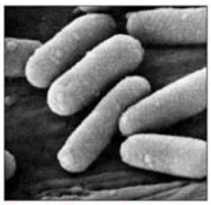
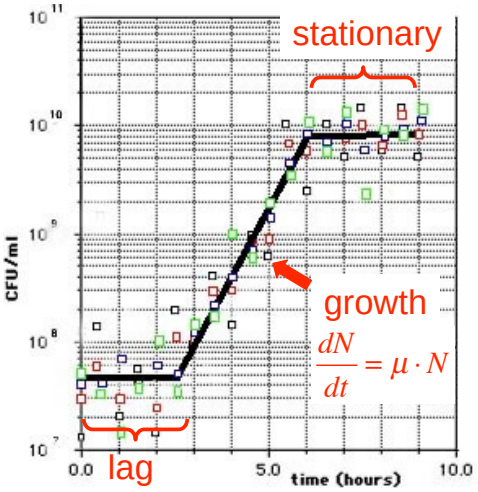
Bacterial Growth





"...the growth of bacterial cultures, despite the immense complexity of the phenomena to which it testifies, generally obeys relatively simple laws, which make it possible to define certain quantitative characteristics of the growth cycle...The accuracy, the ease, the reproducibility of bacterial growth constant determinations is remarkable and probably unparalleled, so far as quantitative biological characteristics are concerned."

-- J. Monod (1949)

JOURNAL OF BACTERIOLOGY, Dec. 1999, p. 7405–7408
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Bacterial Growth: Constant Obsession with *dN/dt*

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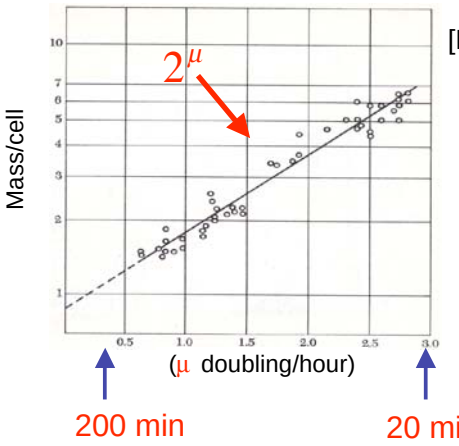
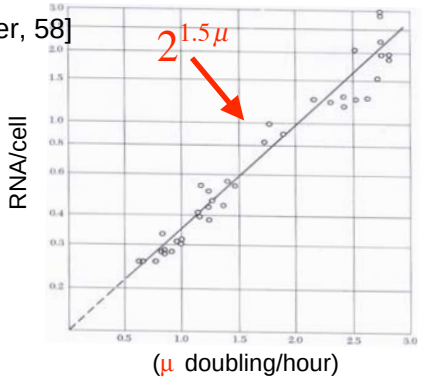
Effect of the growth media

- different growth rates in different media:
  20 min (rich medium) to 200 min (minimal medium)



[M. Schacter, 58]

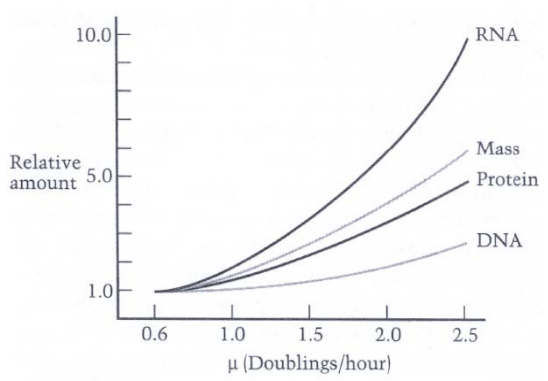


- cell mass ~ cell size can change > 5x
- increases exponentially with growth rate μ ← universal speed limit!
- cellular RNA content ~ ribosome abundance increases more rapidly

➔ dependence on the medium through growth rate μ only -- universal!

➔ macromolecular composition (e.g., RNA:protein ratio) is strongly dependent on the growth rate

growth rate dependence of macromolecular composition

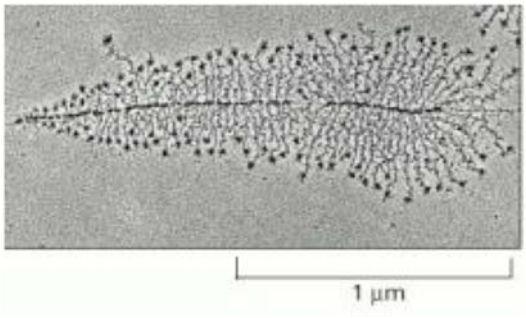


Component	Dry Cell Mass (%)	Molecules /cell	Different types	Copies of each type
Wall	10	1	1	1
Membrane	10	2	2	1
DNA	1.5	1	1	1
mRNA	1	1,500	600	2-3
tRNA	3	200,000	60	>3,000
rRNA	16	38,000	2	19,000
Ribosomal proteins	9	10^6	52	19,000
Soluble proteins	46	2.0×10^6	1,850	>1,000
Small molecules	3	7.5×10^6	800	

- RNA ≈ ribosomal RNA ~ ribosome level
- cell mass ≈ total protein mass (55%)
- a significant fraction of cellular proteins are ribosomal proteins

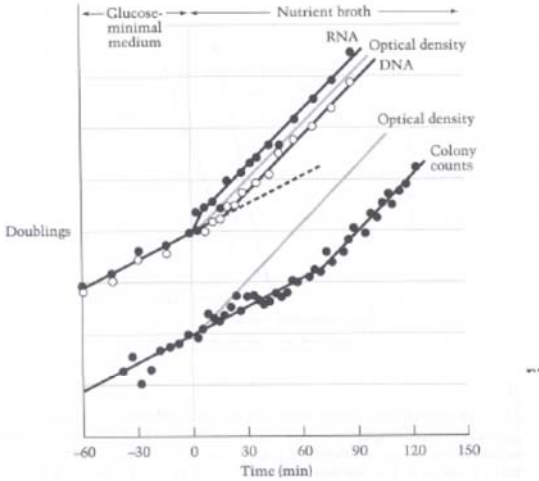
heavy demand on ribosome synthesis
in rapid growth condition (> 20,000/doubling)

max tsx rate (~100/min) x doubling time (20 min)
x 7 copies of rRNA genes x multiplicity of genome

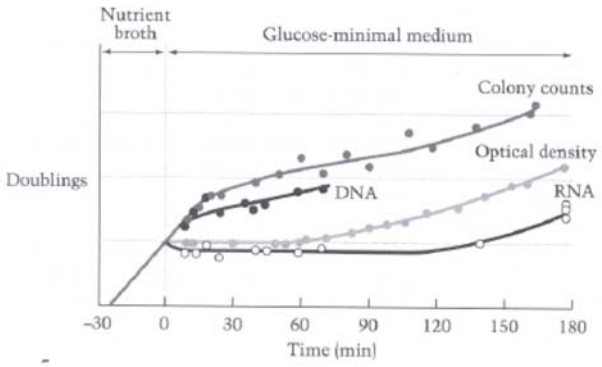


➔ environmental change: macromolecular composition must adjust to the one corresponding to the growth rate of the new environment

nutritional up-shift



nutritional downshift



➔ RNA level (ribosome abundance) responded first during up- and down- shifts, until the new composition was established
➔ efficient usage of ribosome crucial

Phenomenological theory of bacterial growth control

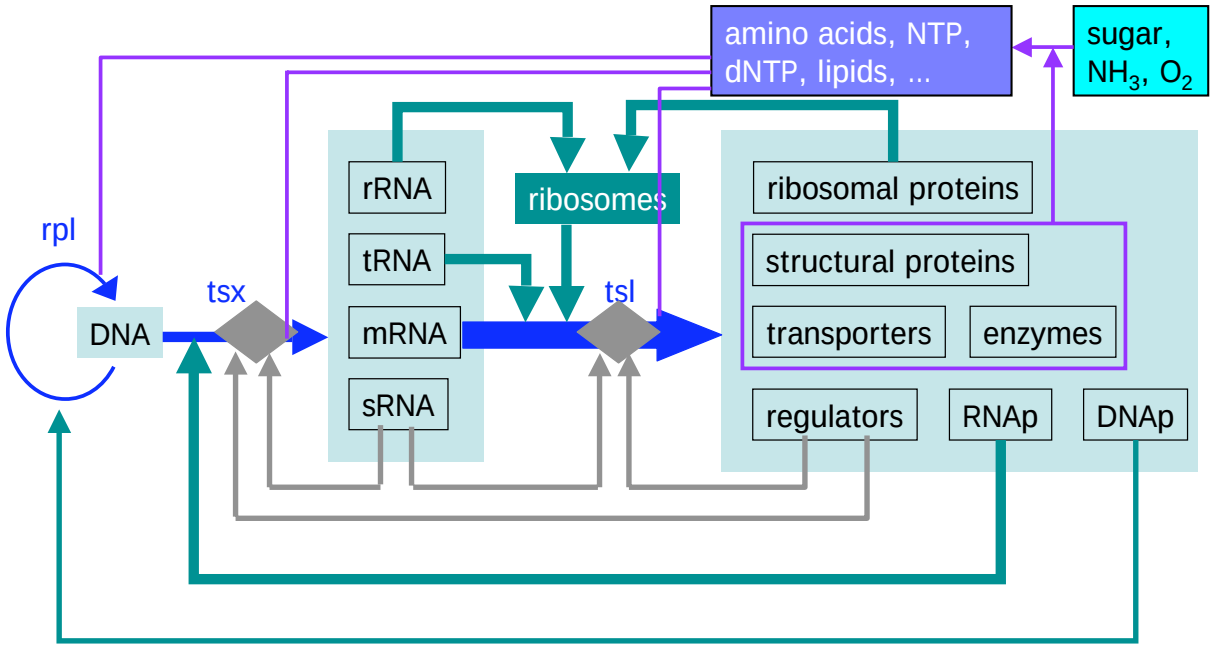
[Eduard Mateescu & TH]

- **focus** on growth in media with various degrees of amino acid abundance
- **input:**
  - qualitative aspects of the known control mechanisms
  - qualitative aspects of the growth data
  - demand on system to maximize growth
  - simplest mathematical description consistent with the known facts
- **output:**
  - parameter-free models
  - quantitative relationship between observables
  - constraints on design of control systems
  - predictions testifiable by genetic expts and quantitative measurements

➔ goal is not to model the amino acid starvation response based on molecular mechanisms

➔ analogous in spirit to van der Vaal's theory of liquid-gas transition

central dogma



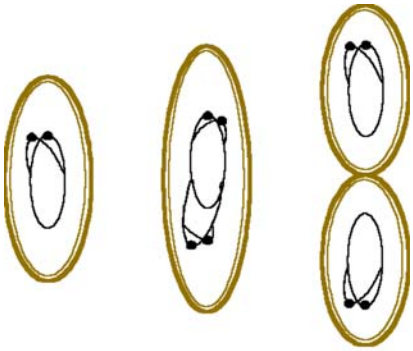
➔ "quantitative cartoon" of the central dogma?

- DNA replication
  - doubling time of *E. coli* can vary over 10x [fastest doubling time: ~20 min]
  - 40 min required to replicate chromosome
  - fixed time of 20 min between completion of one round of replication and cell division

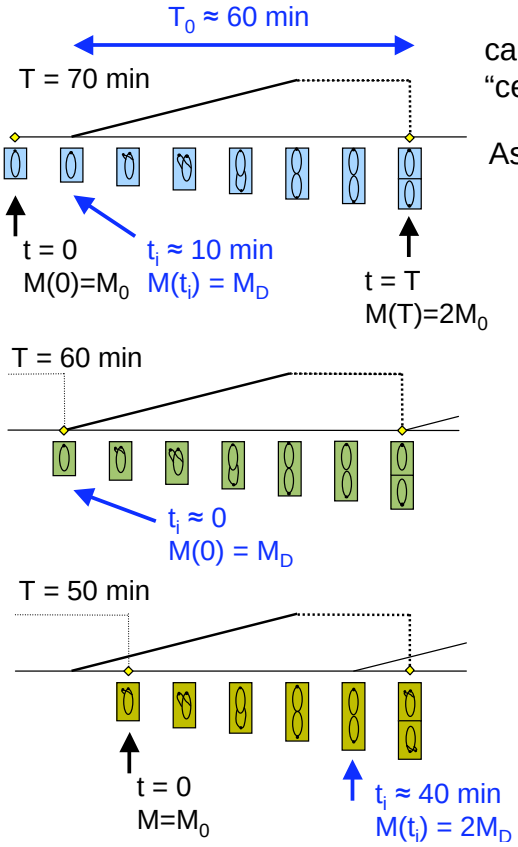
➔ doubling time > 60 min: waiting time between division & replication

➔ doublint time < 60 min: multiple replication forks

➔ empirical observation (Donachie's rule):
initiation of replication if < one replication origin per 1.7 μm



Quantitative relation between cell growth and DNA replication



can work out growth-rate dependence of "cell mass" (e.g., M_0) for **any** growth law $M(t)$

Assume **exponential growth** (within cell cycle)

$$M(t) = M_0 \cdot 2^{t/T}$$

For T > 60 min,

$t_i \approx T - T_0$ and $M(t_i) = M_D = M_0 \cdot 2^{(T-T_0)/T}$

$$\Rightarrow M_0 = (M_D / 2) \cdot 2^{T_0/T}$$

For 60 min > T > 30 min,

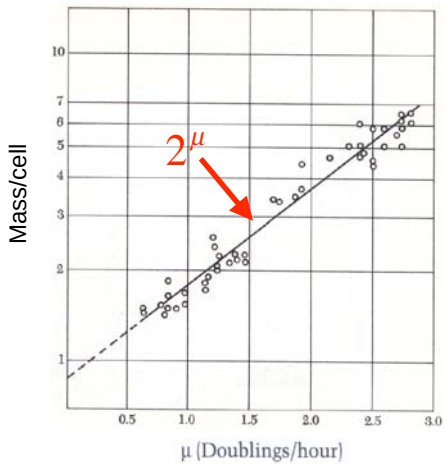
$t_i \approx 2T - T_0$ and $M(t_i) = 2M_D = M_0 \cdot 2^{(2T-T_0)/T}$

$$\Rightarrow M_0 = (M_D / 2) \cdot 2^{T_0/T}$$

For 30 min > T > 20 min,

$t_i \approx 3T - T_0$ and $M(t_i) = 3M_D = M_0 \cdot 2^{(3T-T_0)/T}$

$$\Rightarrow M_0 = (M_D / 2) \cdot 2^{T_0/T}$$



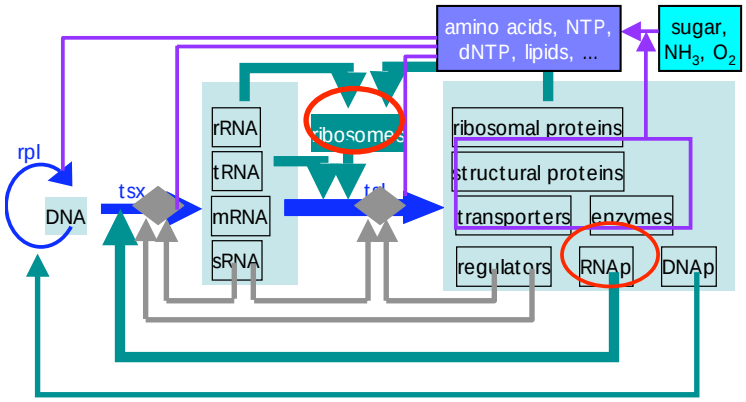
➡ $M_0 = (M_D / 2) \cdot 2^{T_0/T} = M_D \cdot 2^{\mu - 1}$ with $\mu = T_0 / T$

• result consistent with observed exponential dependence of cell mass

• **linear** growth law M(t) = M_0 (1+t/T) gives piecewise linear form of $M_0(\mu)$
• difficult to discriminate between the linear vs exponential form of M(t) directly
• data in favor of the exponential form

➔ will assume exponential form of M(t) below;
➔ DNA replication and cell division "slaved" to M(t)

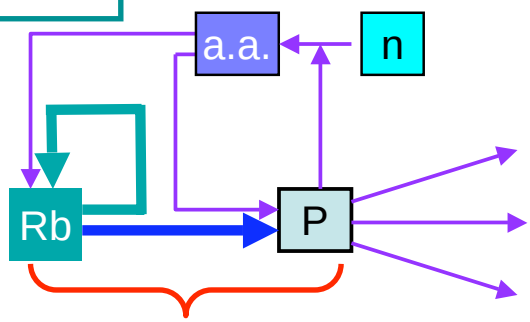
Exponential growth of M(t) ➔ self-replication



candidates:
• RNAp -- abundant
• Rb -- rRNA limiting

➔ focus on Rb
(= rRNA + r-proteins)

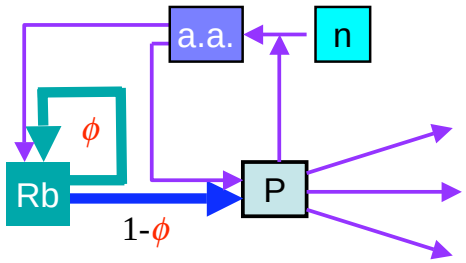
➔ simple model of growth relating nutrient to observables



$M_{RNA} \propto M_{Rb}$
$M(t) \propto M_{Rb} + M_P$

➡ DNA repl & cell size

Quantitative description



ϕ: fraction of Rb synthesizing Rb

$$\frac{d}{dt}N_{Rb} = \phi \cdot \gamma \cdot N_{Rb}$$

γ: rate for one Rb to synthesize Rb

$$= \frac{10 \sim 20 \text{ a.a./sec}}{7336 \text{ a.a./Rb}} = 5 \sim 10 \text{ doubling/hr}$$

- soln: $N_{Rb} \sim 2^{\mu t}$ with $\boxed{\mu = \phi\,\gamma}$

➡ ϕ: master growth control (provided the existence of control mechanisms)

- max possible growth rate: $\mu_{\max} = \gamma$ [cf: for *E. coli*, $\mu_{\max} \approx 3$ db/hr]
- protein synthesis:

M_{RP}: mass of ribosomal proteins
M_P: mass of non-ribosomal proteins

$$\begin{cases} \frac{d}{dt}M_{RP} = \phi \cdot \gamma \cdot M_{RP} \\ \frac{d}{dt}M_P = (1-\phi) \cdot \gamma \cdot M_{RP} \end{cases}$$

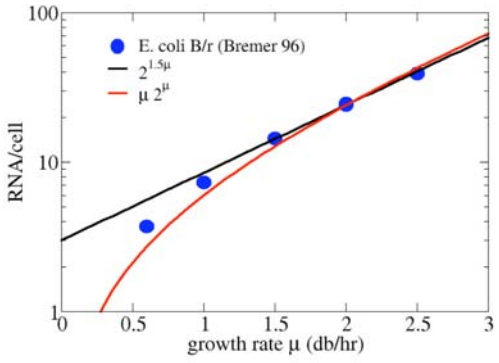
➡ $$r \equiv \frac{M_{RP}}{M_{RP} + M_P} = \phi$$

- relation between observables: $\boxed{r = \mu / \gamma}$

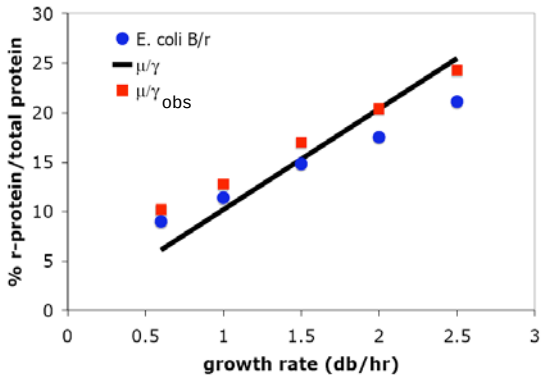
also, $M_{\text{RNA}} \propto M_{Rb} = r \cdot M_{tot} \propto \mu \cdot 2^{\mu}$ [empirical: $M_{\text{RNA}} \sim 2^{1.5\mu}$]

Data consistent with the expected relations:

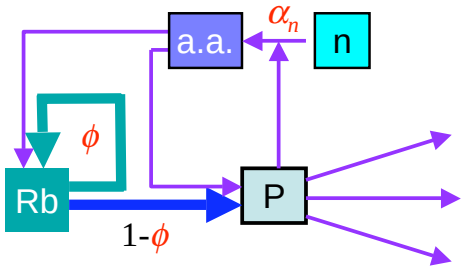
$\boxed{M_{\text{RNA}} \propto M_{Rb} = r \cdot M_{tot} \propto \mu \cdot 2^{\mu}}$



$\boxed{r = \mu / \gamma}$



relate to nutritional input



- steady-state:

  a.a. consumption = a.a. supply

$$\propto \gamma \cdot N_{Rb} \qquad k \cdot N_E \cdot \frac{n}{n+K_n} \propto k \cdot N_P \cdot \frac{n}{n+K_n}$$

$$\gamma M_{RP} = \alpha_n M_P$$

$$r \equiv \frac{M_{RP}}{M_{RP}+M_P} = \phi$$

$$\begin{cases} r = \phi = \dfrac{\alpha_n}{\alpha_n + \gamma} \\ \mu = \dfrac{\alpha_n \cdot \gamma}{\alpha_n + \gamma} \end{cases}$$

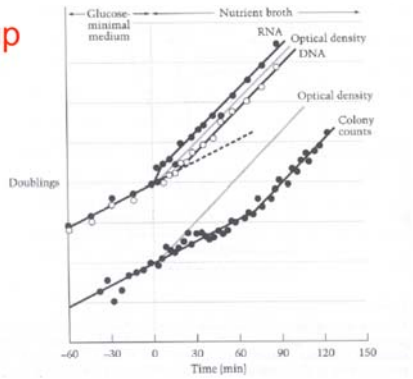
- nutritional shift: change from medium with α to α'

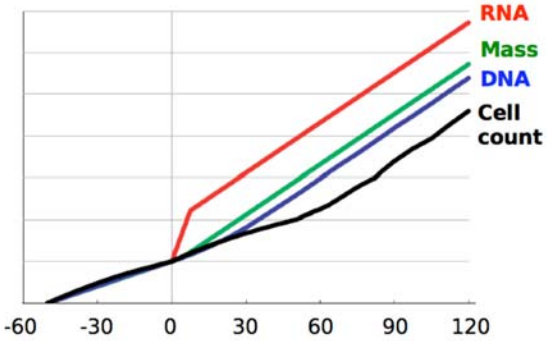
  toy model: Rb makes only (no) Rb if a.a. is in excess (shortage)

$$\phi = \begin{cases} 1 & \text{if} \quad \alpha' \cdot M_P(t) > \gamma \cdot M_{RP}(t) \\ M_{RP}/(M_P + M_{RP}) & \text{if} \quad \alpha' \cdot M_P(t) = \gamma \cdot M_{RP}(t) \\ 0 & \text{if} \quad \alpha' \cdot M_P(t) < \gamma \cdot M_{RP}(t) \end{cases}$$

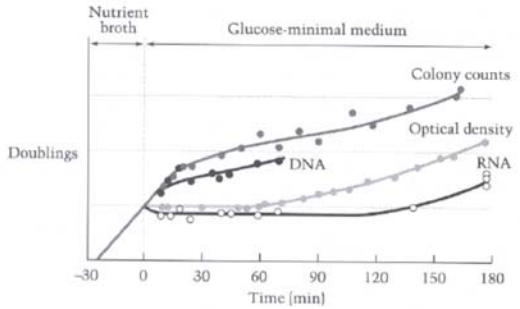
Toy model: Response to nutritional shifts

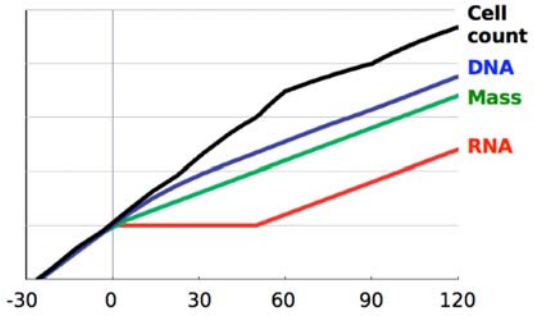
shift-up





shift-down





parameter free!

Biochemical implementation of $\phi(\alpha)$?

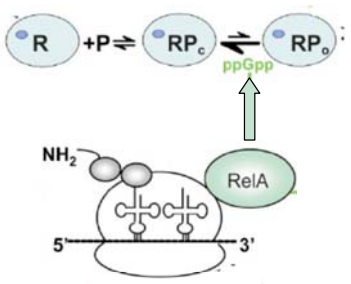
- introduce cellular a.a. level (a) as a dynamic variable
  - translational efficiency
    diminishes due to uncharged tRNA
  - metabolic feedback control
    (allosteric/tsx repression)
  - "stringent response"

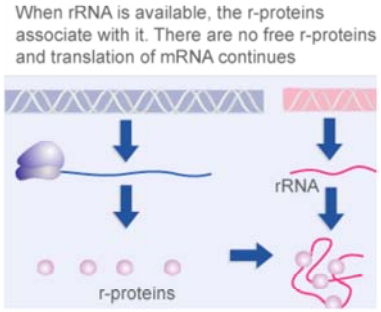
$$\gamma \to \gamma \cdot f_\gamma(a)$$

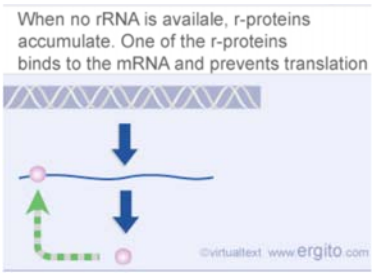
$$\alpha \to \alpha \cdot f_\alpha(a)$$

$$\phi \to \phi(a)$$

uncharged tRNA ⊣ rRNA → r-protein







Q: can $f_\gamma(a)$, $f_\alpha(a)$, $\phi(a)$ be "chosen" to maximize growth for <u>all</u> α ?

- dynamical equations (suppress stringent response $\phi(a)$ for now)

$$\begin{cases} \dot{M}_{RP} = \phi \cdot \gamma \cdot f_\gamma(a) \cdot M_{RP} \\ \dot{M}_P = (1-\phi) \cdot \gamma \cdot f_\gamma(a) \cdot M_{RP} \\ 20\dot{M}_A = \alpha \cdot f_\alpha(a) \cdot M_P - \gamma \cdot f_\gamma(a) \cdot M_{RP} \end{cases}$$

M_A: mass of one free a.a. pool in cell

$a = M_A/(M_P+M_{RP})$: free a.a. conc in cell

$$f_\gamma(a) = (a/K_\gamma)^{h_\gamma} \Big/ \left[1+(a/K_\gamma)^{h_\gamma}\right]$$

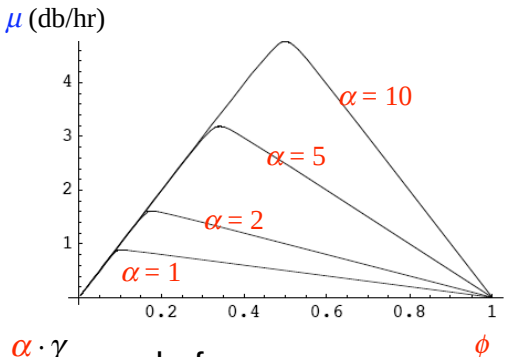
$$f_\alpha(a) = 1 \Big/ \left[1+(a/K_\alpha)^{h_\alpha}\right]$$

- steady exponential growth: $M \propto 2^{\mu t}$

steady a.a. level: $\bar{a}(\alpha,\phi)$

from $\phi = \dfrac{\alpha \cdot f_\alpha(\bar{a})}{\alpha \cdot f_\alpha(\bar{a}) + \gamma \cdot f_\gamma(\bar{a})}$

growth rate from: $\mu = \phi \cdot \gamma \cdot f_\gamma(\bar{a})$



optimal choice $\phi^*(\alpha) = \alpha/(\alpha+\gamma)$ ⟹ $\mu^* = \dfrac{\alpha \cdot \gamma}{\alpha+\gamma}$ as before

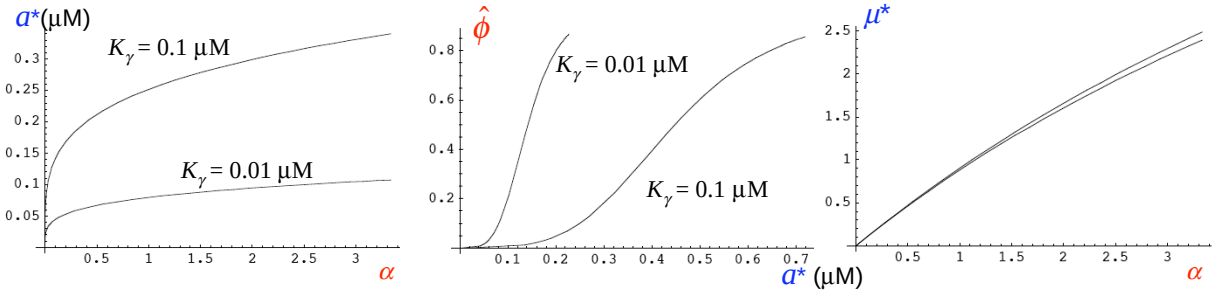
a.a. level at optimal: $a^* = \bar{a}(\alpha,\phi^*)$

$\phi < \phi^*$: growth slow due to the lack of Rb ($a \gg a^*$, $f_\alpha \ll 1$)

$\phi > \phi^*$: growth slow due to the lack of a.a. ($a \ll a^*$, $f_\gamma \ll 1$)

How to realize $\phi^*(\alpha)$ from the stringent response function $\phi(a)$?

-- one strategy: choose $\hat{\phi}(a)$ such that $\boxed{\hat{\phi}\left(a^*(\alpha)\right) = \phi^*(\alpha)}$



-- can be done for any given $f_\gamma(a)$, but would need different $\phi(a)$ for each a.a.

-- alternatively, fix $\phi(a)$ and find optimal feedback function $f_\alpha(a)$
[note: $f_\alpha(a)$ can be individually chosen for each pathway.]

but empirically, find $\mu \approx \mu^*$ for a broad range of parameters!

How?

want $\phi = \dfrac{\alpha \cdot f_\alpha(\bar{a})}{\alpha \cdot f_\alpha(\bar{a}) + \gamma \cdot f_\gamma(\bar{a})} \Rightarrow \phi^* = \dfrac{\alpha}{\alpha + \gamma}$

just need to have $f_{\gamma,\alpha}(\bar{a}) \approx 1$

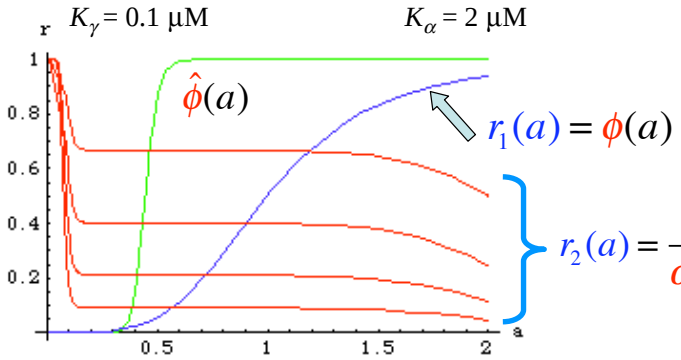
Steady-state solution

$$\dot{r} = \phi(a) \cdot \gamma \cdot f_\gamma(a) \cdot r - \gamma \cdot f_\gamma(a) \cdot r^2$$

$$20\dot{a} = \alpha \cdot f_\alpha(a) \cdot (1 - r) - \gamma \cdot f_\gamma(a) \cdot r$$

$r = M_{RP}/(M_P + M_{RP})$

$a = M_A/(M_P + M_{RP})$



$r_1(a) = \phi(a)$

$r_2(a) = \dfrac{\alpha \cdot f_\alpha(a)}{\alpha \cdot f_\alpha(a) + \gamma \cdot f_\gamma(a)}$

➔ maximal growth insensitive to form of stringent response $\hat{\phi}(a)$ provided $K_\gamma \ll K_\phi \ll K_\alpha$

- $K_\gamma \ll K_\phi$ expected: Rb synthesis time given by

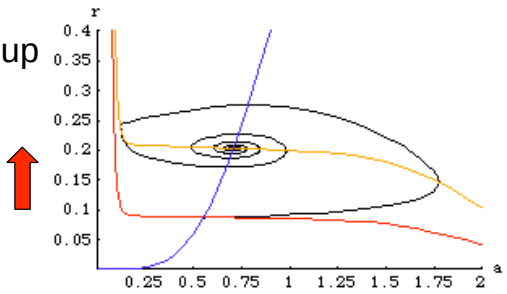
$$\gamma^{-1} = \sum_{i \in \{\text{a.a.}\}} \nu_i / \left(\gamma_0 \cdot q_i(a_i)\right)$$

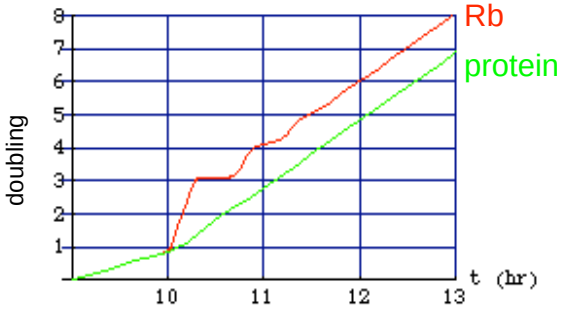
abundance of tRNA-aa$_i$ ~ $\phi(a_i)$

- $K_\phi \ll K_\alpha$: from separate sensing of charged tRNA and a.a.

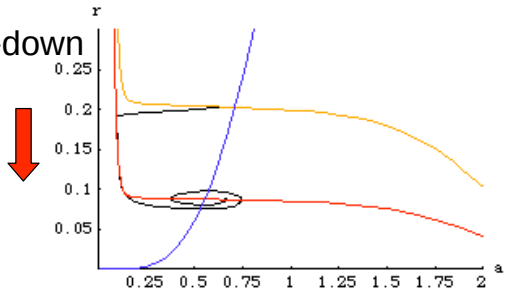
transient response

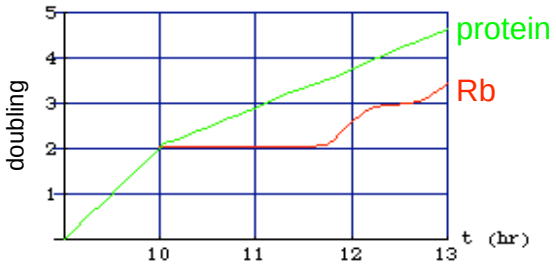
shift-up





shift-down





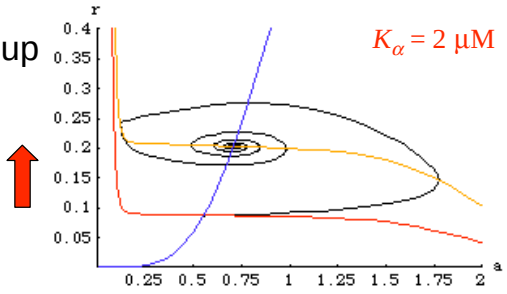
➔ damped oscillation generically expected

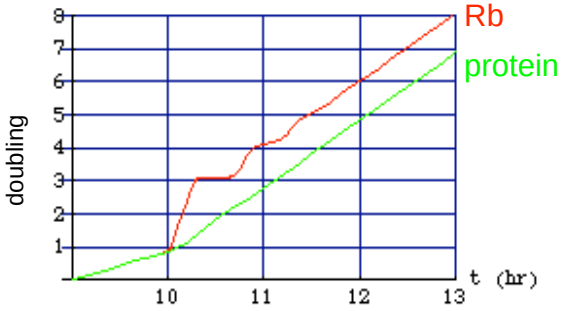
– amplitude set by K_γ, K_α

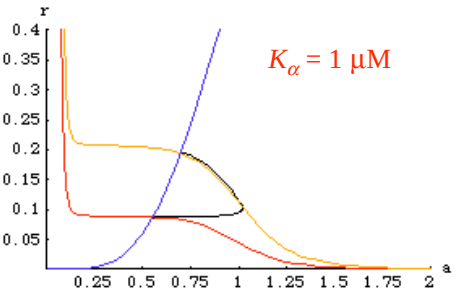
– frequency $\approx \mu\sqrt{n_\phi K_\phi / \bar{a}}/(2\pi)$ -- not simply doubling time

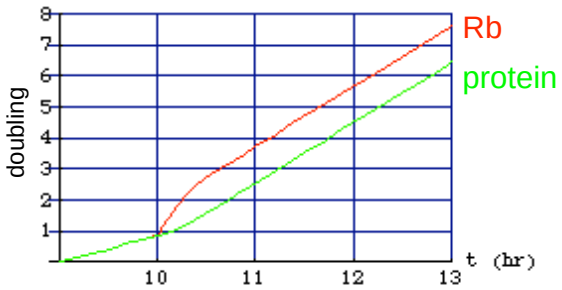
Role of metabolic feedback control $f_\alpha(a)$?

shift-up





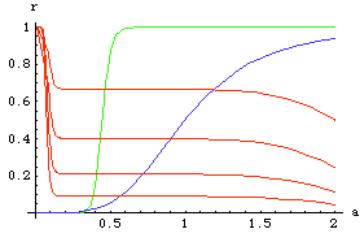




as $K_\alpha \to K_\phi$, oscillation disappeared;
however growth rate reduced!

Summary:

- Simple, versatile model of growth and control
  - built on known phenomenology (efficient usage of Rb)
  - insensitive to parameters and forms of control functions in the buffer zone $K_\gamma \ll K_\phi \ll K_\alpha$
    [analogous to "first order transition"]
  - predicts transient oscillation in Rb level and hence in generic protein expression



  - suggests role of metabolic feedback control to limit oscillation
  - testable by modifying stringent responseand other regulatory functions
- Many applications
  - top-down approach to metabolism
  - precision in cell division
  - codon usage
  - antibiotics and resistance
  - chemotaxis? temperature response?

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Bacterial Growth: Constant Obsession with *dN/dt*

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Some of my closest scientific colleagues -- geneticists, many of them -- have never constructed a microbial growth curve. Nor, for that matter, have many microbial biochemists, ecologists, structural biologists, and even some physiologists. I would hope, however, that current students will soon recognize the usefulness that growth measurements can play in the coming era of functional genomics and proteomics. And they may then understand what Moselio Schaechter declares about the special source of satisfaction and inspiration available to bacterial physiologists: when we meet a dry time, we can always go into the lab and construct a growth curve.

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related publications: *http://matisse.ucsd.edu/~hwa/pub*