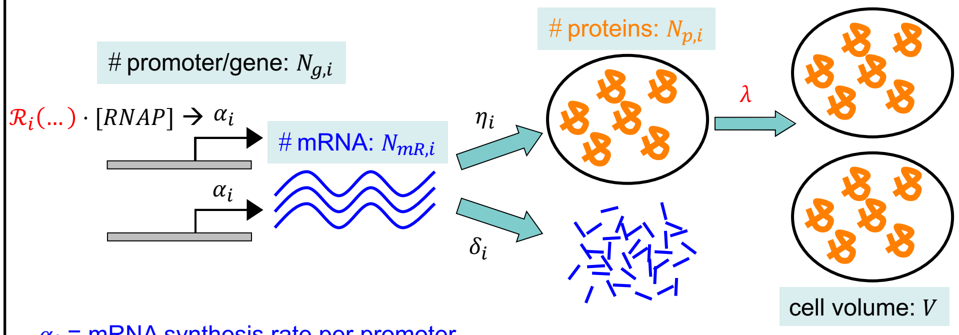
Topic 5: Global effects on gene expression



α_i = mRNA synthesis rate per promoter
δ_i = mRNA degradation rate
η_i = protein synthesis rate per mRNA
λ = cell growth rate (protein assumed stable)

steady-state protein conc

$$\boxed{[P_i]^* = [g_i]\frac{\alpha_i\eta_i}{\delta_i\lambda}} \propto \cancel{\frac{1}{\lambda}}$$

Q: growth-rate dependence for constitutive gene expression? $\sum_i [P_i] \approx const.$

conc of genes and gene products

$$[g_i] = N_{g,i}/V$$
$$[mR_i] = N_{mR,i}/V$$
$$[P_i] = N_{p,i}/V$$

$$\begin{cases} \dfrac{d}{dt}[mR_i] = \alpha_i[g_i] - \delta_i[mR_i] \\ \dfrac{d}{dt}[P_i] = \eta_i[mR_i] - \lambda\,[P_i] \end{cases}$$

Alternative formulation of protein synthesis :

$$\frac{d}{dt}[P_i] = \underbrace{\eta_i\psi_i/\bar{\eta}}_{\chi_i\text{, allocation of synthesis flux}} \cdot \underbrace{(\varepsilon/\ell)[Rb^*]}_{\text{total flux of protein synthesis}} - \lambda\,[P_i]$$

in steady state, in term of protein mass M_i:

$$\overbrace{(\varepsilon/l_{Rb})}^{\text{rate to translate r-proteins} \approx 8/h}\overbrace{(M^*_{Rb}/M_{Rb})}^{\text{active Rb fraction} \approx 90\% \text{ until slow growth}} = \text{tsl capacity}$$

$$\lambda M_i = \lambda\ell[P_i]V = \chi_i \cdot (\varepsilon/\ell_{Rb})M^*_{Rb} = \chi_i \cdot \gamma M_{Rb}$$

residues of all r-proteins (ℓ_{Rb}); total mass of r-proteins (M^*_{Rb})

➔ Apply to r-proteins (i.e., $i = R$): [Maaloe et al]

$$\lambda M_{Rb} = \chi_R \cdot \gamma M_{Rb} \Rightarrow \boxed{\lambda = \chi_R \cdot \gamma} \Rightarrow \boxed{\frac{M_{Rb}}{M_{tot}} \equiv \phi_R = \lambda/\gamma}$$

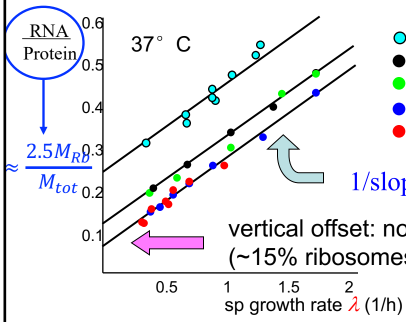
χ_R = fraction of ribosomes making ribosomes

- absolute max growth rate ($\chi_R = 1$) set by γ (5-6 min/dbl)
  [note: max doubling rate of E. coli = 20min/dbl ($\lambda = 2/h$)]
- change growth rate via χ_R (capitalism) or γ (socialism)

(cf: ribo-seq)

➔ direct estimate of χ_R: $\sum_i \lambda M_i \equiv \lambda M_{tot} = \gamma M_{Rb} \Rightarrow \boxed{\chi_R = \frac{M_{Rb}}{M_{tot}}}$

growth law: RNA/protein = $a \bullet \lambda + b$



- ● Aerobacter aerogenes (XXXV – Fraenkel & Neidhardt, 1961)
- ● Escherichia coli (B/r – Bremer & Dennis, 1996)
- ● Escherichia coli (15τ-bar – Forchhammer & Lindahl, 1970)
- ● Escherichia coli (B – Bennett & Maaløe, 1974)
- ● Escherichia coli (K12 – this study)

$1/\text{slope} \approx$ 8 Rb/hr $\approx$ 16 aa/sec $\approx \gamma$

vertical offset: non-translating ribosomes
(~15% ribosomes in 30S/50S forms)

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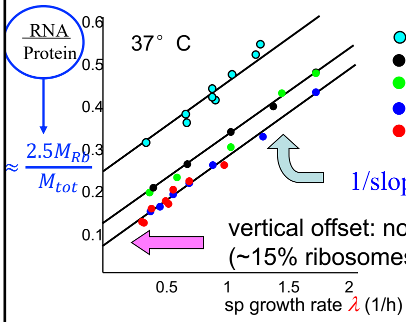
growth law: $M_{Rb}/M_{tot} \equiv \phi_R = \lambda/\gamma_0 + \phi_0$



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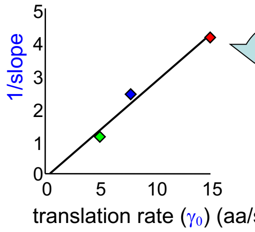
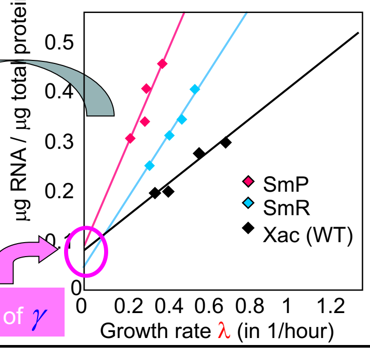
[Ruusala et al, 1984]



Translational mutant (S12)

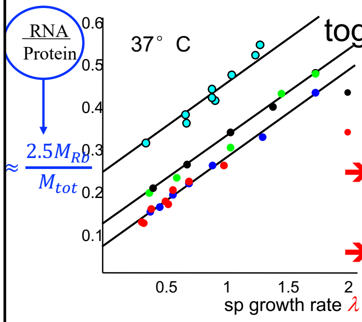


intercept (ϕ_0) independent of γ

growth law: $M_{Rb}/M_{tot} \equiv \phi_R = \lambda/\gamma_0 + \phi_0$

together with $M_{Rb}/M_{tot} = \lambda/\gamma$

$$\gamma = \gamma_0 \frac{\lambda}{\lambda + \phi_0\gamma_0}$$

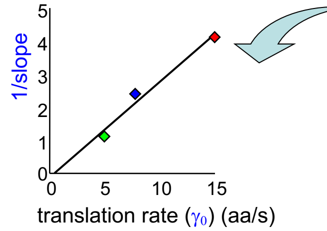


- $\gamma \approx \gamma_0$ and intercept (ϕ_0) negligible for $\lambda \gtrsim 0.5/h$
- $M_{Rb}/M_{tot} \approx \lambda/\gamma_0$

➔ ribosomes mostly engaged in translation
i.e,. capitalism at fast to med growth ($\lambda \geq 0.5/h$)

➔ reg. mechanisms exist to set χ, ϕ according to λ

[Ruusala et al, 1984]



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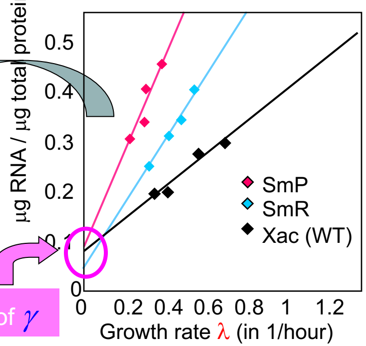
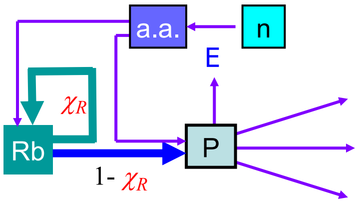


intercept (r_0) independent of γ

What should χ_R be set to for a given nutrient?



$$\lambda \cdot M_{Rb} = \chi_R \cdot \gamma \cdot M_{Rb}$$

$$\Rightarrow \boxed{\lambda = \chi_R \cdot \gamma}$$

$$\lambda \cdot M_{tot} = \gamma \cdot M_{Rb}$$

$$\Rightarrow \boxed{\phi_R \equiv \frac{M_{Rb}}{M_{tot}} = \frac{\lambda}{\gamma}}$$

- include non-Rb proteins P
  (provides for transport & metabolism)
- assume Rb all active ($\gamma \approx \gamma_0$)
- include nutrient input (e.g., a.a. limited growth)

➔ flux balance at steady-state:

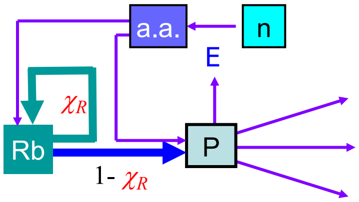
$$\boxed{\gamma \cdot M_{Rb} = \nu \cdot M_P}$$

a.a. consumption = a.a. supply

E: bottleneck enzyme

$$\gamma \cdot M_{Rb} \qquad k_E^{cat} \cdot M_E \cdot \frac{n}{n+K_E} = \underbrace{\left(k_E^{cat} \cdot \frac{M_E}{M_P} \cdot \frac{n}{n+K_n}\right)}_{\nu} \cdot M_P$$

What should χ_R be set to for a given nutrient?



$$\lambda \cdot M_{Rb} = \chi_R \cdot \gamma \cdot M_{Rb}$$

$$\Rightarrow \boxed{\lambda = \chi_R \cdot \gamma}$$

$$\lambda \cdot M_{tot} = \gamma \cdot M_{Rb}$$

$$\Rightarrow \boxed{\phi_R \equiv \frac{M_{Rb}}{M_{tot}} = \frac{\lambda}{\gamma}} \qquad \boxed{\phi_P = \frac{\lambda}{\nu}}$$

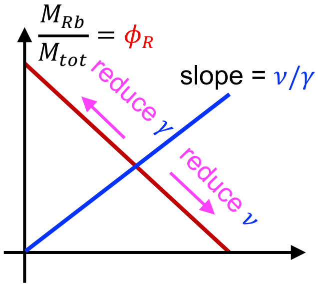
- include non-Rb proteins P (provides for transport & metabolism)
- assume Rb all active ($\gamma \approx \gamma_0$)

Q: Do γ and ν act as independent 'variables'?

$$\lambda = \phi_R \cdot \gamma = \frac{\gamma \cdot \nu}{\gamma + \nu}$$

$$\boxed{\frac{M_{Rb}}{M_{tot}} \equiv \phi_R = \frac{\nu}{\gamma + \nu}}$$

$$\boxed{\frac{M_P}{M_{tot}} \equiv \phi_P = \frac{\gamma}{\gamma + \nu}}$$

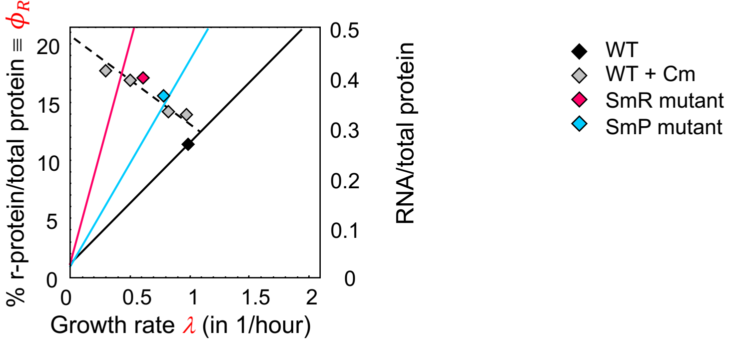


$$\frac{M_P}{M_{tot}} = \phi_P$$

$$\boxed{\gamma \cdot M_{Rb} = \nu \cdot M_P}$$

$$\boxed{\frac{M_{Rb}}{M_{tot}} + \frac{M_P}{M_{tot}} = 1}$$

modulate translation rate γ for fixed nutrient



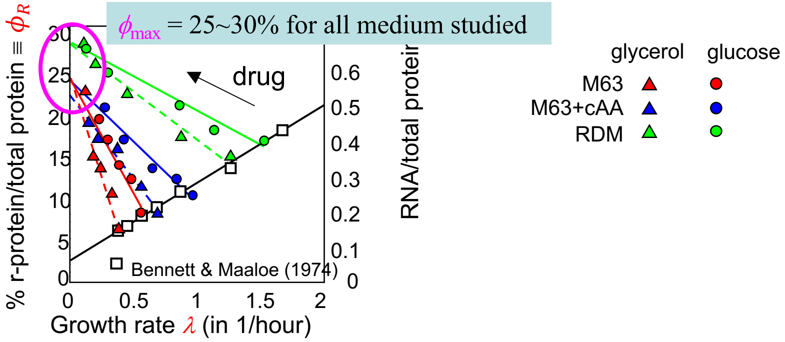
for expt with saturating nutrients: $\nu \approx \nu_0 = k_E^{cat} M_E / M_P$

➔ empirical determination of ν from $\boxed{\phi_R = 1 - \phi_P = 1 - \lambda/\nu}$

– vary γ while keeping nutrient fixed

– obtain $\nu(\gamma)$ from correlation plot of ϕ_R vs λ

modulate translation rate γ for fixed nutrient



$\phi_{\max}$ = 25~30% for all medium studied

- similar effects from sublethal dose of Cm
- <u>linear</u> relation obtained: $\phi_R = \phi_{\max} - \lambda/\nu$

for expt with saturating nutrients: $\nu \approx \nu_0 = k_E^{cat} M_E / M_P$

➔ empirical determination of ν from $\boxed{\phi_R = 1 - \phi_P = 1 - \lambda/\nu}$

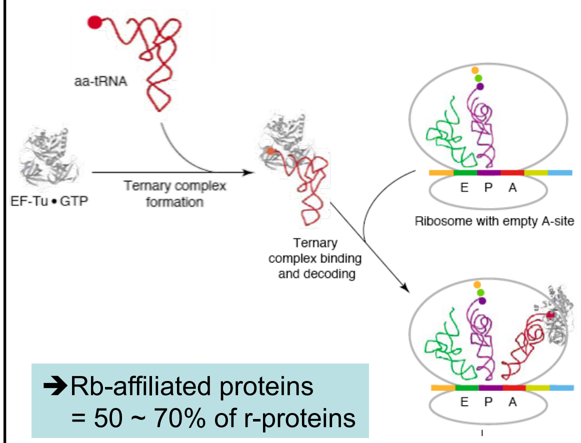
  – vary γ while keeping nutrient fixed
  – obtain $\nu(\gamma)$ from correlation plot of ϕ_R vs λ

➔ ν only weakly affected by γ (“orthogonal” perturbation)
➔ empirically quantifies “nutrient quality” ν; note $M_E \propto M_P$
➔ however, y-intercept < 1

➔ $\phi_{\max}$ = 25~30%: importance of other proteins

$\phi = \phi_{\max} - \lambda / \nu$

e.g., EF-TU needed to escort every charged-tRNA
(~5 TU/Rb @ 40min doubling)

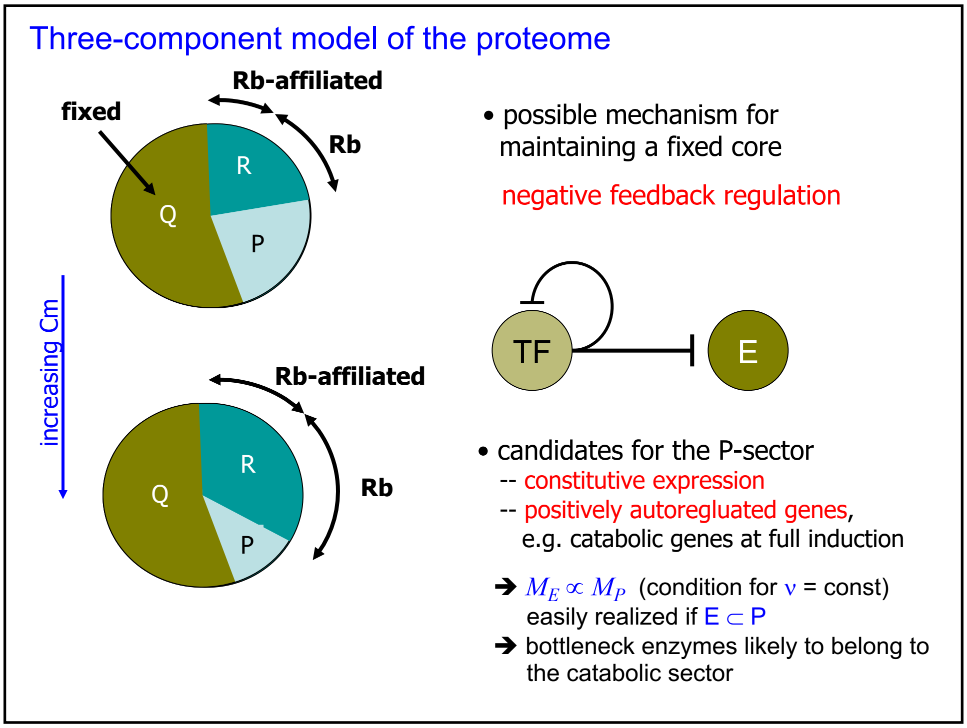


➔Rb-affiliated proteins
= 50 ~ 70% of r-proteins

➔ other nonribosomal core proteins

essential translational proteins

Protein	Mol wt (10^3)	α_i^a ($t = 40$ m (%)
r-Protein	850	13.5
L7/L12	12	0.81
EF-Tu	42	5.55
EF-G	84	1.66
EF-Ts	31	0.13
IF1	8	0.04
IF2	115	0.52
IF3	20	0.07
Leu S	100	0.12
Phe S-β	94	0.21
Lys S	58	0.11
Arg S	58	0.08
Gly S	77	0.17
Val S	106	0.14
Glu S-β	48	0.10
Ile S	107	0.24
Phe S-α	36	0.11
Gln S	61	0.11
Thr S	65	0.09
RNA polymerase β	150	0.52
RNA polymerase α	39	0.37
RNA polymerase, core	375	1.30



Three-component model of the proteome

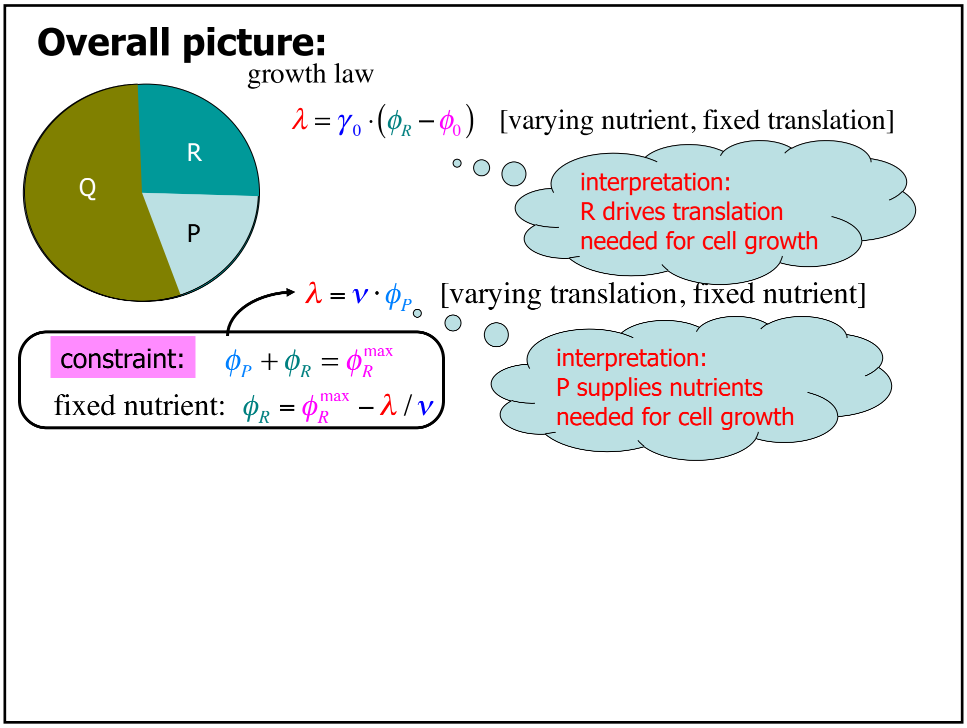
- possible mechanism for maintaining a fixed core

negative feedback regulation

- candidates for the P-sector
  - -- constitutive expression
  - -- positively autoregluated genes, e.g. catabolic genes at full induction

➔ $M_E \propto M_P$ (condition for ν = const) easily realized if $E \subset P$

➔ bottleneck enzymes likely to belong to the catabolic sector



Overall picture:

growth law

$\lambda = \gamma_0 \cdot (\phi_R - \phi_0)$ [varying nutrient, fixed translation]

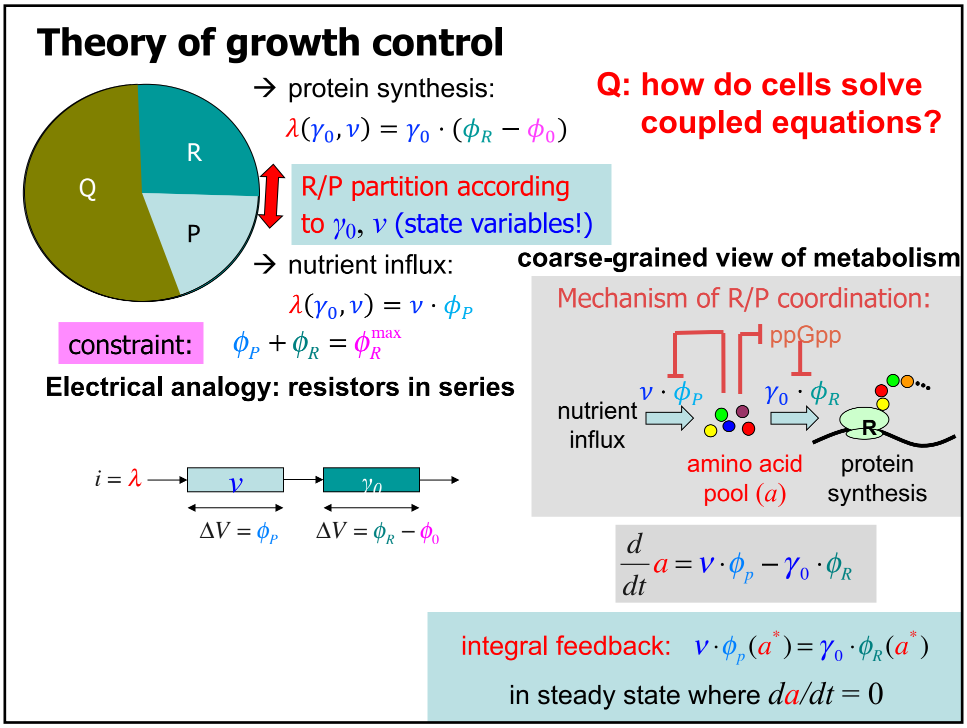
interpretation:
R drives translation
needed for cell growth

$\lambda = \nu \cdot \phi_P$ [varying translation, fixed nutrient]

interpretation:
P supplies nutrients
needed for cell growth

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

fixed nutrient: $\phi_R = \phi_R^{\max} - \lambda / \nu$



Theory of growth control

→ protein synthesis:

$\lambda(\gamma_0,\nu) = \gamma_0 \cdot (\phi_R - \phi_0)$

R/P partition according to γ_0, ν (state variables!)

→ nutrient influx:

$\lambda(\gamma_0,\nu) = \nu \cdot \phi_P$

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

Electrical analogy: resistors in series

Q: how do cells solve coupled equations?

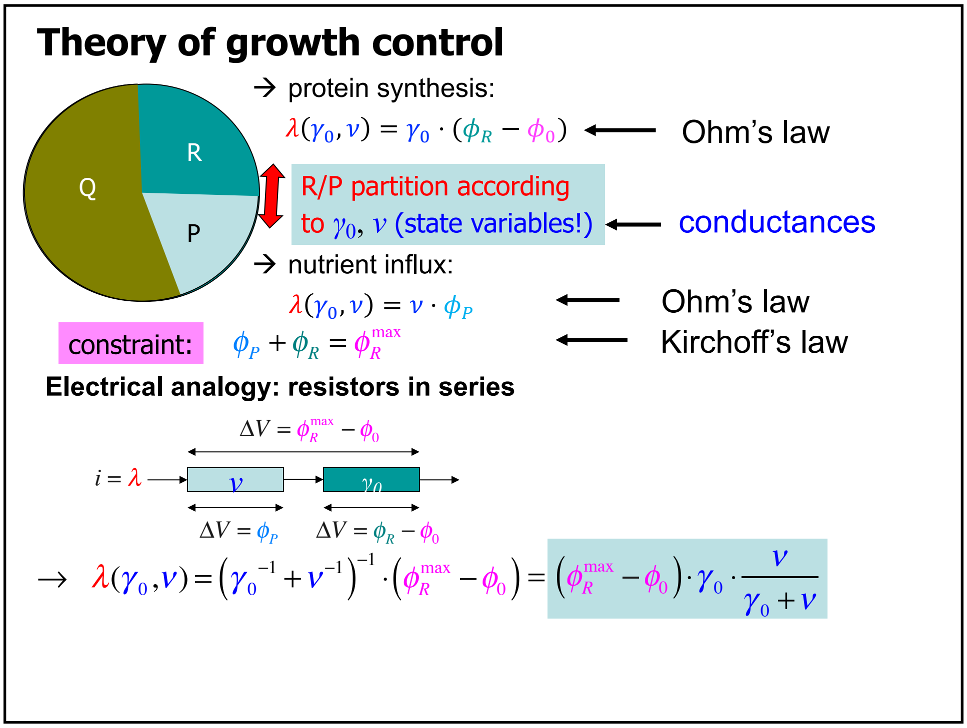
coarse-grained view of metabolism

Mechanism of R/P coordination:

$$\frac{d}{dt}a = \nu \cdot \phi_p - \gamma_0 \cdot \phi_R$$

integral feedback: $\nu \cdot \phi_p(a^*) = \gamma_0 \cdot \phi_R(a^*)$

in steady state where $da/dt = 0$



Theory of growth control

→ protein synthesis:

$\lambda(\gamma_0,\nu) = \gamma_0 \cdot (\phi_R - \phi_0)$ ⟵ Ohm's law

R/P partition according to γ_0, ν (state variables!) ⟵ conductances

→ nutrient influx:

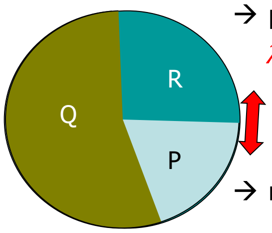
$\lambda(\gamma_0,\nu) = \nu \cdot \phi_P$ ⟵ Ohm's law

constraint: $\phi_P + \phi_R = \phi_R^{\max}$ ⟵ Kirchoff's law

Electrical analogy: resistors in series

$$\rightarrow \quad \lambda(\gamma_0,\nu) = \left(\gamma_0^{-1} + \nu^{-1}\right)^{-1} \cdot \left(\phi_R^{\max} - \phi_0\right) = \left(\phi_R^{\max} - \phi_0\right) \cdot \gamma_0 \cdot \frac{\nu}{\gamma_0 + \nu}$$

Theory of growth control



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→ nutrient influx:

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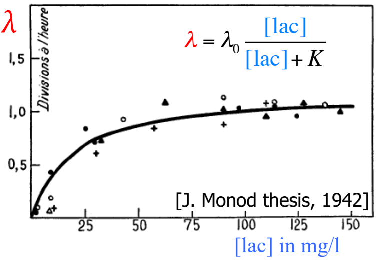
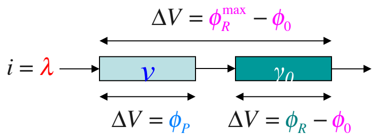


$$\lambda = \lambda_0 \frac{[\text{lac}]}{[\text{lac}] + K}$$

[J. Monod thesis, 1942]

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

Electrical analogy: resistors in series



$\Delta V = \phi_R^{\max} - \phi_0$

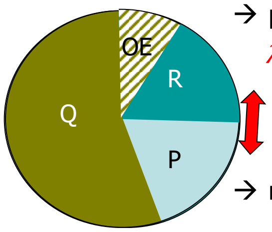
$i = \lambda$

$\Delta V = \phi_P$ $\quad \Delta V = \phi_R - \phi_0$

Monod growth formula

$$\rightarrow \lambda(\gamma_0, \nu) = \left(\gamma_0^{-1} + \nu^{-1}\right)^{-1} \cdot \left(\phi_R^{\max} - \phi_0\right) = \underbrace{\left(\phi_R^{\max} - \phi_0\right) \cdot \gamma_0}_{\lambda_{\max} \approx 3.5 \text{ dbl/hr}} \cdot \frac{\nu}{\gamma_0 + \nu}$$

Test: cost of protein overexpression



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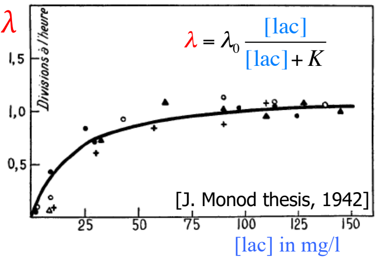

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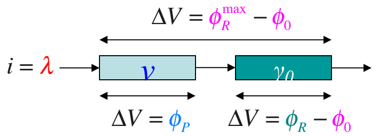

$\Delta V = \phi_R^{\max} - \phi_0$

$i = \lambda$

$\Delta V = \phi_P$ $\quad \Delta V = \phi_R - \phi_0$

Monod growth formula

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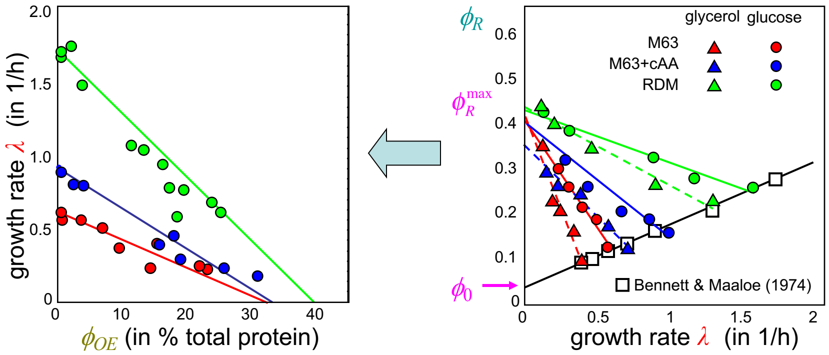
$\rightarrow$ protein overexpression: $\phi_R^{\max} \rightarrow \phi_R^{\max} - \phi_{OE}$

$$\lambda(\phi_{OE}; \gamma_0, \nu) = \lambda(0; \gamma_0, \nu) \cdot \left[1 - \phi_{OE} / (\phi_R^{\max} - \phi_0)\right]$$

Test: cost of protein overexpression

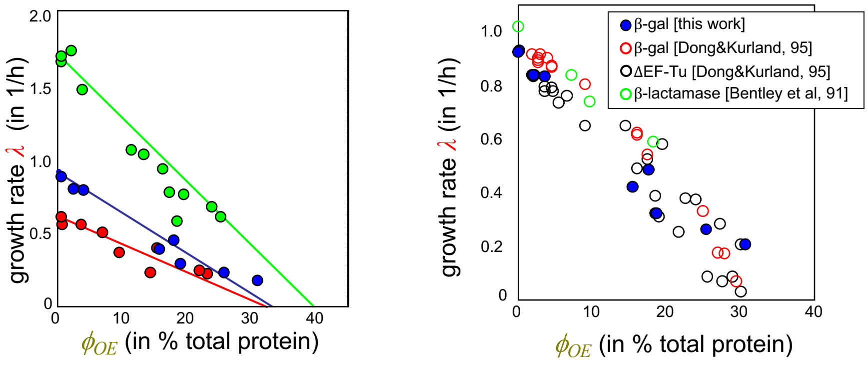


$$\rightarrow \lambda(\gamma_0,\nu) = \left(\gamma_0^{-1}+\nu^{-1}\right)^{-1}\cdot\left(\phi_R^{\max}-\phi_0\right) = \left(\phi_R^{\max}-\phi_0\right)\cdot\gamma_0\cdot\frac{\nu}{\gamma_0+\nu}$$

→ protein overexpression: $\phi_R^{\max} \rightarrow \phi_R^{\max}-\phi_{OE}$

$$\lambda(\phi_{OE};\gamma_0,\nu)=\lambda(0;\gamma_0,\nu)\cdot\left[1-\phi_{OE}/(\phi_R^{\max}-\phi_0)\right]$$

Test: cost of protein overexpression



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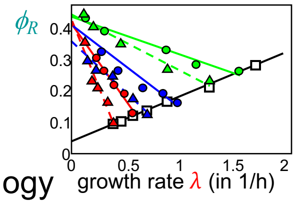
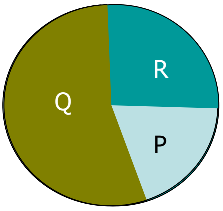
→ protein overexpression: $\phi_R^{\max} \rightarrow \phi_R^{\max}-\phi_{OE}$

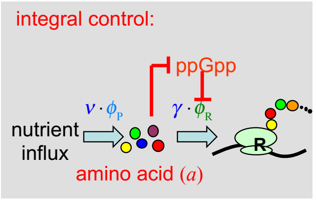
$$\lambda(\phi_{OE};\gamma_0,\nu)=\lambda(0;\gamma_0,\nu)\cdot\left[1-\phi_{OE}/(\phi_R^{\max}-\phi_0)\right]$$

Summary:

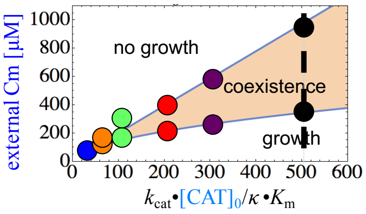


- quantitative laws exist in biology
- result from ribosome allocation constraint
- 3-component model captures phenomenology
- R/P coordination from integral feedback control
- laws lead to new understanding and novel predictions (useless proteins, antibiotic response, metabolic overflow, …)





[Deris et al, Science 2013]



➔ further applications: catabolite repression, growth shift kinetics

➔ how does a cell sense its growth rate?