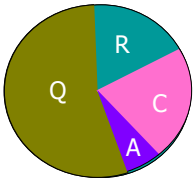
When C-influx is high, trade catabolic proteins for the ribosomal and anabolic proteins which are necessary for fast growth

25

Strategy of proteome-metabolome coordination



constraint: $\phi_R + \phi_C + \phi_A = \phi_{\max}$



protein synthesis:

$\lambda = \gamma \cdot \phi_R$

carbon influx:

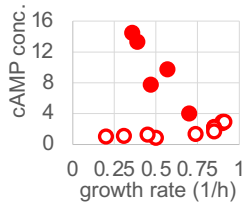
$j_C = c_0 \lambda = k_C \cdot \phi_C$

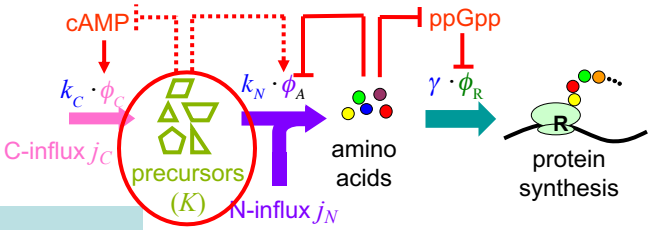
nitrogen influx:

$j_N = n_0 \lambda = k_N \cdot \phi_A$

enzyme-limited

Coarse-grained metabolic flux flow





$$\frac{d}{dt}K = j_C - j_N$$

← integral feedback

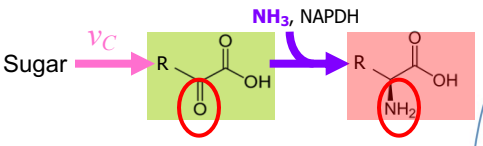
($j_C = j_N$ in steady state)

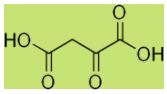
26

Strategy of proteome metabolic coordination

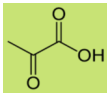
Simplest scenario: feedback by α-ketoacids (αKA)

All amino acids synthesized from amination of αKAs

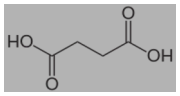




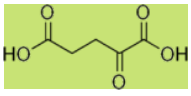
oxaloacetate



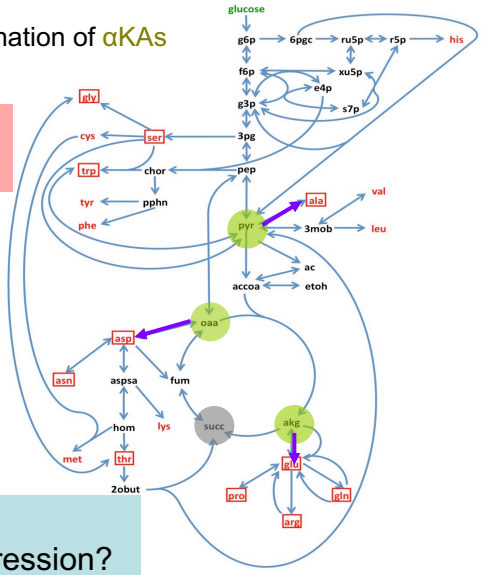
pyruvate



succinate



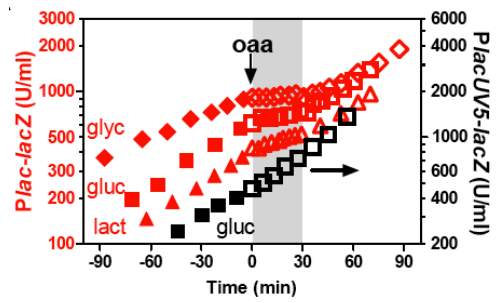
α-ketoglutarate

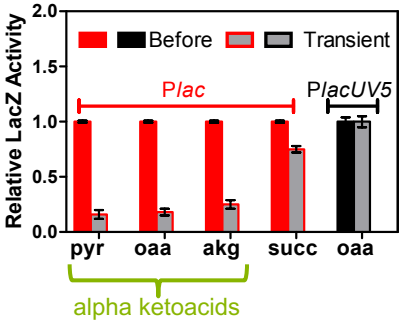


Test: does the addition of αKA affect catabolic gene expression?

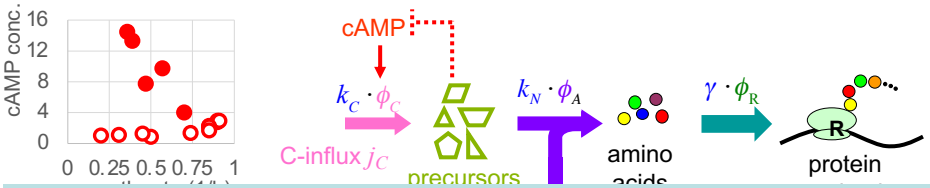
27

Testing the carbon precursor feedback model





Coarse-grained metabolic flux flow



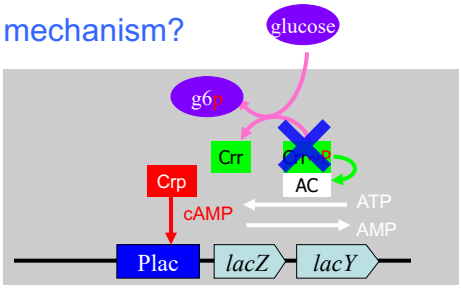
Q2: What are the "catabolites?

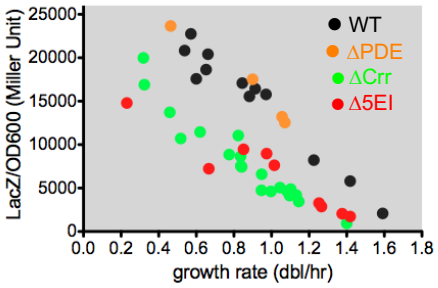
A: alpha ketoacids

28

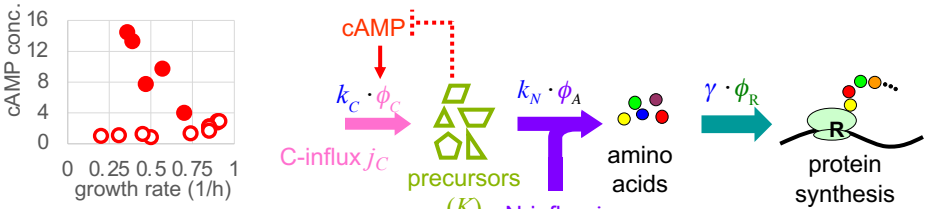
Testing the carbon precursor feedback model

mechanism?





Coarse-grained metabolic flux flow



$$\frac{d}{dt}K = j_C - j_N$$

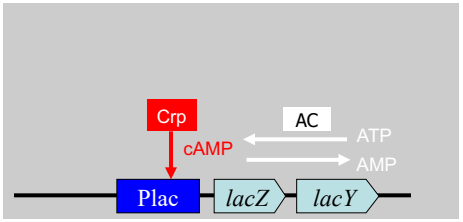
← integral feedback

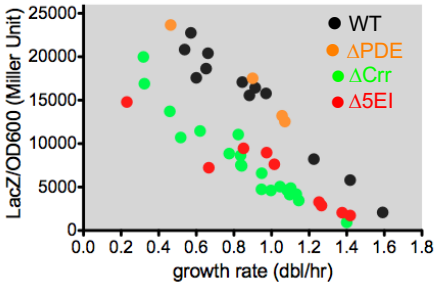
($j_C = j_N$ in steady state)

29

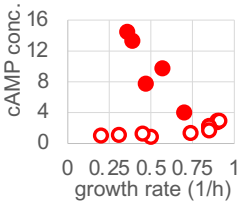
Testing the carbon precursor feedback model

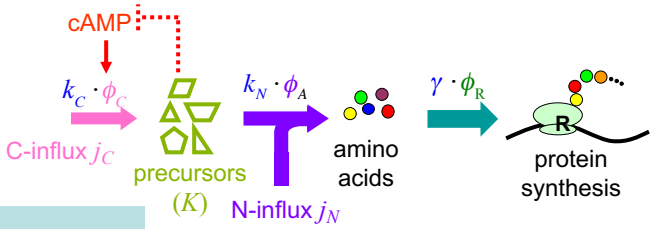
mechanism? PTS not necessary





Coarse-grained metabolic flux flow





$$\frac{d}{dt}K = j_C - j_N$$

← integral feedback

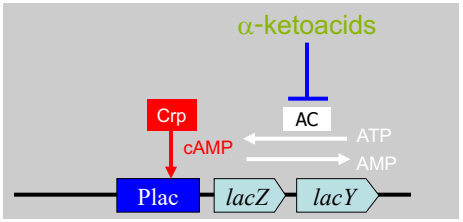
($j_C = j_N$ in steady state)

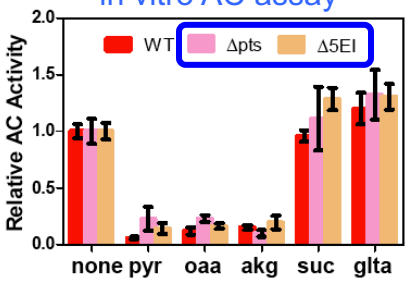
30

Testing the carbon precursor feedback model

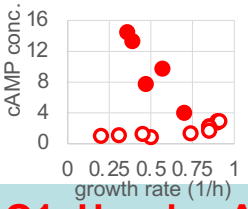
mechanism? PTS not necessary

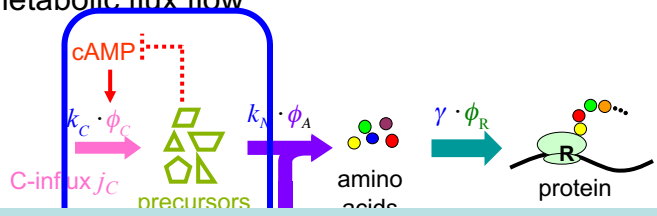
in vitro AC assay





Coarse-grained metabolic flux flow





Q1: How is cAMP level controlled?

A: direct inhibition of AC activity by alpha ketoacids

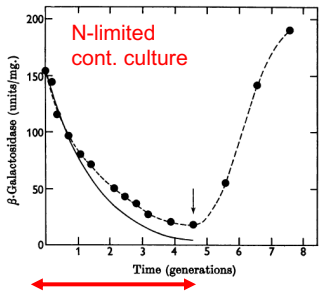
31

Catabolite repression

➔ "catabolites" formed rapidly from glucose would accumulate and repress the formation of enzymes whose activity would augment the already large intracellular pools of these compounds [B. Magasanik, 1961]

➔ qualitative effect of N-, S-, P- limitations known already in the early 60s

[Mandelstam, Magasanik, …]



➔ **Molecular Physiology**

32

Proc. Natl. Acad. Sci. USA
Vol. 73, No. 10, pp. 3476–3479, October 1976
Biochemistry

Catabolite modulator factor: A possible mediator of catabolite repression in bacteria

(physiological repression and derepression/β-galactosidase/adenosine 3′:5′-cyclic monophosphate)

AGNES ULLMANN, FRANCOISE TILLIER, AND JACQUES MONOD

ABSTRACT Water soluble extracts of *Escherichia coli* cells have been found to exert an extremely strong repressive effect upon the expression of catabolite sensitive operons. The compound responsible for this activity has been partially purified and proves to be of low molecular weight and heat stable. The effect of this compound, hereafter designated as catabolite modulator factor, is only partially antagonized by adenosine 3′:5′-cyclic monophosphate. The possible role of catabolite modulator factor in the physiological regulation of catabolite repression is discussed.

Citing Articles

Author(s): KOLB, A; BUSBY, S; BUC, H; GARGES, S; ADHYA, S
Title: TRANSCRIPTIONAL REGULATION BY CAMP AND ITS RECEPTOR PROTEIN
Source: ANNUAL REVIEW OF BIOCHEMISTRY, 62: 749-795 1993

33

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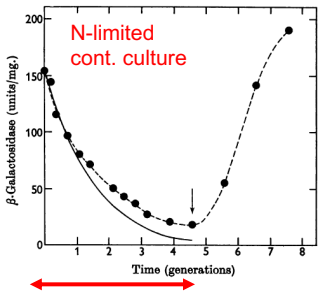
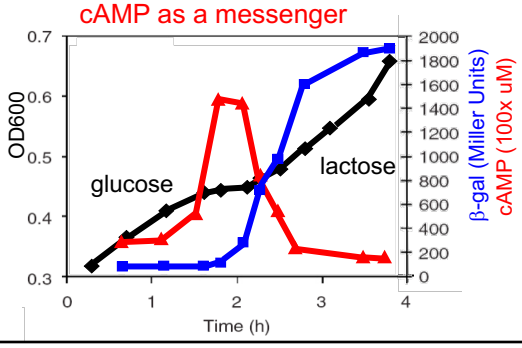


Molecular Physiology

cAMP as a messenger



34

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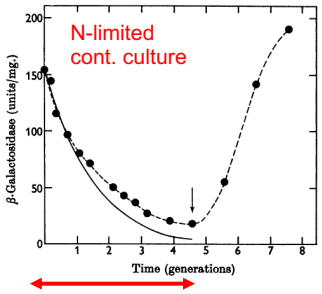
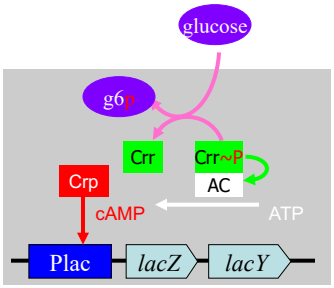


Molecular Physiology

Molecular Zoology



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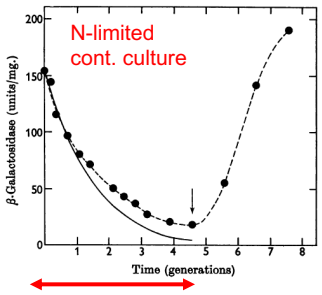
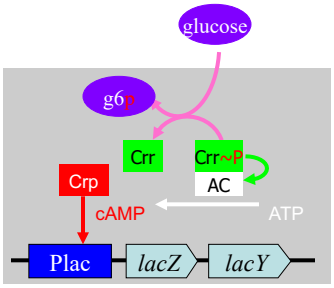
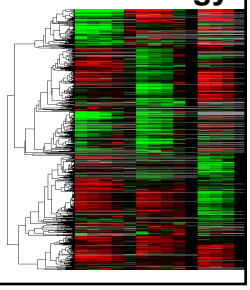


Molecular Physiology

Molecular Zoology ▪▪▪▪▪▶ **Systems Zoology**





36

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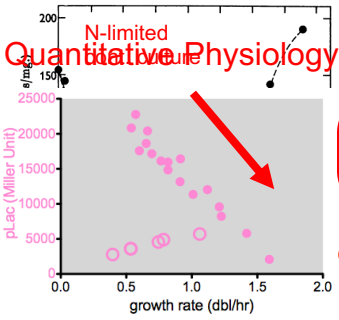
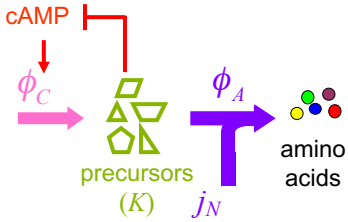


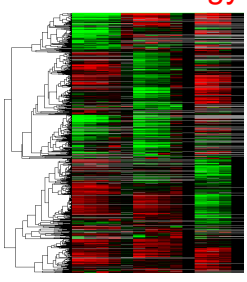
Quantitative Physiology

Molecular Physiology

Quantitative Systems Biology

HTP methodolgy





37