Biological Evolution:

- small changes: observed all the time
- large changes (e.g., creation of species): rarely seen

Theory of biological evolution:

- large changes from accumulation of small changes
- main difficulty: how to hold on to the desired small changes?
- easy if every small changes gives a fitness benefit

function of a circuit/device requires
coordinated activities of multiple components

➔ many small changes before fitness benefit realized
(cf development of eyes)

at least a plateau landscape

function of a circuit/device requires
coordinated activities of multiple components

➔ many small changes before fitness benefit realized

➔ how to get to the edge of the plateau
given the huge genome space?

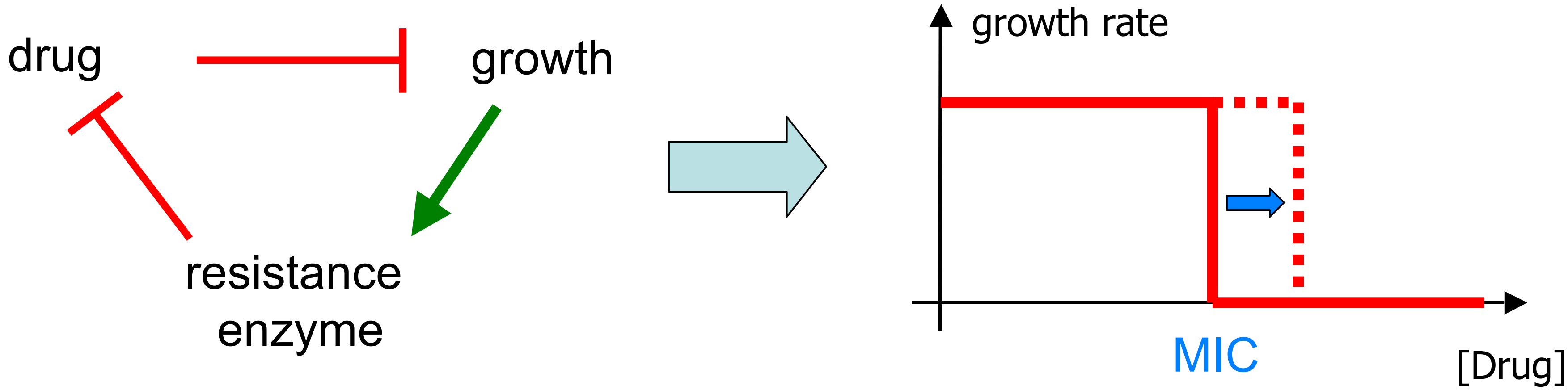
How does evolution overcome the severe entropy problem?

get inspiration from biology

rapid evolution of antibiotic resistance

- emerging medical crisis: bacteria resistant to multiple antibiotics
- drug resistance emerged over just the last 30 years
- attributed to wide usage of antibiotics in hospitals and on farms

This talk: theory of drug resistance evolution



- growth-dependence of constitutive gene expression
- positive feedback w/o need of gene regulation

[Scott et al, Science 2010]
[Klumpp et al, Cell 2009]

- abrupt response to drug levels
- increased MIC for higher resistance enzyme expression

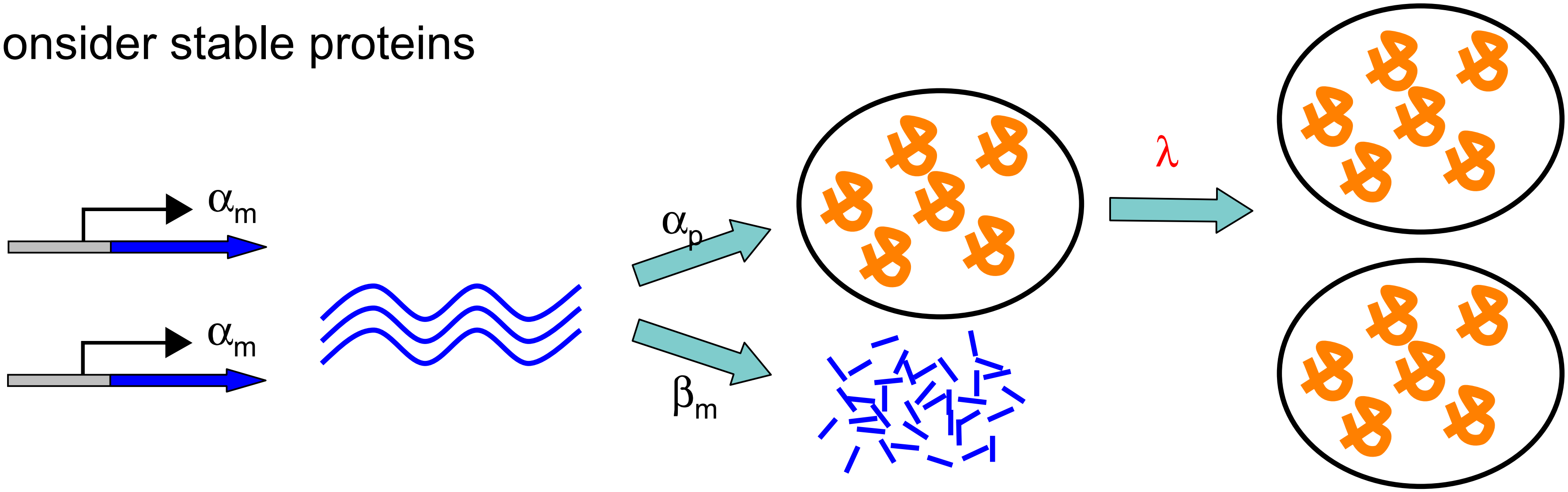
[Deris et al, in prep]

➔ **recipe for rapid evolution of drug resistance** [Hermsen & TH, PRL 2010]

➔ **possible lesson for the evolution of more complex systems**

Growth-rate dependence of constitutive gene expression?

Consider stable proteins



factors expected to increase with growth rate:

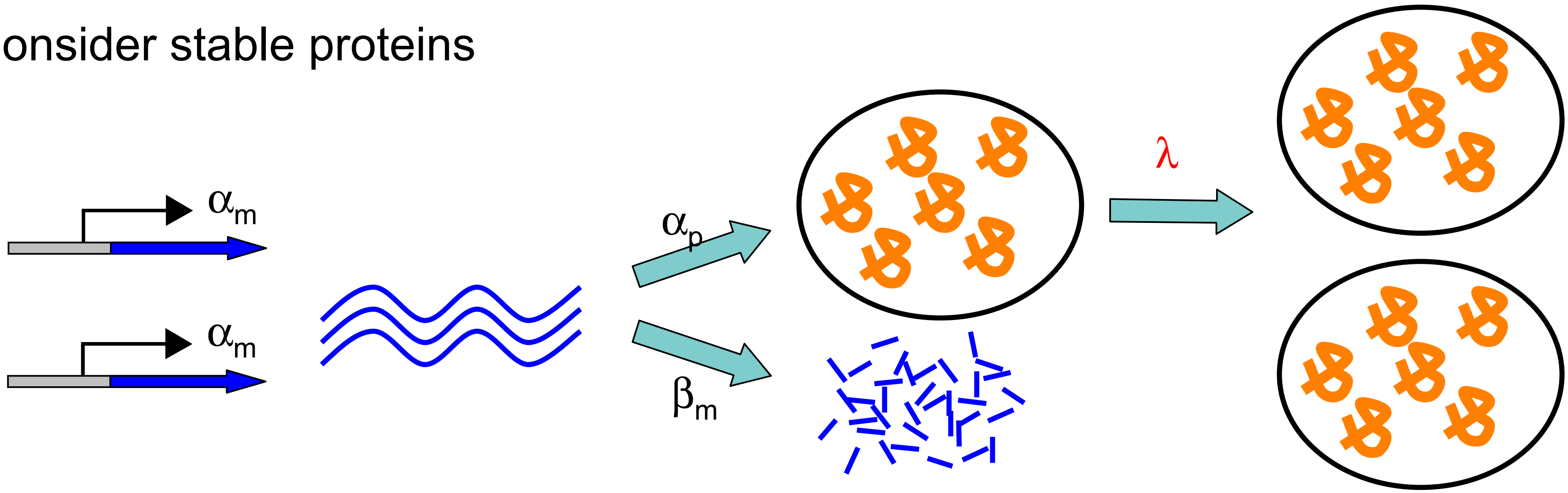
– chromosome copy number ➔ gene dose (g)
– ribosome conc ➔ translational initiation (α_p)
– dilution rate (λ)
– cell volume (V)

steady state protein conc

$$[p] = \frac{g\alpha_m\alpha_p}{\beta_m \lambda V} \propto \frac{1}{\lambda}$$

Growth-rate dependence of constitutive gene expression?

Consider stable proteins



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- cell volume (V)

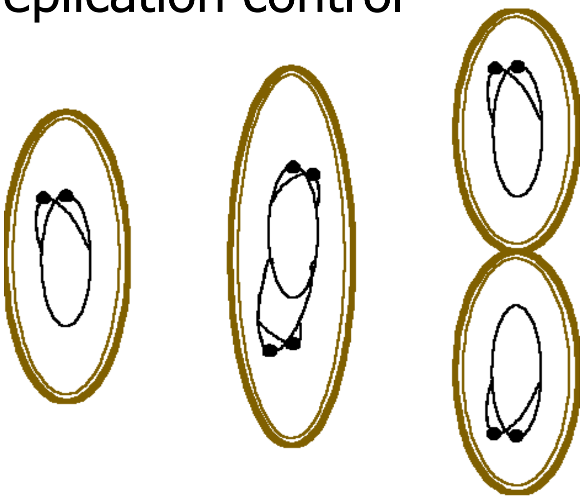
steady state protein conc

$$[p] = \frac{g\alpha_m\alpha_p}{\beta_m \lambda V} \propto \frac{1}{\lambda}$$

- growth-rate dependence of gene expression may be complex
- growth-rate dependence of genetic circuits even more complex

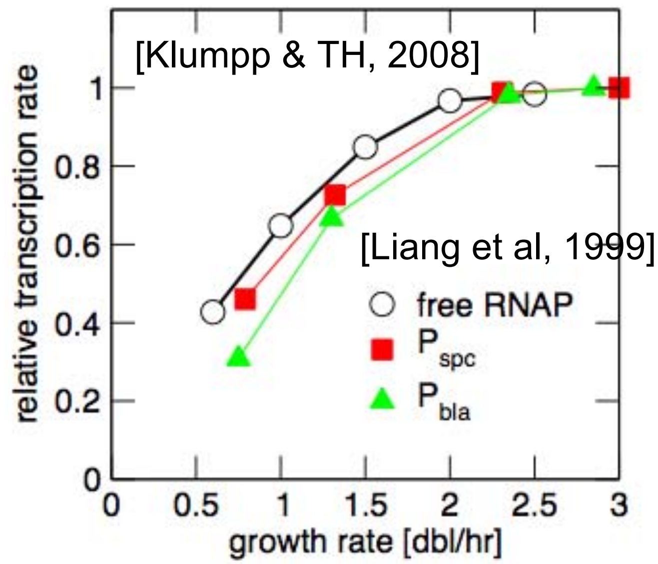
[Klumpp et al, Cell 2009]

gene dose from mass-dependent DNA replication control

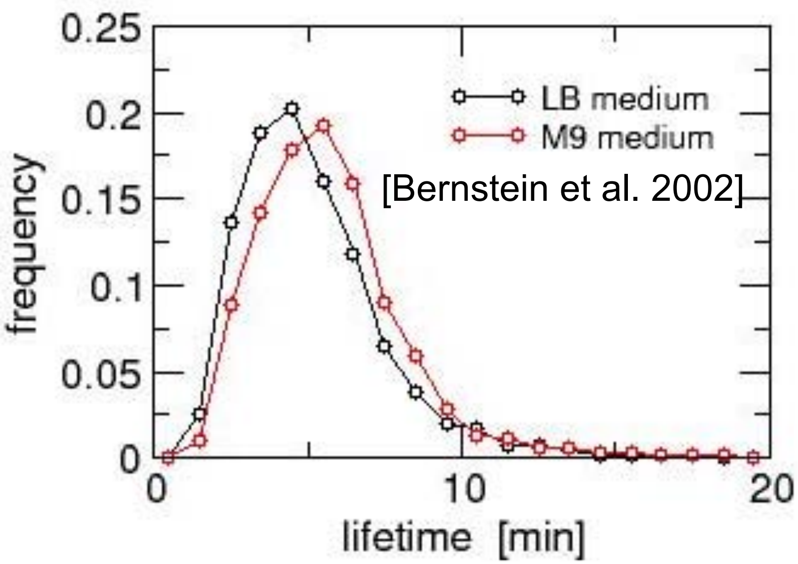


[Cooper & Helmstetter, JMB 1968]

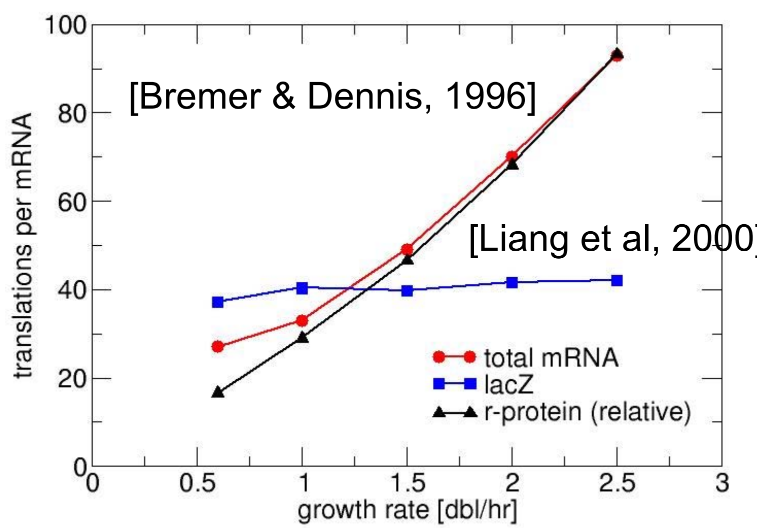
RNAp abundance from tsx studies



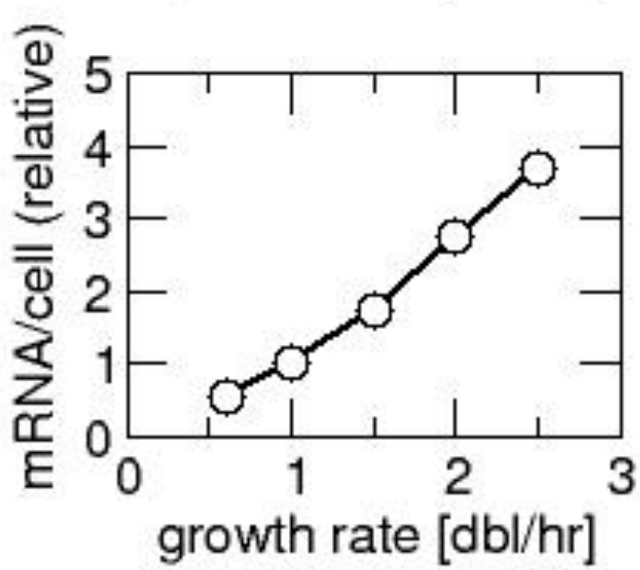
mRNA stability from microarray

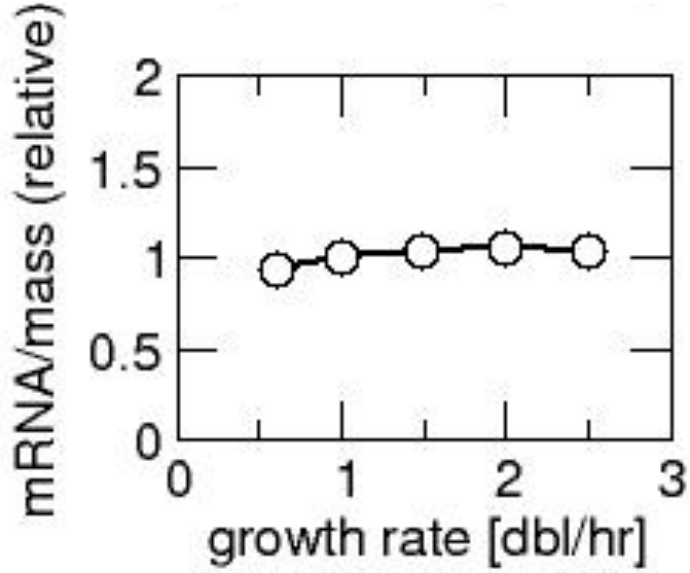


Translational burstiness

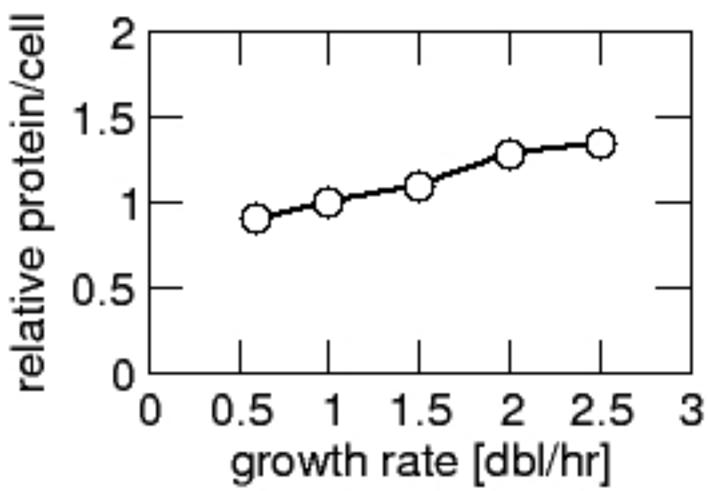


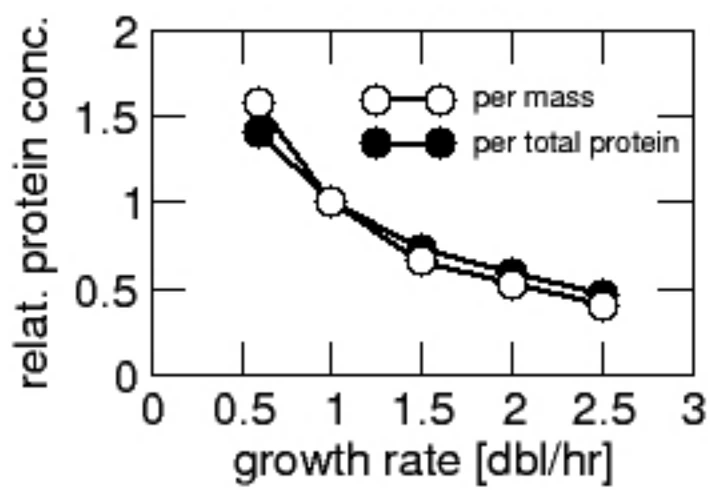
➔ growth rate dependence of mRNA "levels"





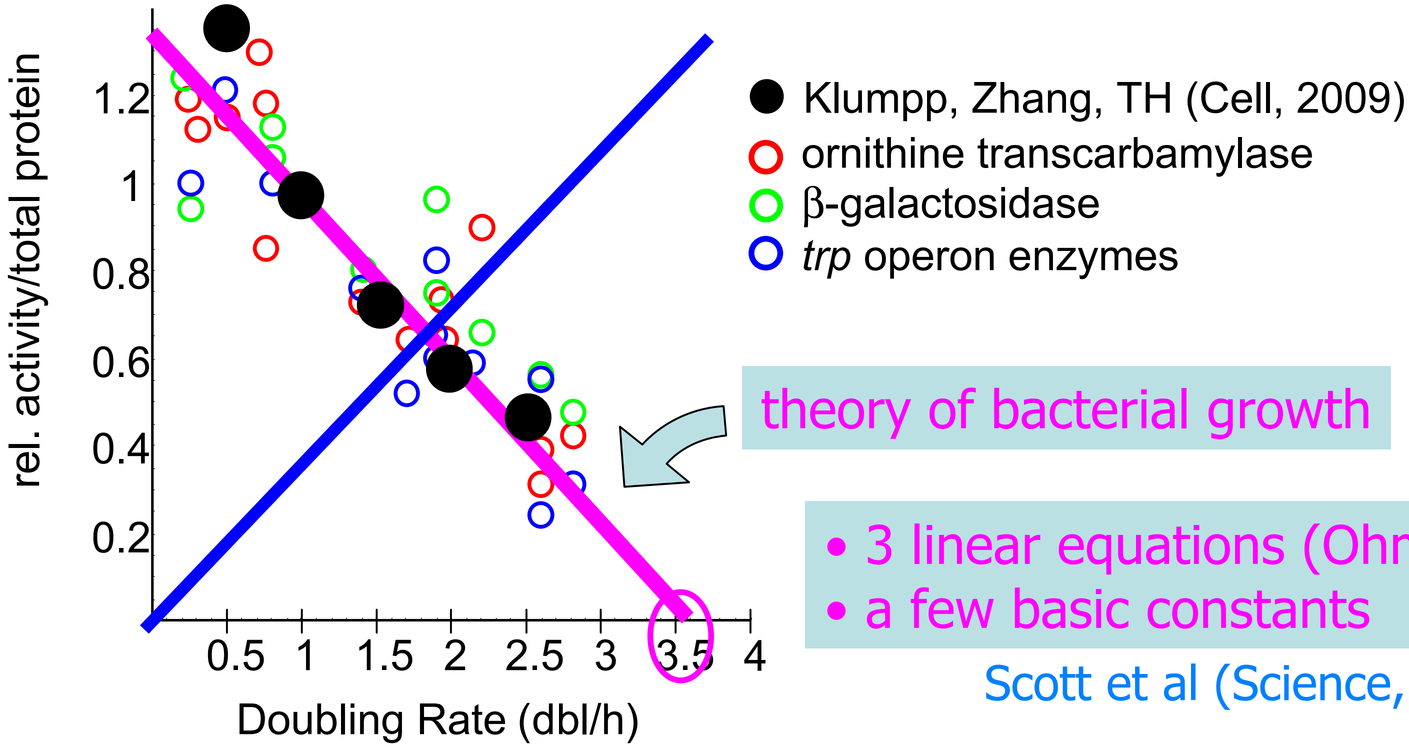
➔ growth rate dependence of protein "levels"





[stronger dependences for genes expressed from plasmids]

Growth-rate dependence of constitutive gene expression



- 3 linear equations (Ohm's laws)
- a few basic constants

Scott et al (Science, 2010)

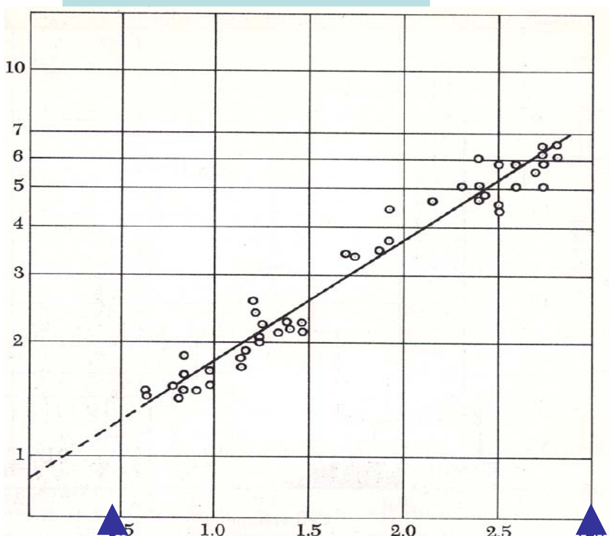
- can be derived quantitatively from theory of bacterial growth
- based on empirical growth laws + model of proteome partition
- some applications:
  - effect of (sub-lethal) antibiotics on gene expression
  - fitness cost of unnecessary protein expression
  - catabolite repression, metabolic coordination, …

SCHAECHTER, M., MAALØE, O. & KJELDGAARD, N. O. (1958). *J. gen. Microbiol.* 19,

Dependency on Medium and Temperature of Cell Size and Chemical Composition during Balanced Growth of *Salmonella typhimurium*

1st growth law

Mass/cell

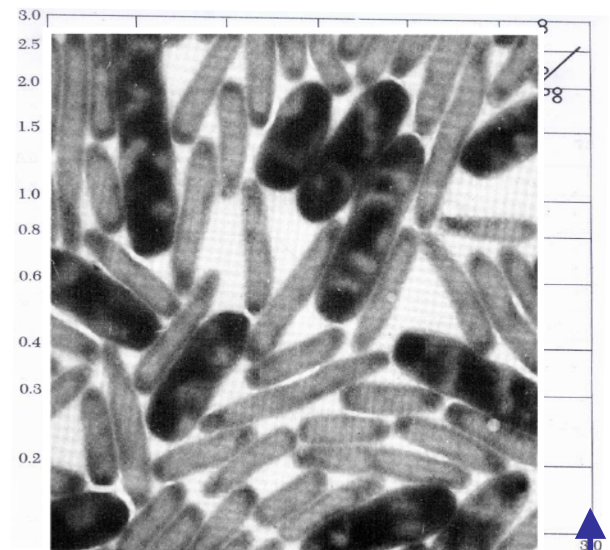


(doubling/hour)

100 min

20 min

RNA/cell



(doubling/hour)

100 min

20 min

• dependence on the medium through growth rate only!

• cell mass (∝ cell size) increases exponentially with growth rate

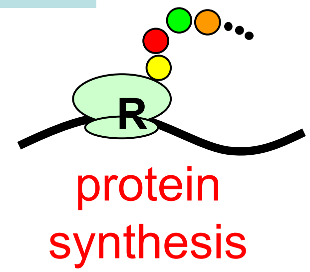
• similar dependences seen in other bacteria

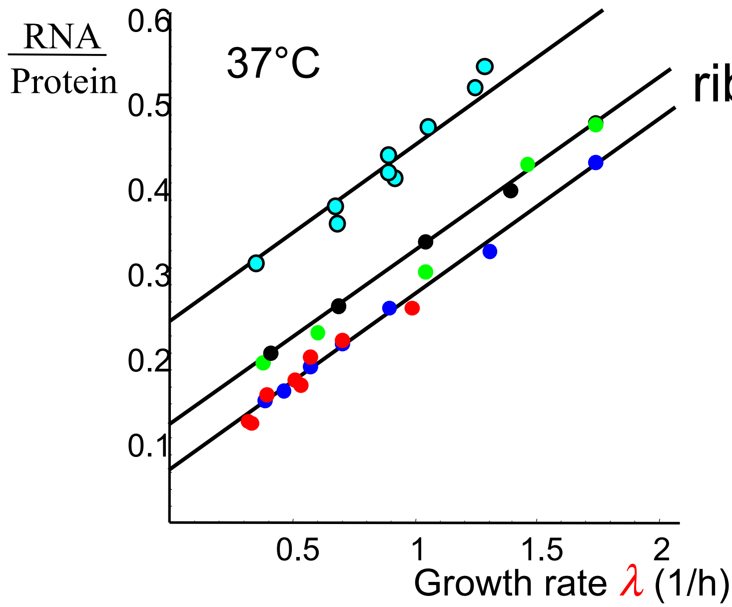
➔ understood from mass-dependent DNA replication control

[Cooper & Hemstetter, 1968; Donachie, 1968]

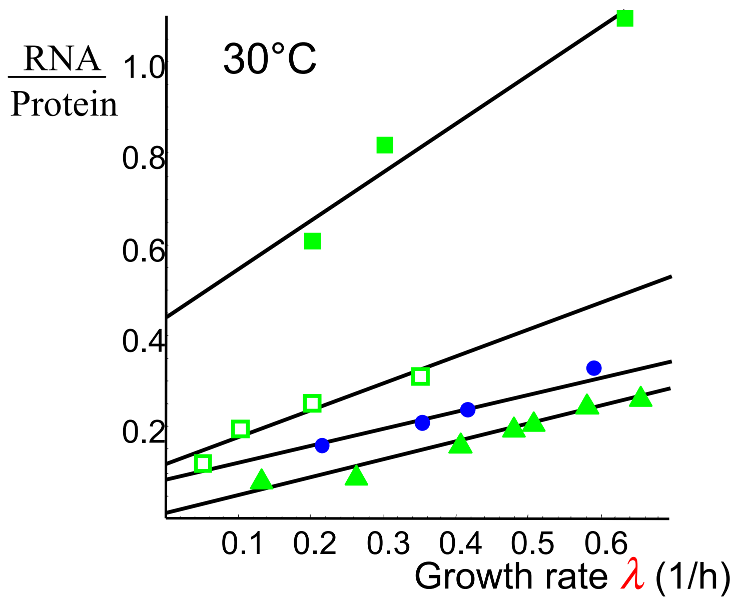
2nd growth law: RNA/protein = $a \cdot \lambda + b$ ∝ ribosome conc

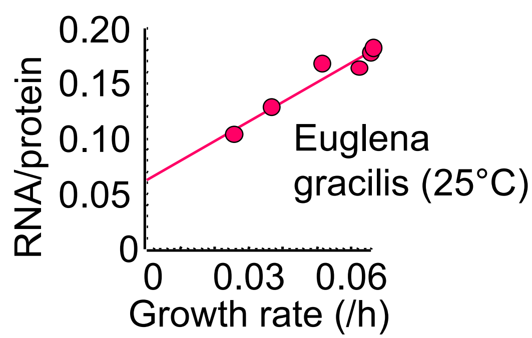
ribosome   cell volume





- 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)



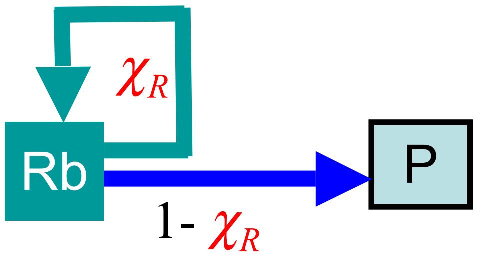


- Saccharomyces cerevisiae (5105D – Wehr & Parks, 1969)
- Candida utilis (NCYC 321 – Brown & Rose, 1969)
- Neurospora crassa (74A – Alberghina et al., 1975)
- Escherichia coli (ML308 – Rosset et al., 1964)

Simple two-component model of bacterial growth [Maaloe et al]

Focus on the ribosomes as the growth-limiting resource

- let χ_R be the fraction of Rb synthesizing Rb



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

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

λ : specific growth rate

γ : Rb elongation rate (~20 aa/s or 10 Rb/hr)

➔ absolute max growth rate ($\chi_R = 1$) set by γ (10/hr or 5 min/doubling)
[note: maximal doubling rate of E. coli = 20min/doubling]

➔ can change growth rate by changing χ_R (capitalism) or γ (socialism)

- ribosomes efficiently used in protein synthesis
- synthesized proteins predominantly stable

rate protein mass accum. = rate Rb elongation

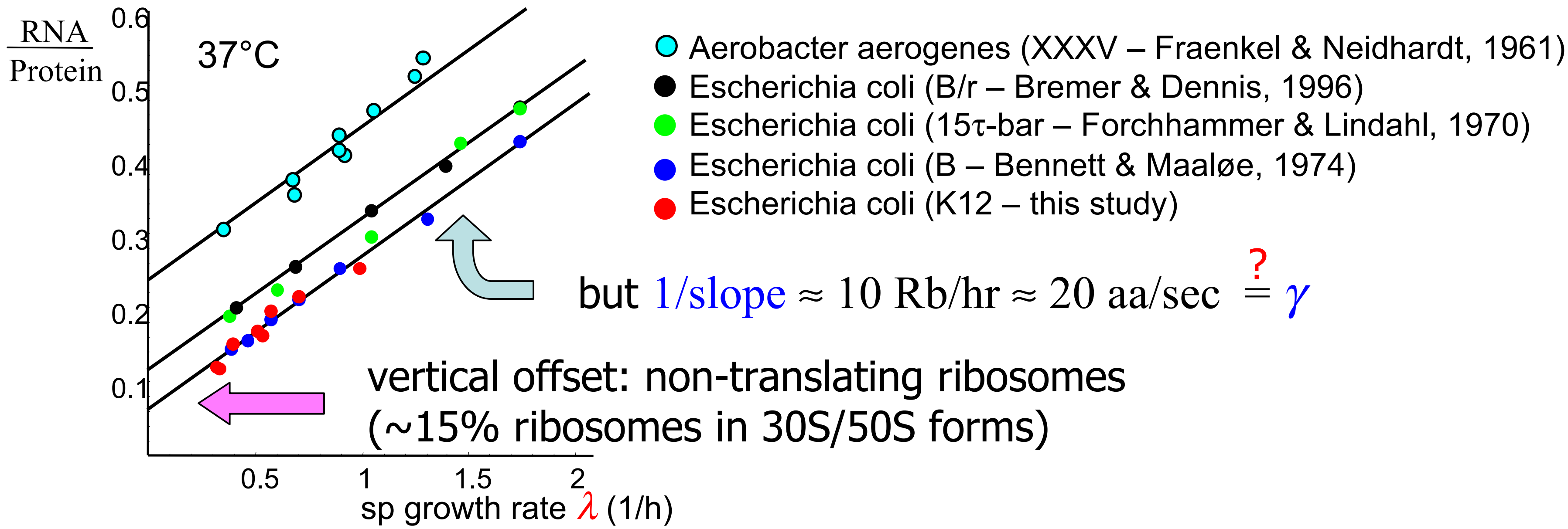
$\Uparrow$ $\qquad\qquad$ $\Uparrow$

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

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

➡ $\chi_R(\lambda) = M_{Rb}/M_{tot}$ growth control strategy revealed by r vs λ plots

2nd growth law: RNA/protein = $a \cdot \lambda + b$



but 1/slope ≈ 10 Rb/hr ≈ 20 aa/sec $\stackrel{?}{=} \gamma$

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

- ribosomes efficiently used in protein synthesis
- synthesized proteins predominantly stable

rate protein mass accum. = rate Rb elongation

$\Uparrow$ $\qquad$ $\Uparrow$

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

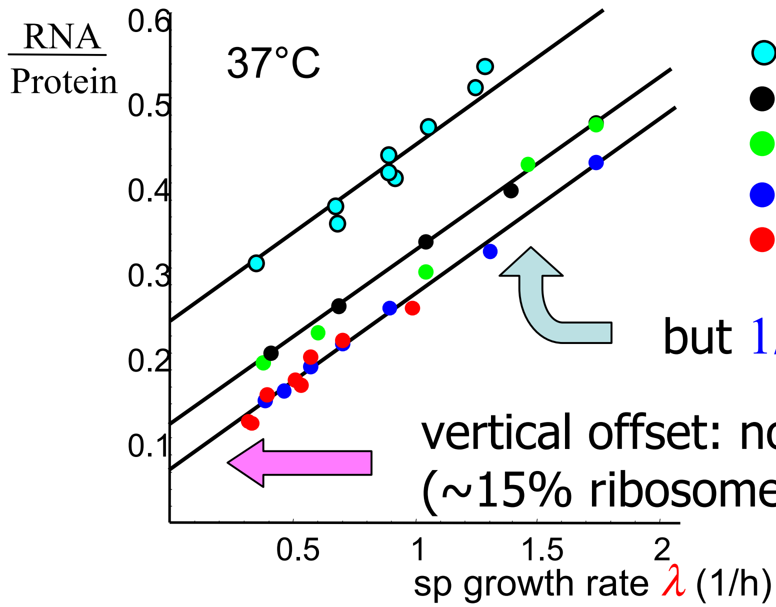
$\Rightarrow$ $r \equiv \dfrac{M_{Rb}}{M_{tot}} = \lambda / \gamma$

λ : specific growth rate

γ : Rb elongation rate
(~20 aa/s or 10 Rb/hr)

$\chi_R(\lambda) = M_{Rb}/M_{tot}$ growth control strategy revealed by r vs λ plots

2nd growth law: $M_{Rb}/M_{tot} \equiv r = \lambda/\gamma + r_0$



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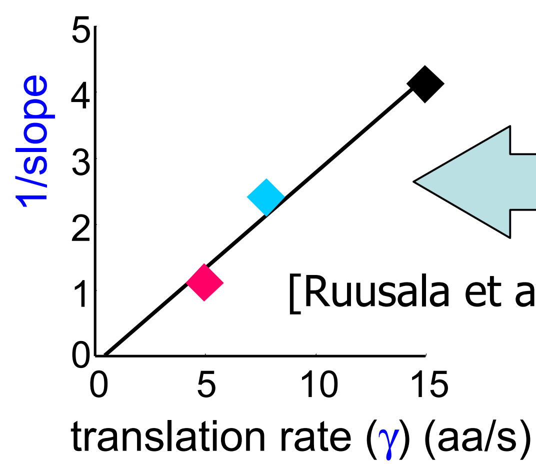
- Escherichia coli (K12 – this study)

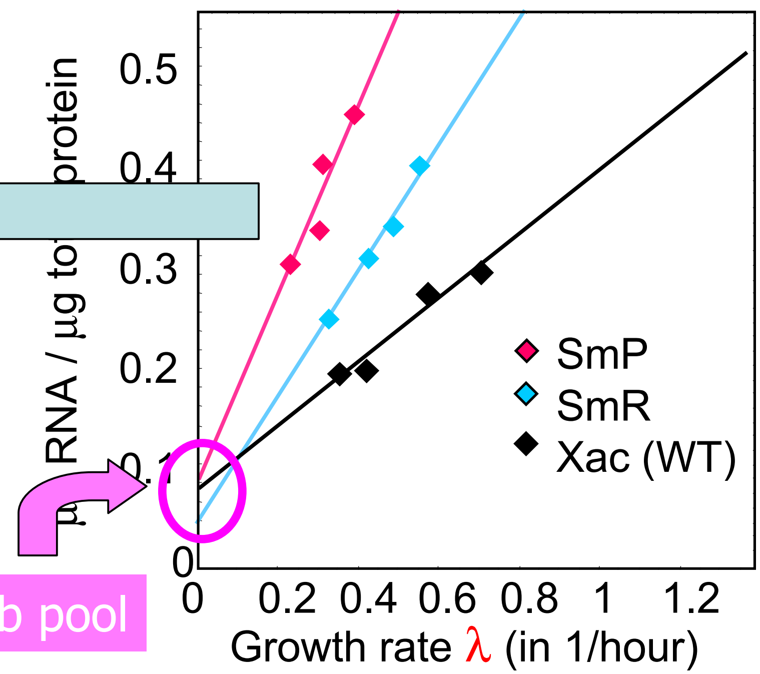
but $1/\text{slope} \approx 10$ Rb/hr ≈ 20 aa/sec $= \gamma$

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

Translational mutant (S12)

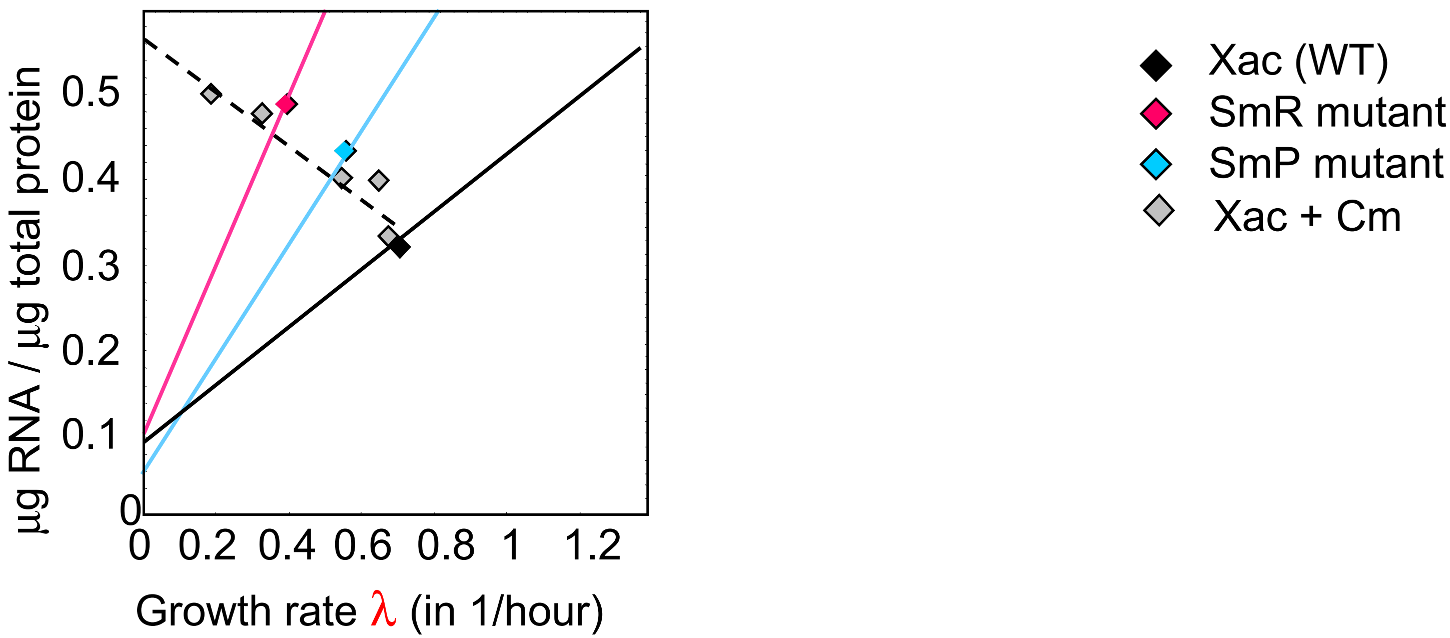


[Ruusala et al, '84]



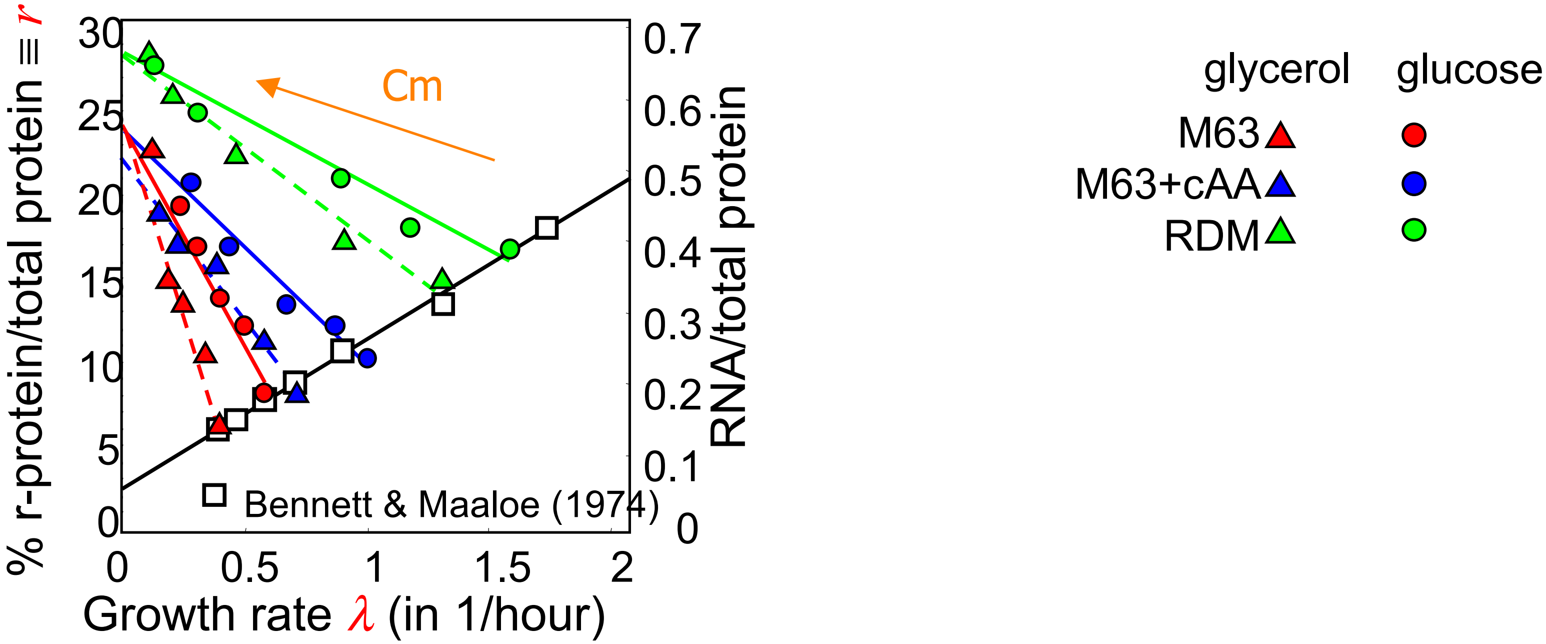
intercept (r_0) from free Rb pool

modulate translation rate γ for fixed nutrients

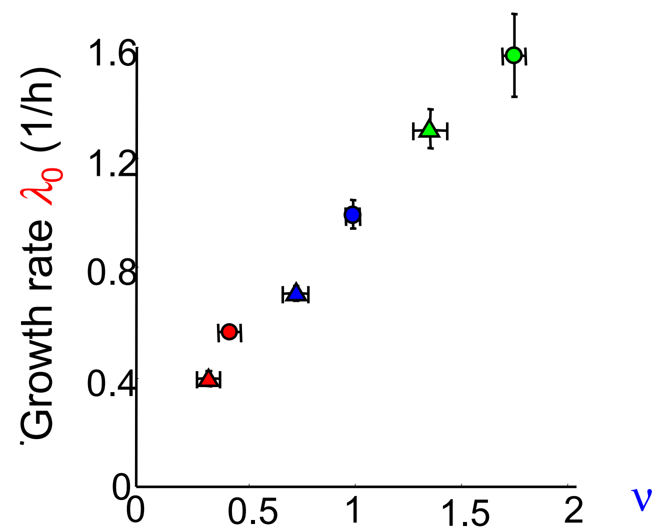


- similar effects from tsl mutants and sublethal dose of Cm
- linear relation obtained: $r = r_{max} - \lambda / \nu$

modulate translation rate γ for fixed nutrients

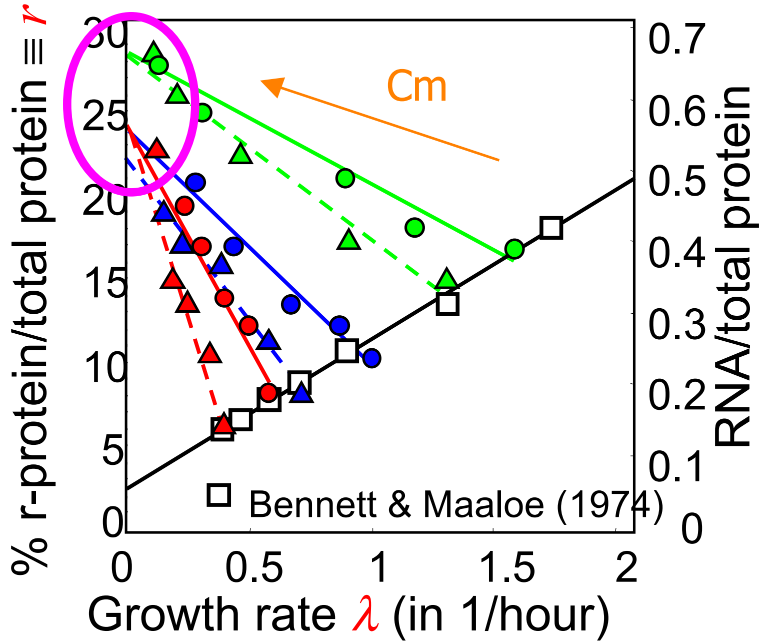


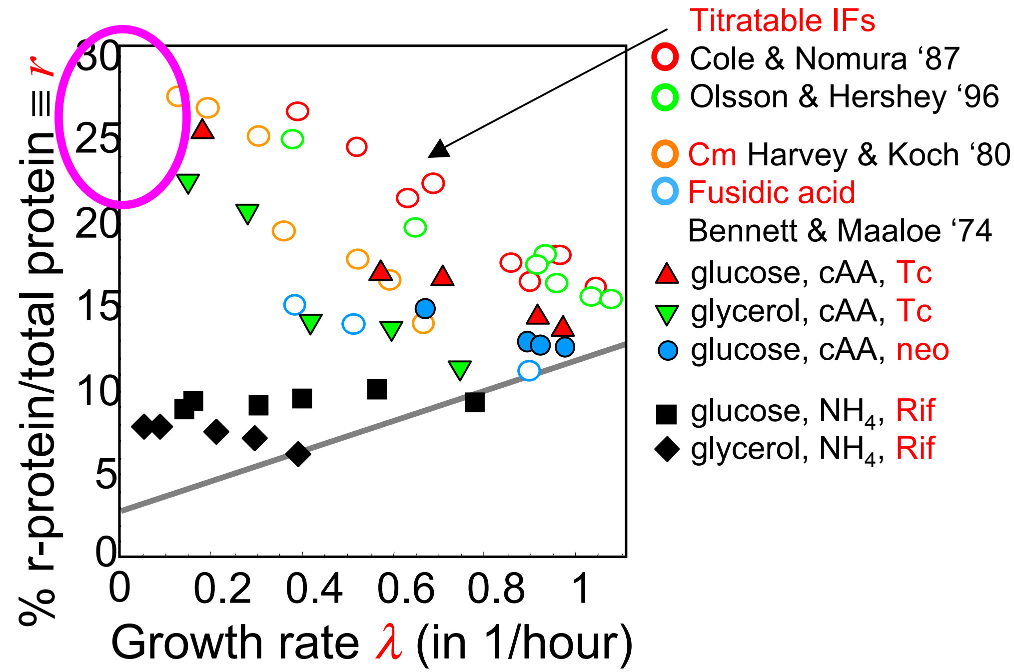
- similar effects from tsl mutants and sublethal dose of Cm
- linear relation obtained: $r = r_{max} - \lambda / \nu$
- seen for all media studied
- ν ~ "nutrient quality"



Significance of the 3rd law?

r_{max} ~ 25%: importance of other proteins





- similar effects from tsl mutants and sublethal dose of Cm
- linear relation obtained:

$$r = r_{max} - \lambda / \nu$$

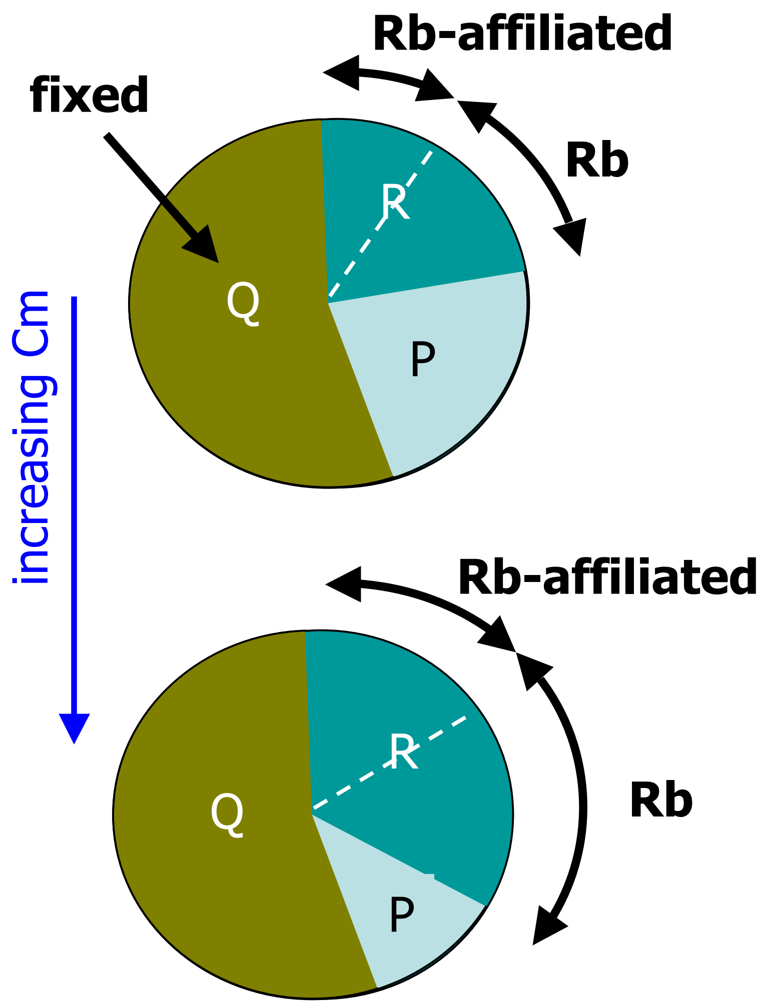
← 3rd growth law

- seen for all media studied
- ν ~ "nutrient quality"

(inverse r-λ correlation expected qualitatively from ppGpp-mediated rRNA control)

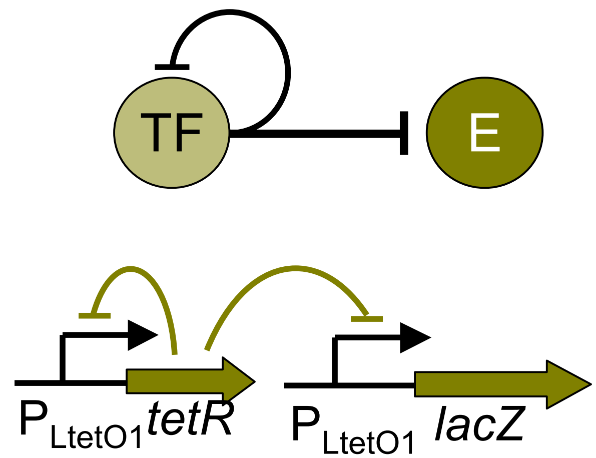
- also seen for other tsl inhibiting drugs (Tc, neo, FA, …), and variable induction of tsl initiators IF2/IF3 but not tsx inhibiting drug (Rif)

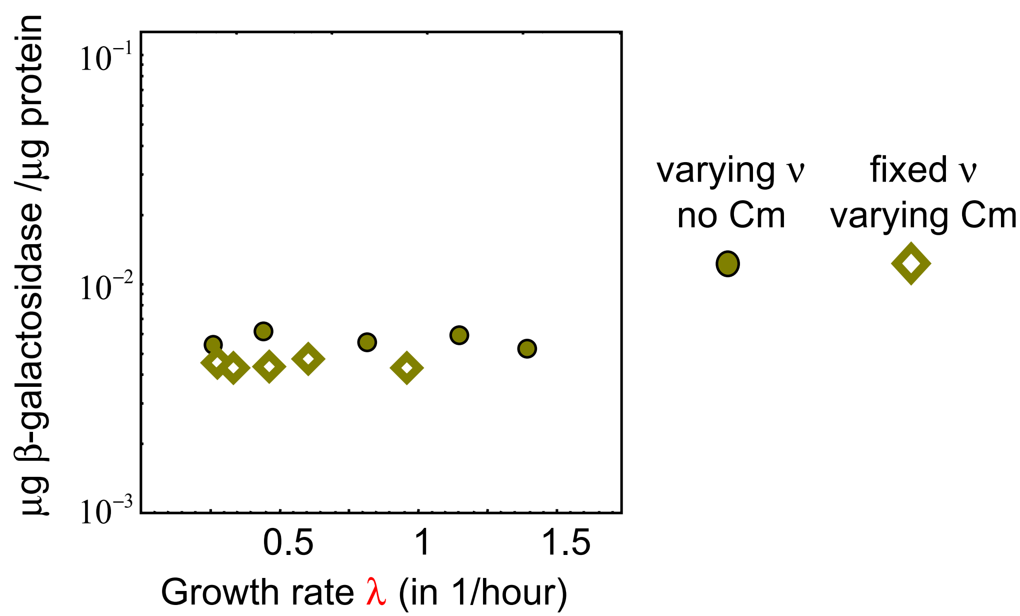
Three-component model of the proteome



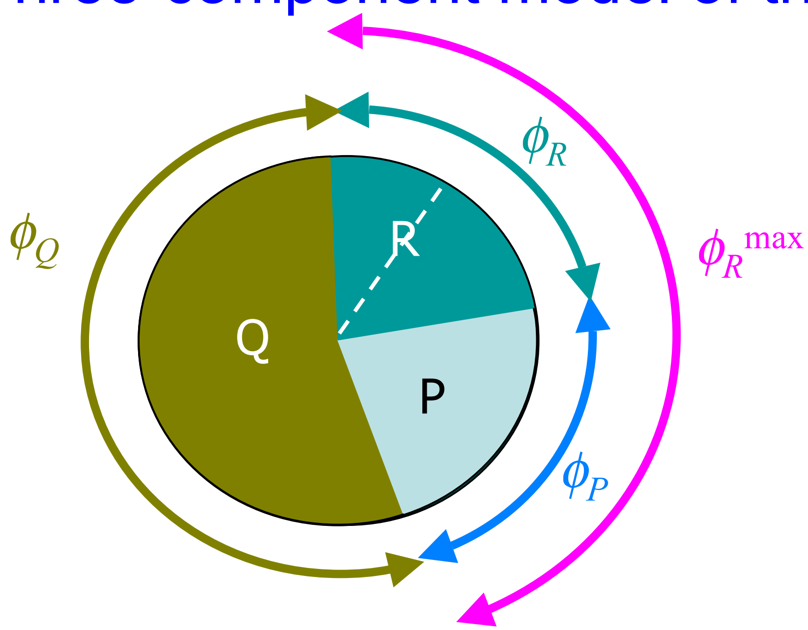
- maintenance of a fixed core (Q)

negative feedback regulation





Three-component model of the proteome



Mass fraction: $\phi_R + \phi_Q + \phi_P = 1$

$$\Downarrow \quad \phi_R^{max} + \phi_Q = 1,$$

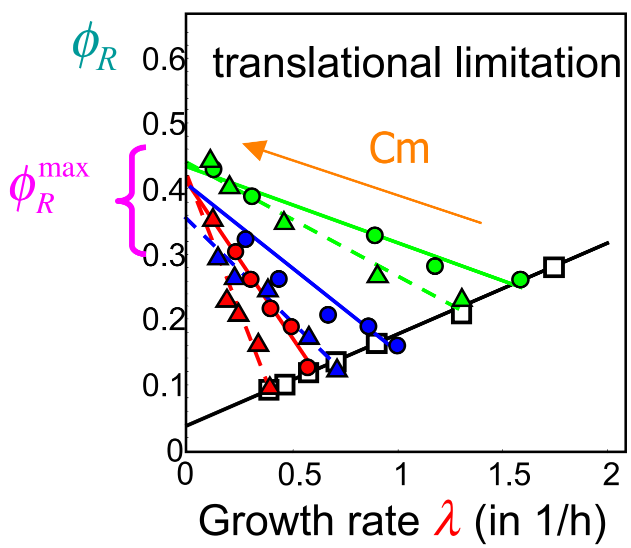
$$\boxed{\phi_P + \phi_R = \phi_R^{max}}$$

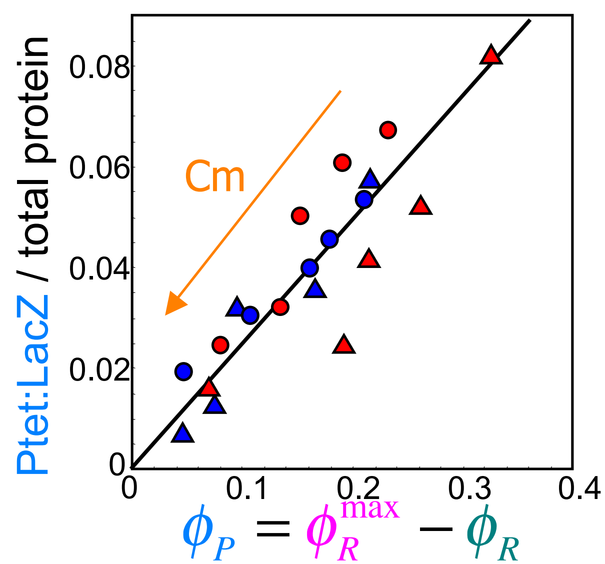
$$\Rightarrow \boxed{\phi_P = \lambda / \nu} \quad \text{for tsl limitation}$$

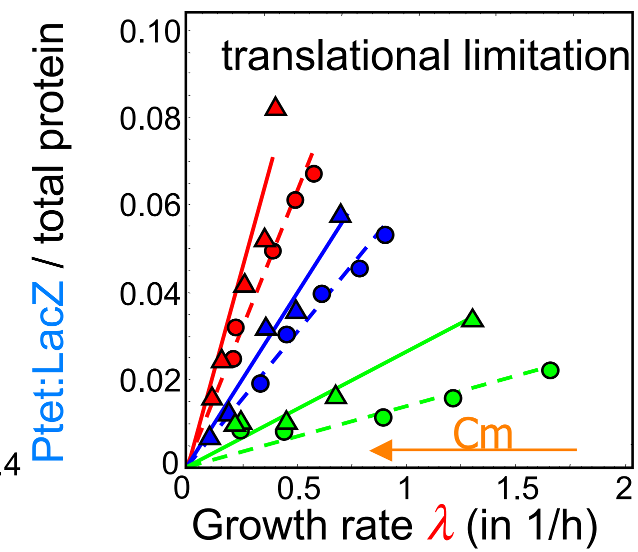
✓ constitutive expression $\propto \phi_P$

✓ linear increase with GR

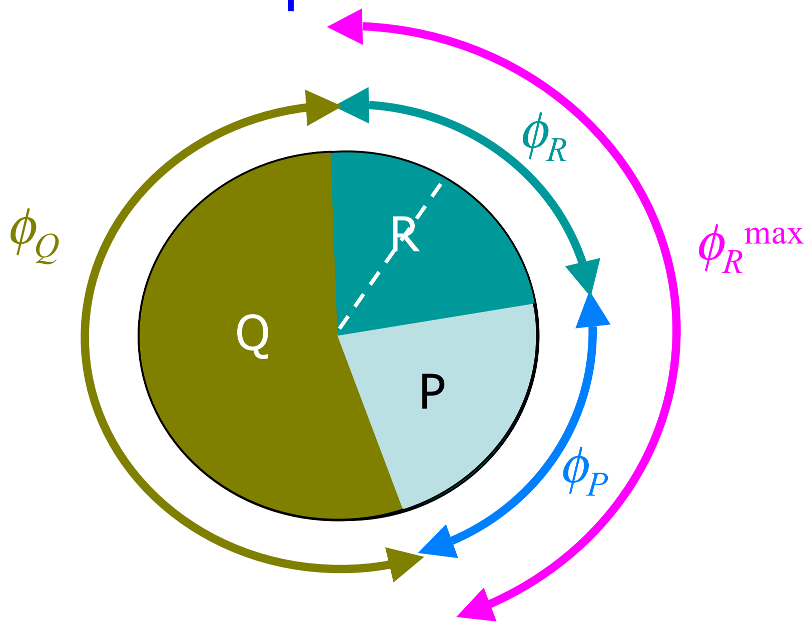
combine with $\phi_R = \phi_R^{max} - \lambda / \nu$







Three-component model of the proteome



Mass fraction: $\phi_R + \phi_Q + \phi_P = 1$

$$\Downarrow \quad \phi_R^{\max} + \phi_Q = 1,$$

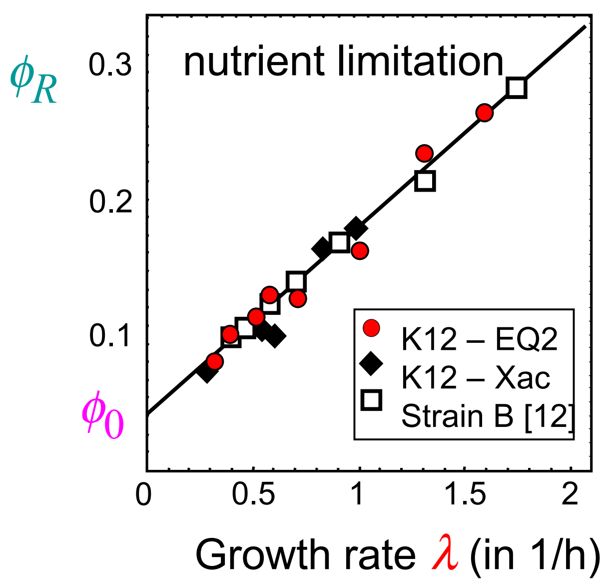
$$\phi_P + \phi_R = \phi_R^{\max}$$

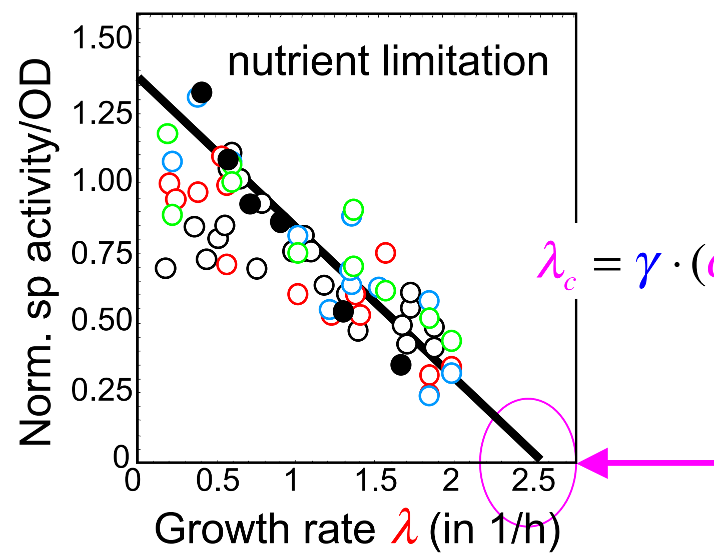
$\Rightarrow$ $\phi_P = \lambda / \nu$ for tsl limitation

$\Rightarrow$ $\phi_P = \left(\phi_R^{\max} - \phi_0\right) - \lambda / \gamma$

for nutrient limitation

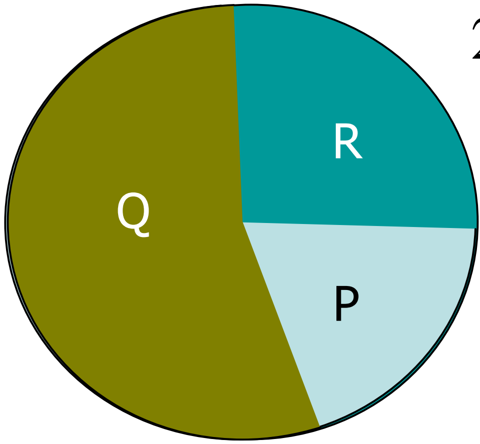
combine with $\phi_R = \lambda / \gamma + \phi_0$





$$\lambda_c = \gamma \cdot (\phi_R^{\max} - \phi_0)$$

Overall picture:



2nd growth law:

$$\lambda = \gamma \cdot (\phi_R - \phi_0)$$

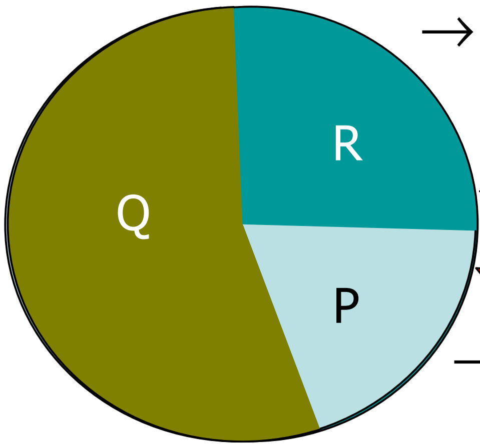
$$\lambda = \nu \cdot \phi_P$$

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

3rd growth law: $\phi_R = \phi_R^{\max} - \lambda / \nu$

Theory of growth-dependent gene expression

[Scott et al, Science 2010]



→ protein synthesis:

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

R/P partition according to ν,γ (state variables!) ← conductances

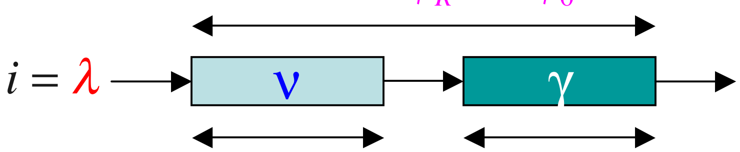
→ nutrient influx:

$$\lambda(\gamma,\nu) = \nu \cdot \phi_P$$ ← Ohm's law

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

Electrical analogy: resistors in series

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



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

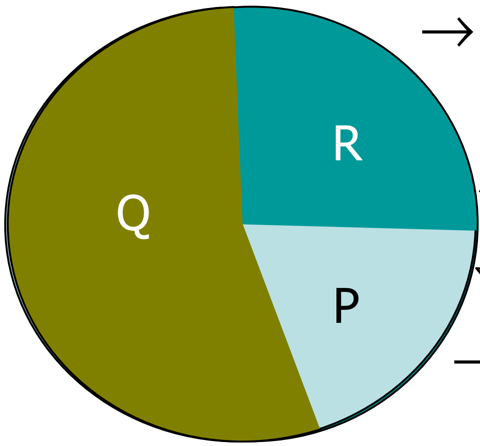
$\lambda_{\max} \approx 3.5$ dbl/hr

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

[J. Monod, '42]

Michaelis formula for cell growth!

Theory of growth-dependent gene expression



→ protein synthesis:

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

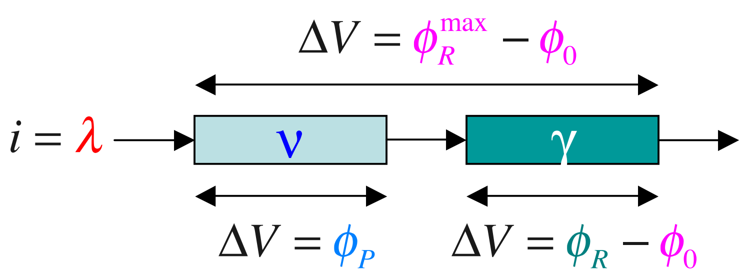
R/P partition according to ν,γ (state variables!)

→ nutrient influx:

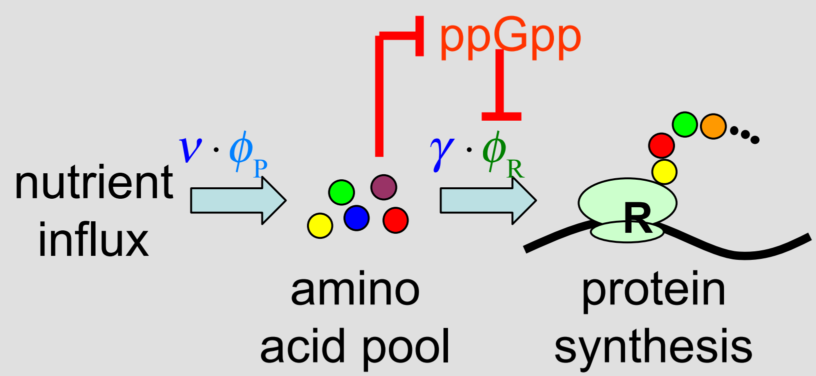
$$\lambda(\gamma,\nu) = \nu \cdot \phi_P$$

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

Electrical analogy: resistors in series



Mechanism of R/P coordination:



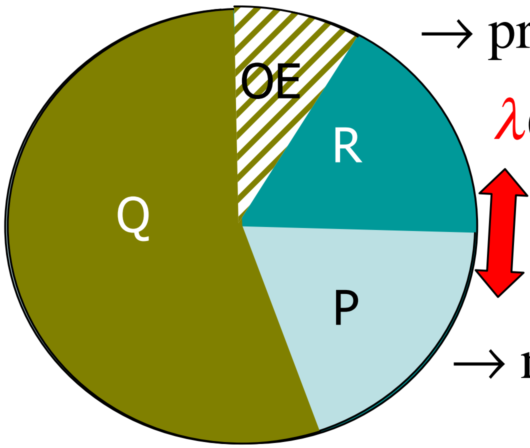
$\lambda_{max} \approx 3.5$ dbl/hr

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

[J. Monod, '42]

Michaelis formula for cell growth!

Test: cost of protein overexpression



$\rightarrow$ protein synthesis:

$$\lambda(\gamma,\nu)=\gamma\cdot\left(\phi_R-\phi_0\right)$$

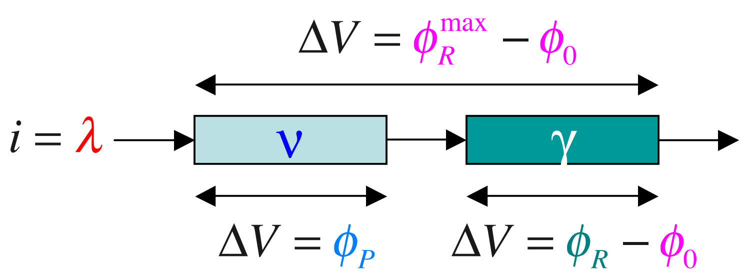
R/P partition according to ν,γ (state variables!)

$\rightarrow$ nutrient influx:

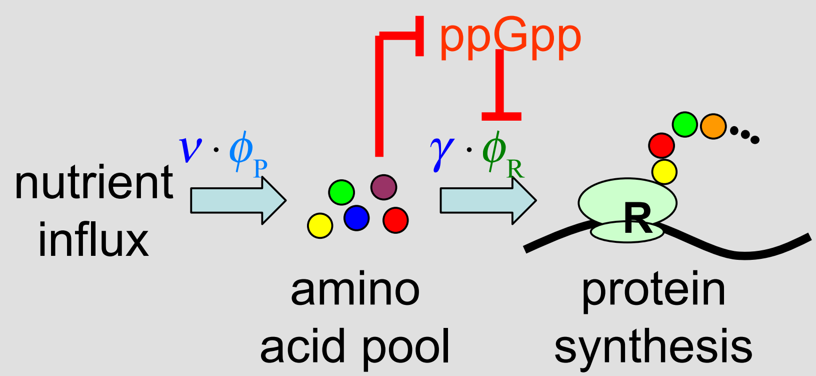
$$\lambda(\gamma,\nu)=\nu\cdot\phi_P$$

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

Electrical analogy: resistors in series



Mechanism of R/P coordination:

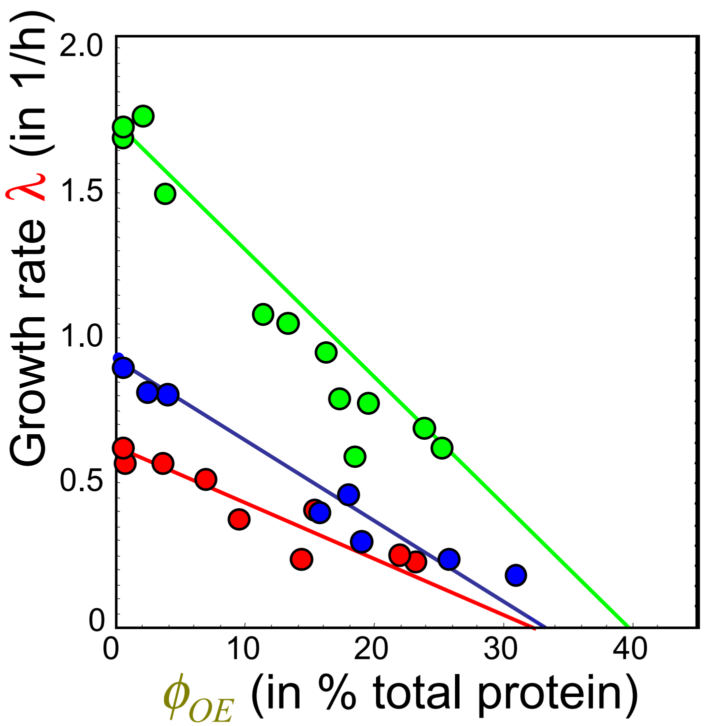


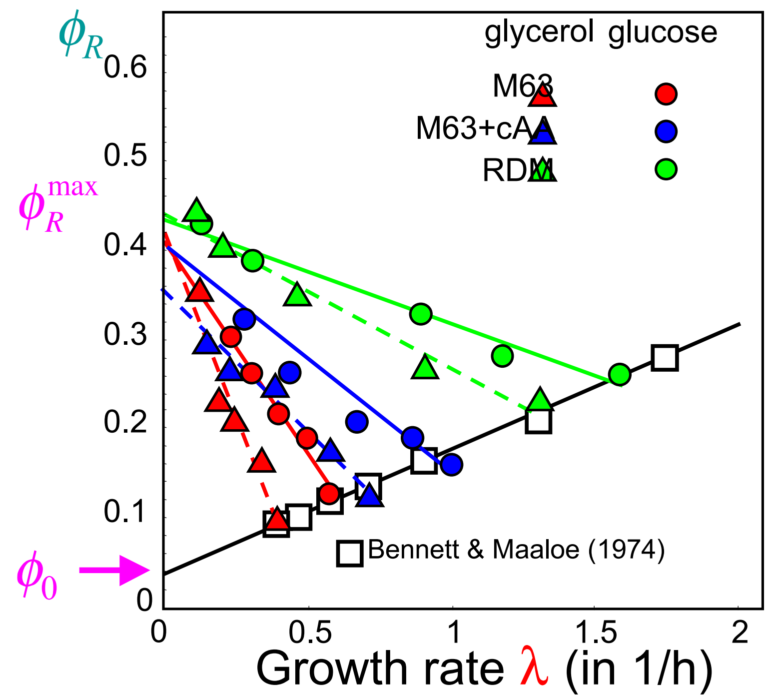
$$\rightarrow\quad \lambda(\gamma,\nu)=\left(\gamma^{-1}+\nu^{-1}\right)^{-1}\cdot\left(\phi_R^{\max}-\phi_0\right)=\underbrace{\left(\phi_R^{\max}-\phi_0\right)\cdot\gamma}_{\lambda_{\max}\approx 3.5\ \mathrm{dbl/hr}}\cdot\frac{\nu}{\gamma+\nu}$$

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

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

Test: cost of protein overexpression





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

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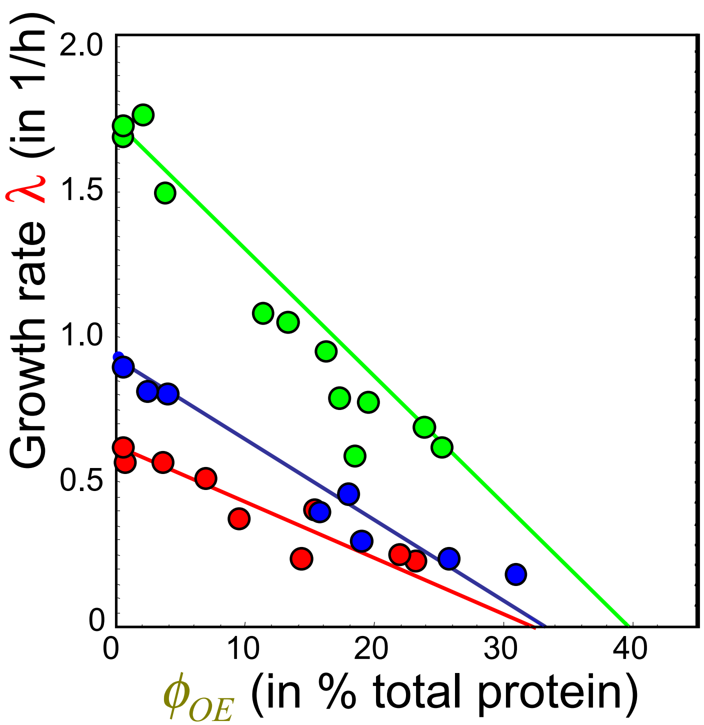

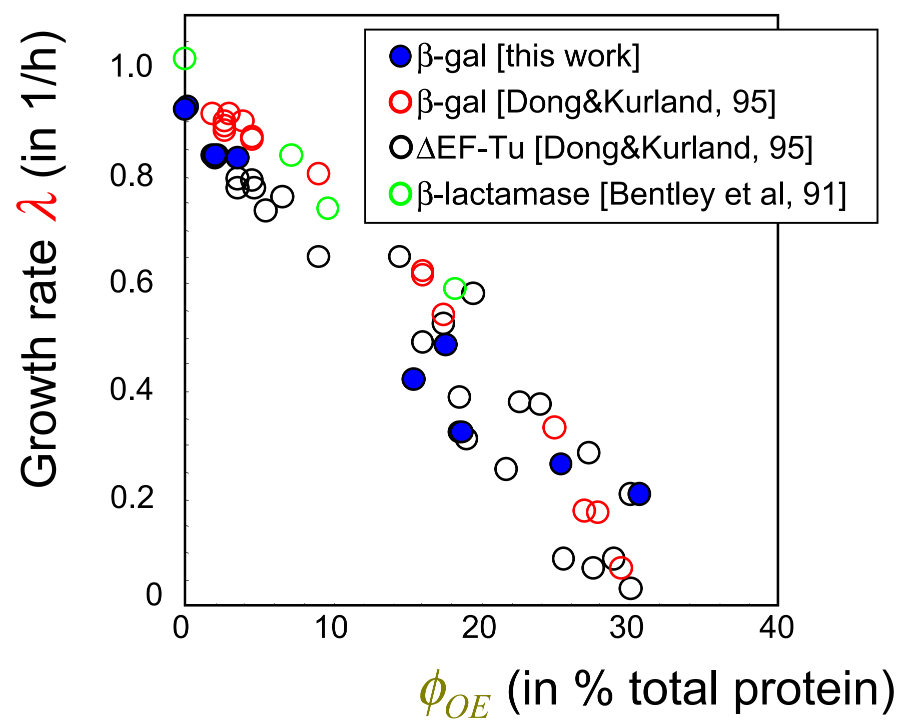

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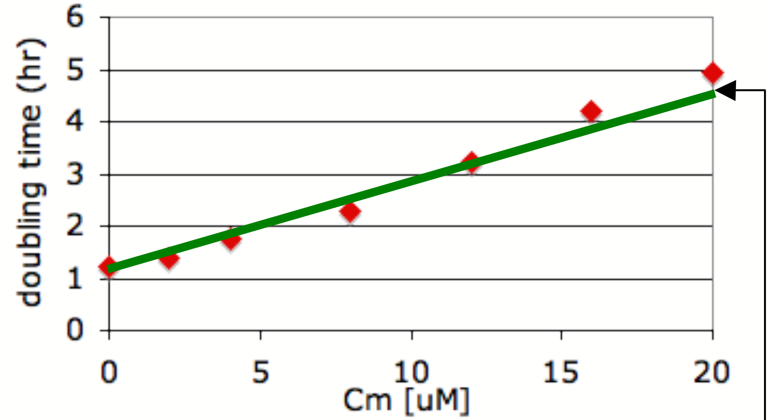
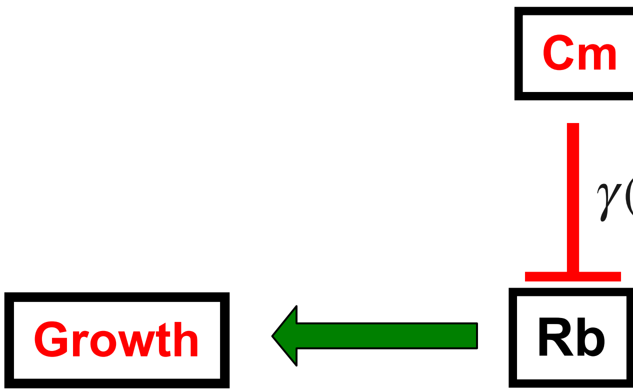
Application: Effect of antibiotics on cell growth

- consider a translation-inhibiting antibiotics (e.g., chloramphenicol)





$$\gamma(C) = \frac{\gamma_0}{1 + C / K_D}$$

[Harvey/Koch, 1980]

$$C_{50} \equiv K_D \cdot \left(\lambda_{\max} / \lambda_0\right)$$

$$K_D \approx 2 \mu M$$

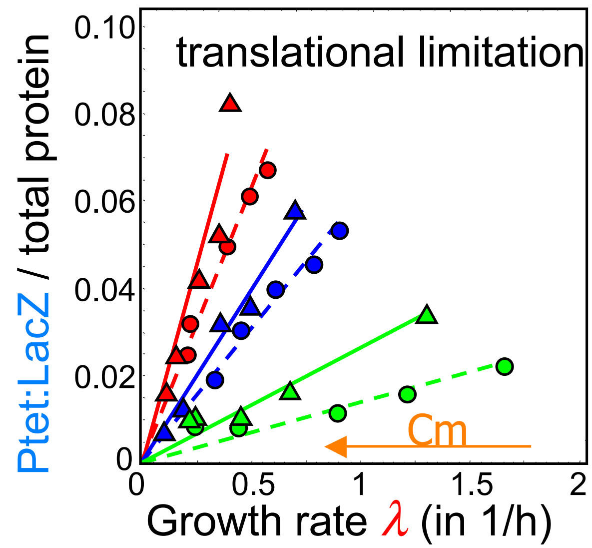
$$\Rightarrow \ C_{50} \approx 7 \mu M$$

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

$$\lambda^{-1} = \lambda_0^{-1} \cdot \left(1 + C / C_{50}\right)$$

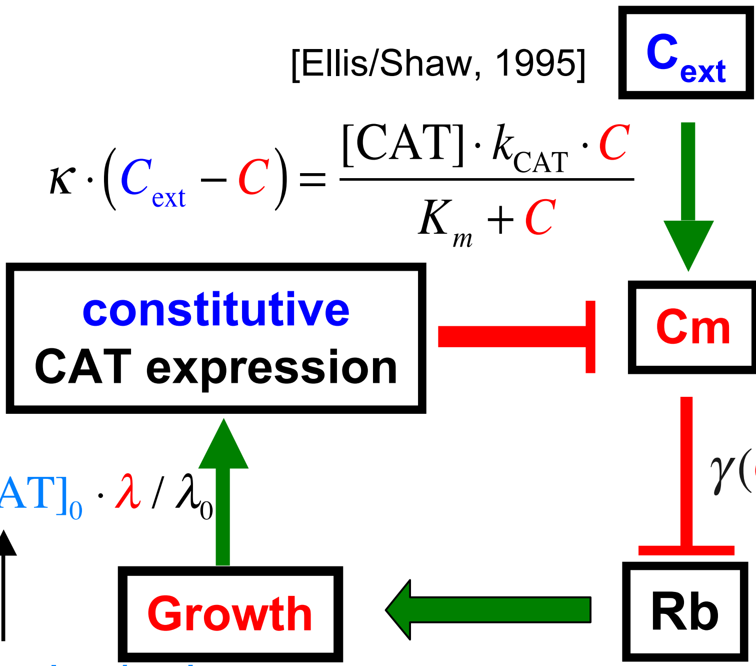
Application: Effect of antibiotics on cell growth

- consider a translation-inhibiting antibiotics (e.g., chloramphenicol)
- expression of Cm resistance (CAT)



[Ellis/Shaw, 1995]

$$\kappa \cdot (C_{ext} - C) = \frac{[\mathrm{CAT}] \cdot k_{\mathrm{CAT}} \cdot C}{K_m + C}$$



$$\gamma(C) = \frac{\gamma_0}{1 + C / K_D}$$

[Harvey/Koch, 1980]

$$[\mathrm{CAT}] = [\mathrm{CAT}]_0 \cdot \lambda / \lambda_0$$

CAT level w/o drug

$$\lambda(C, \nu) = (\phi_R^{max} - \phi_0) \cdot \gamma(C) \frac{\nu}{\gamma(C) + \nu}$$

$$\lambda^{-1} = \lambda_0^{-1} \cdot (1 + C / C_{50})$$

➔ positive feedback without need for specific regulation!

Application: Effect of antibiotics on cell growth

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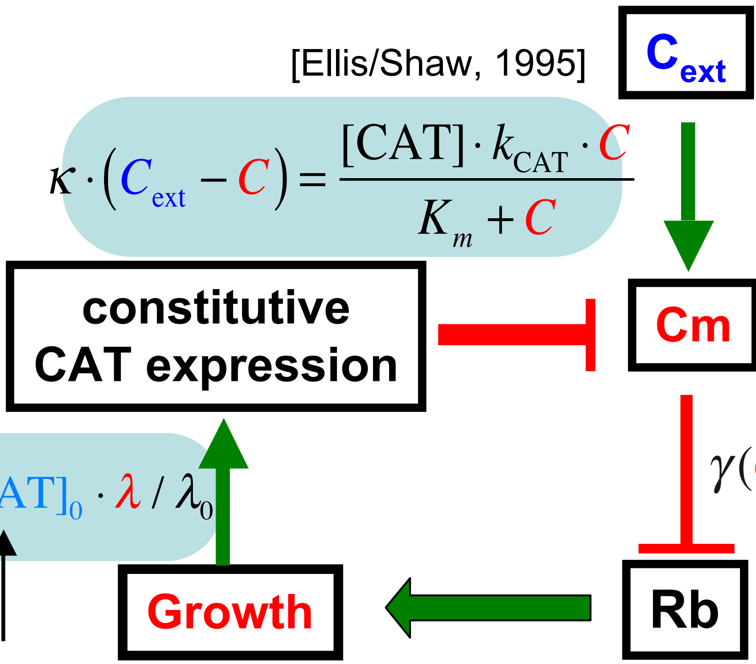

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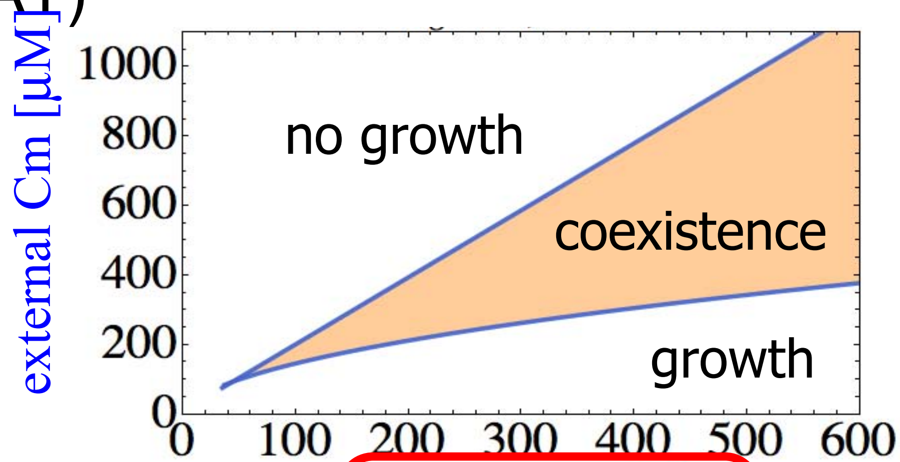


$k_{\text{cat}} \bullet [\text{CAT}]_0 / \kappa \bullet K_{\text{m}}$

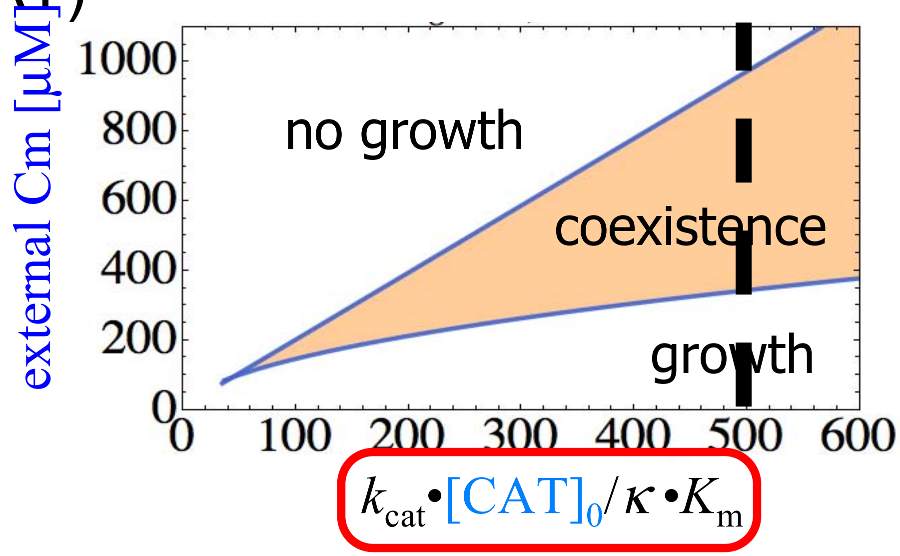
➔ positive feedback without need for specific regulation!

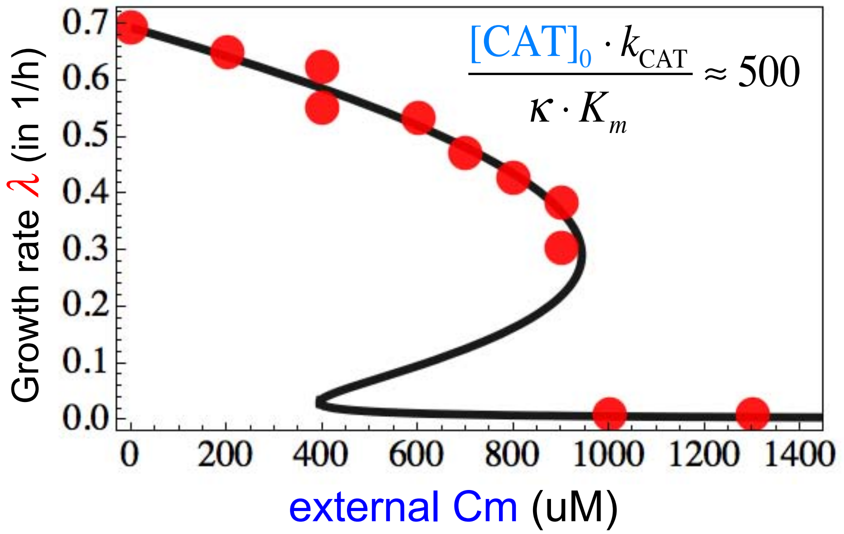
➔ generically expect abrupt transition and bimodality

➔ one dimensionless parameter (resistance efficacy)

Application: Effect of antibiotics on cell growth

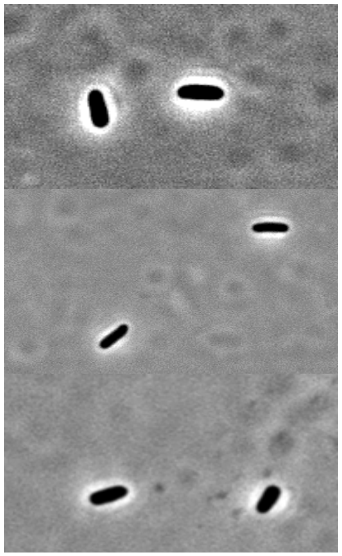
- consider a translation-inhibiting antibiotics (e.g., chloramphenicol)
- expression of Cm resistance (CAT)

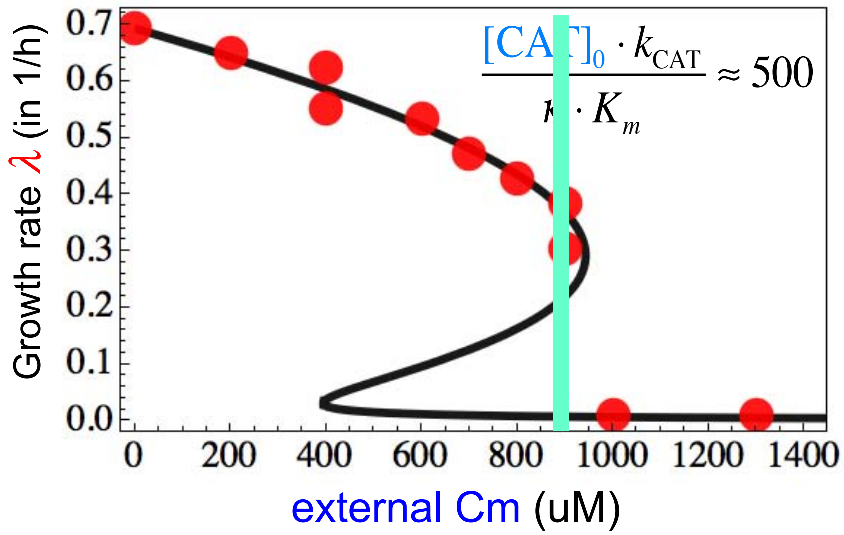




Occurrence of growth bimodality in the transition region

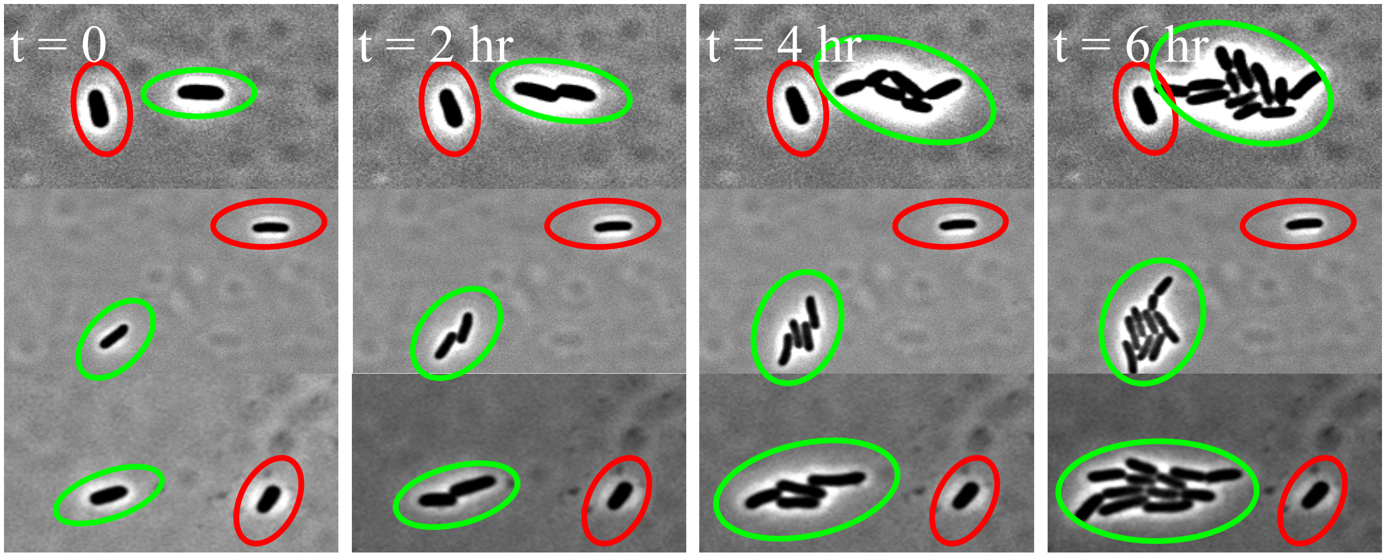
Observe cell growth in microfluidic chamber at 0.9mM Cm



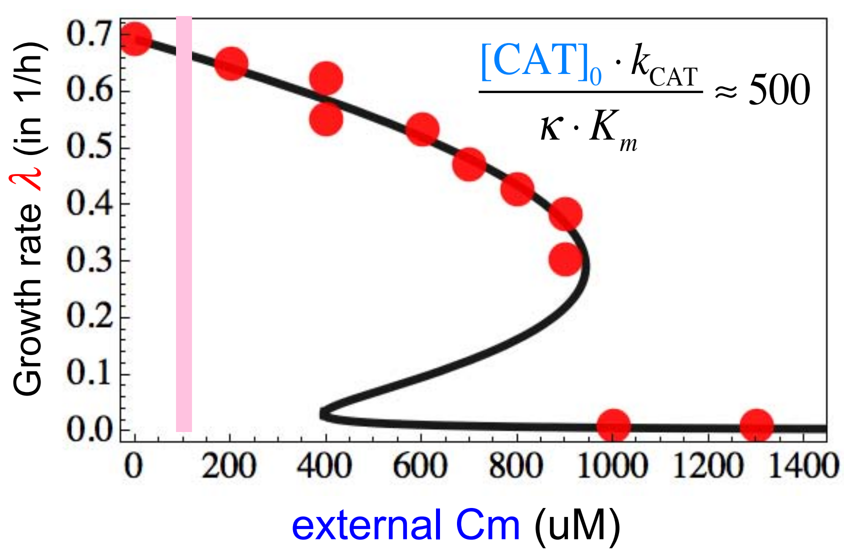


Occurrence of growth bimodality in the transition region

30% of seeded cells grew in microfluidic chamber at 0.9mM Cm



Switch back to 0.1mM Cm



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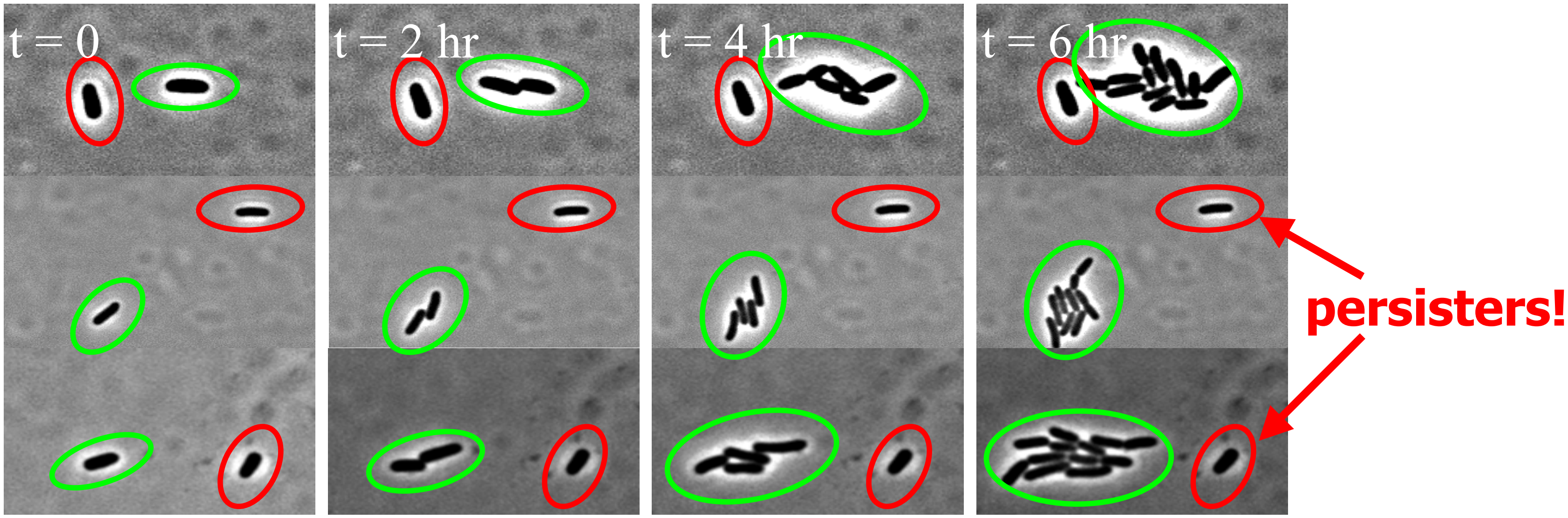


non-growers resumed growth 10hr after downshift to 0.1mM Cm

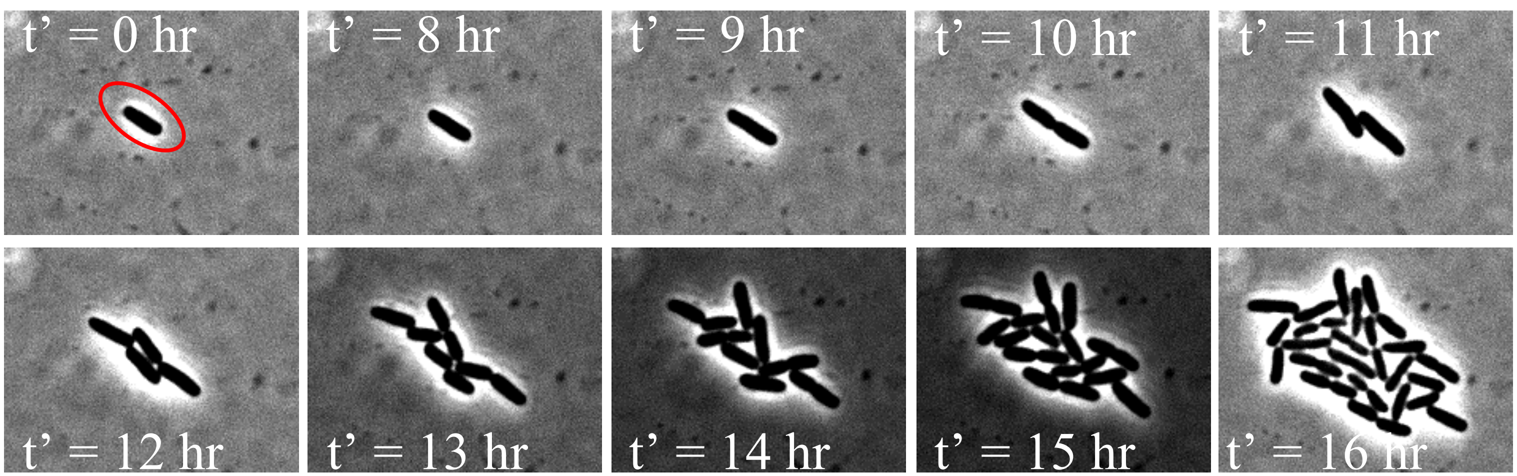
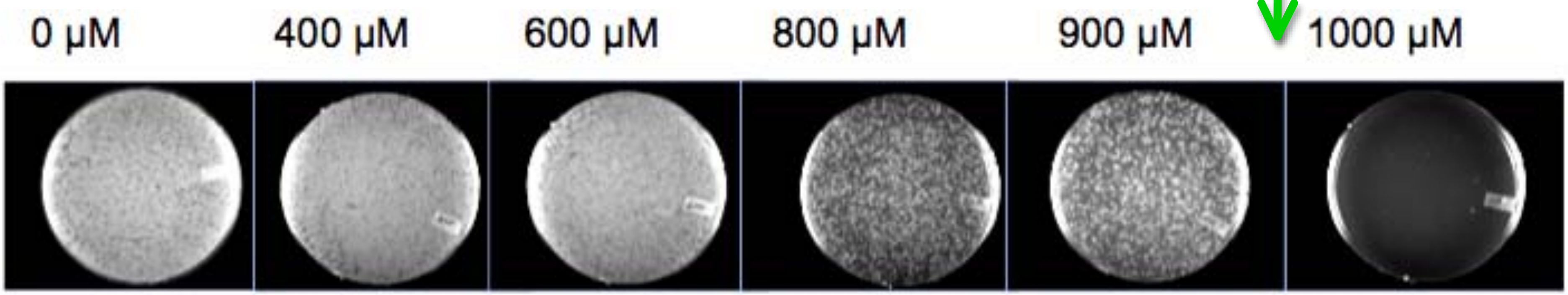


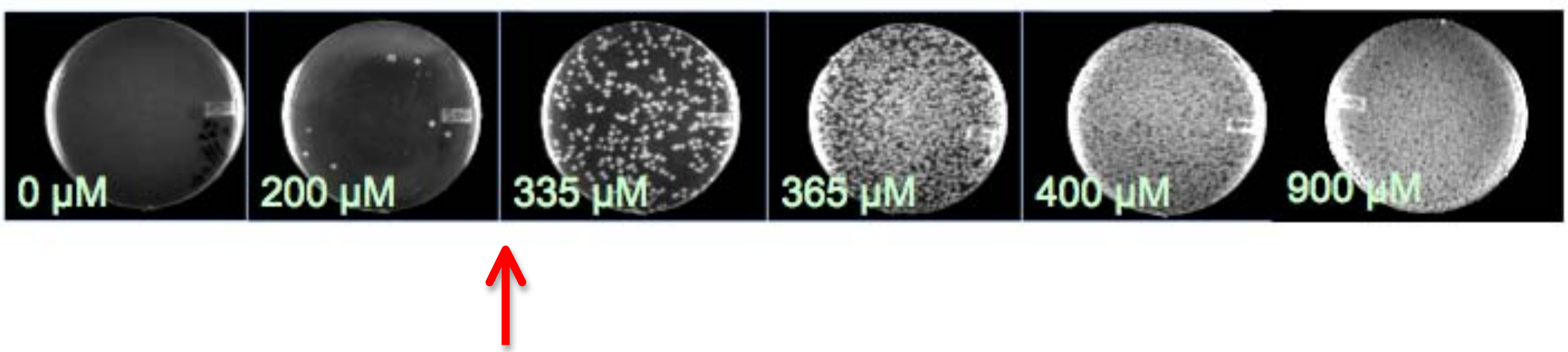
Plate assays to determine upper/lower transitions

Upper transition: Cm sensitivity assay

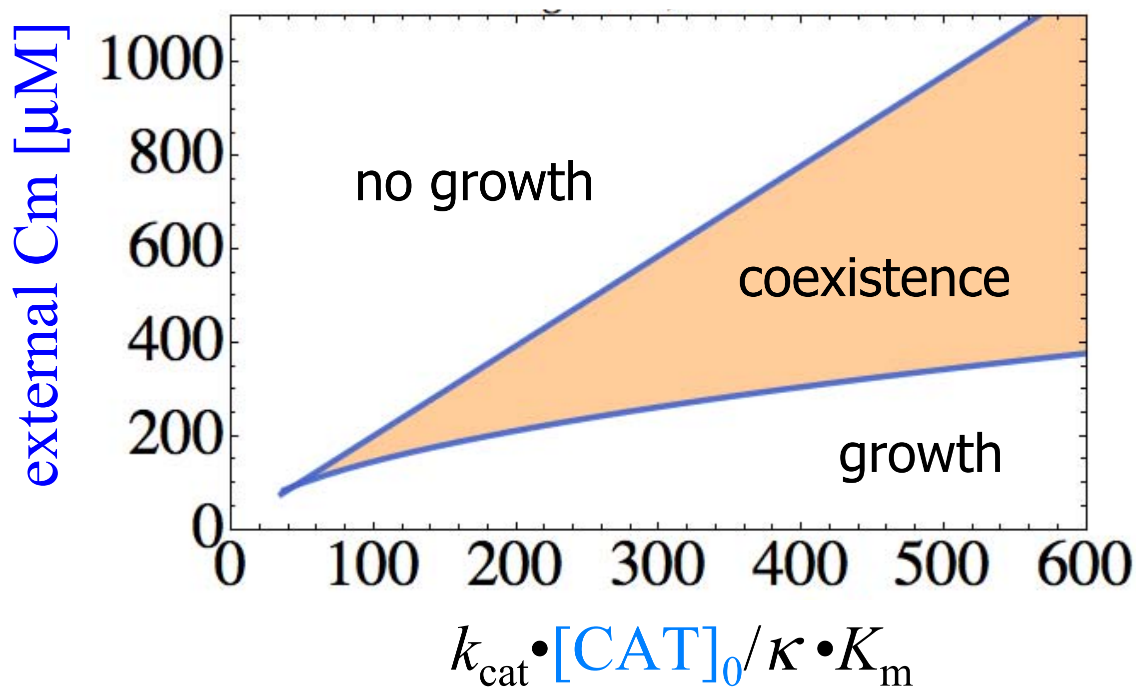


Lower transition:

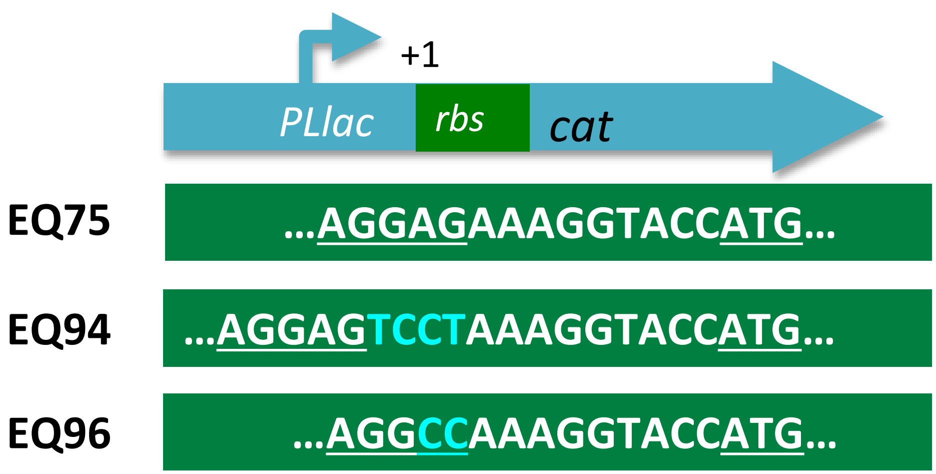
1. Batch culture growth in medium with Cm+Amp
2. Plate on LB plates with no drugs

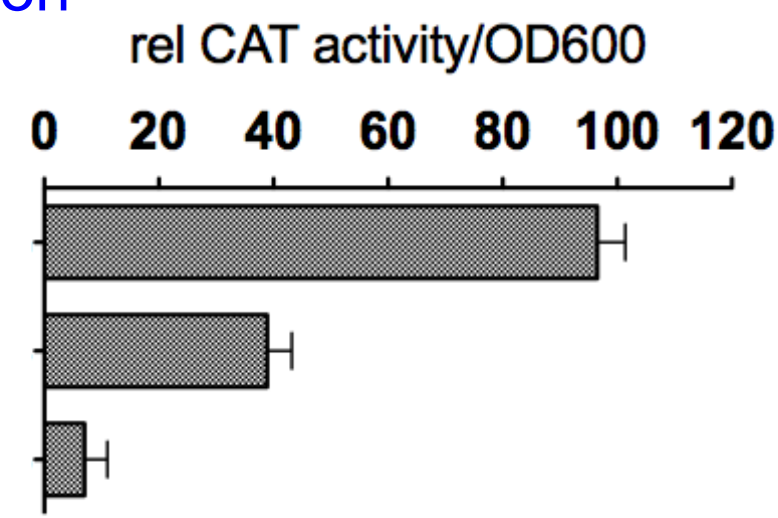


Predicted phase diagram



Probe by varying basal CAT expression





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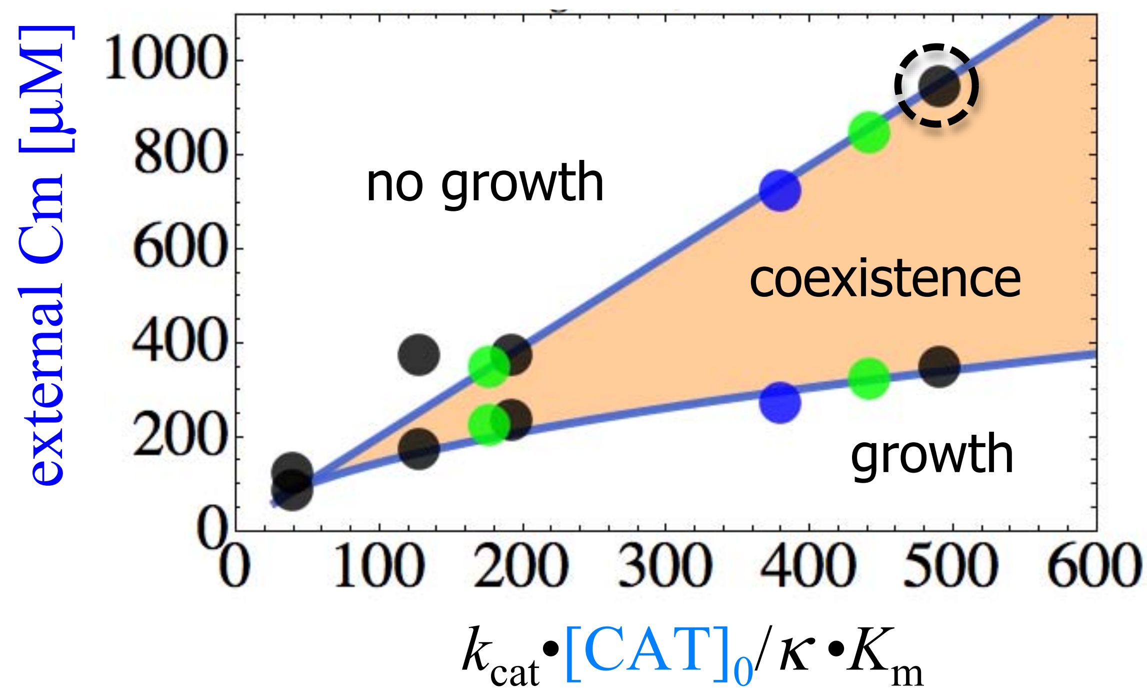

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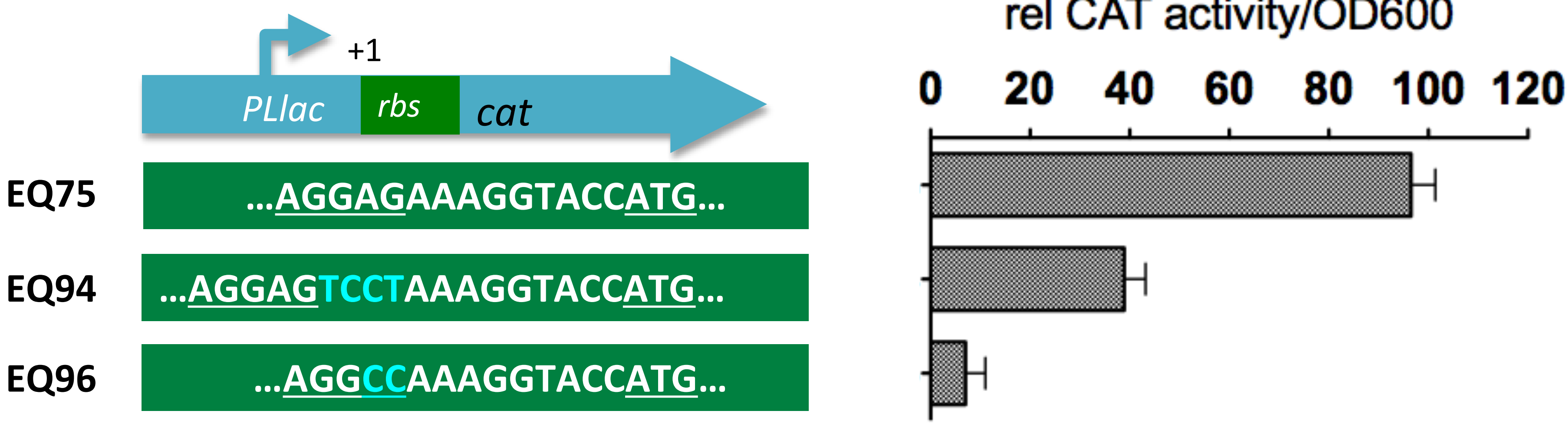
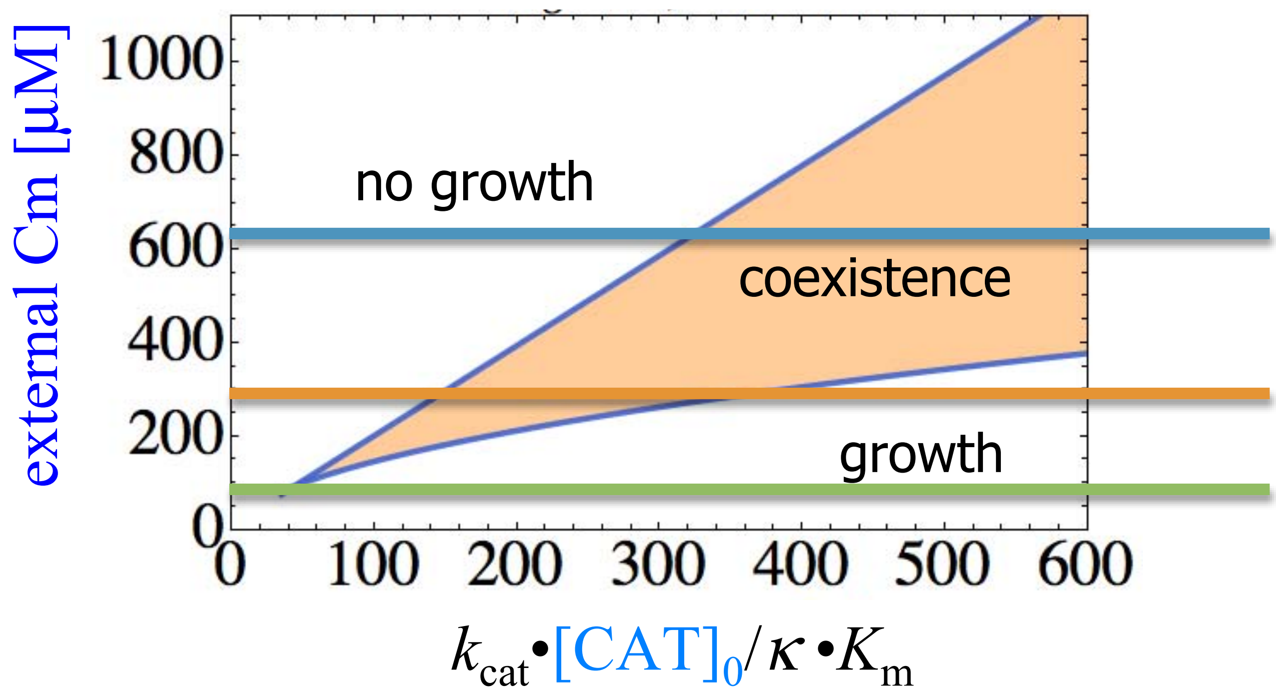
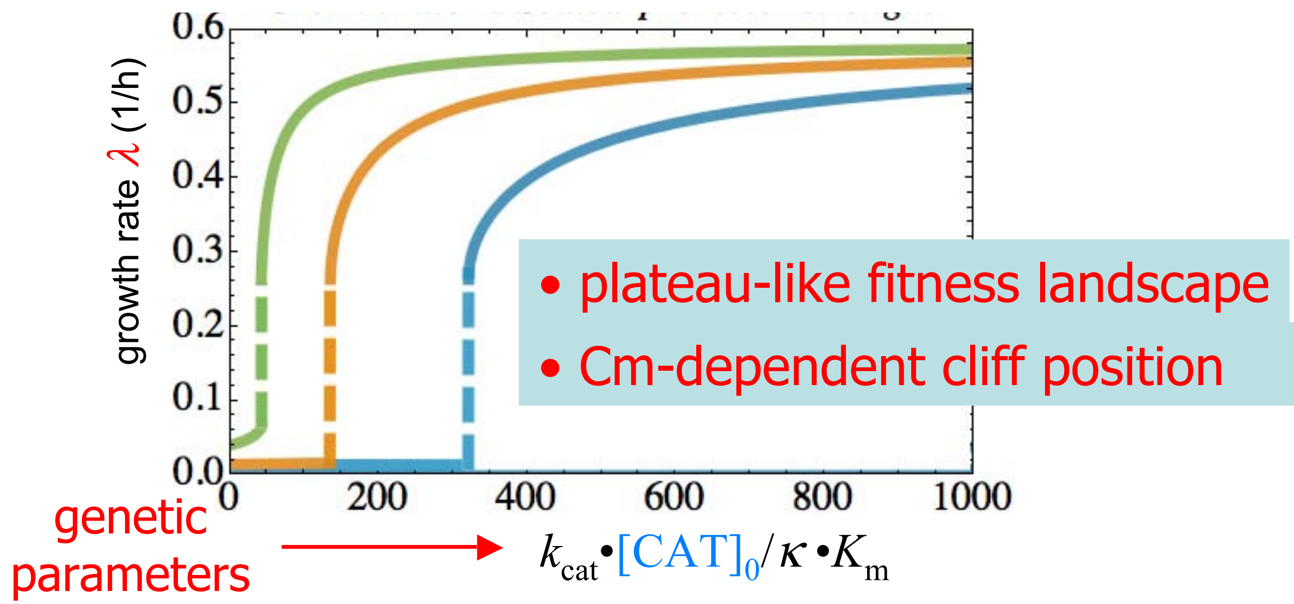
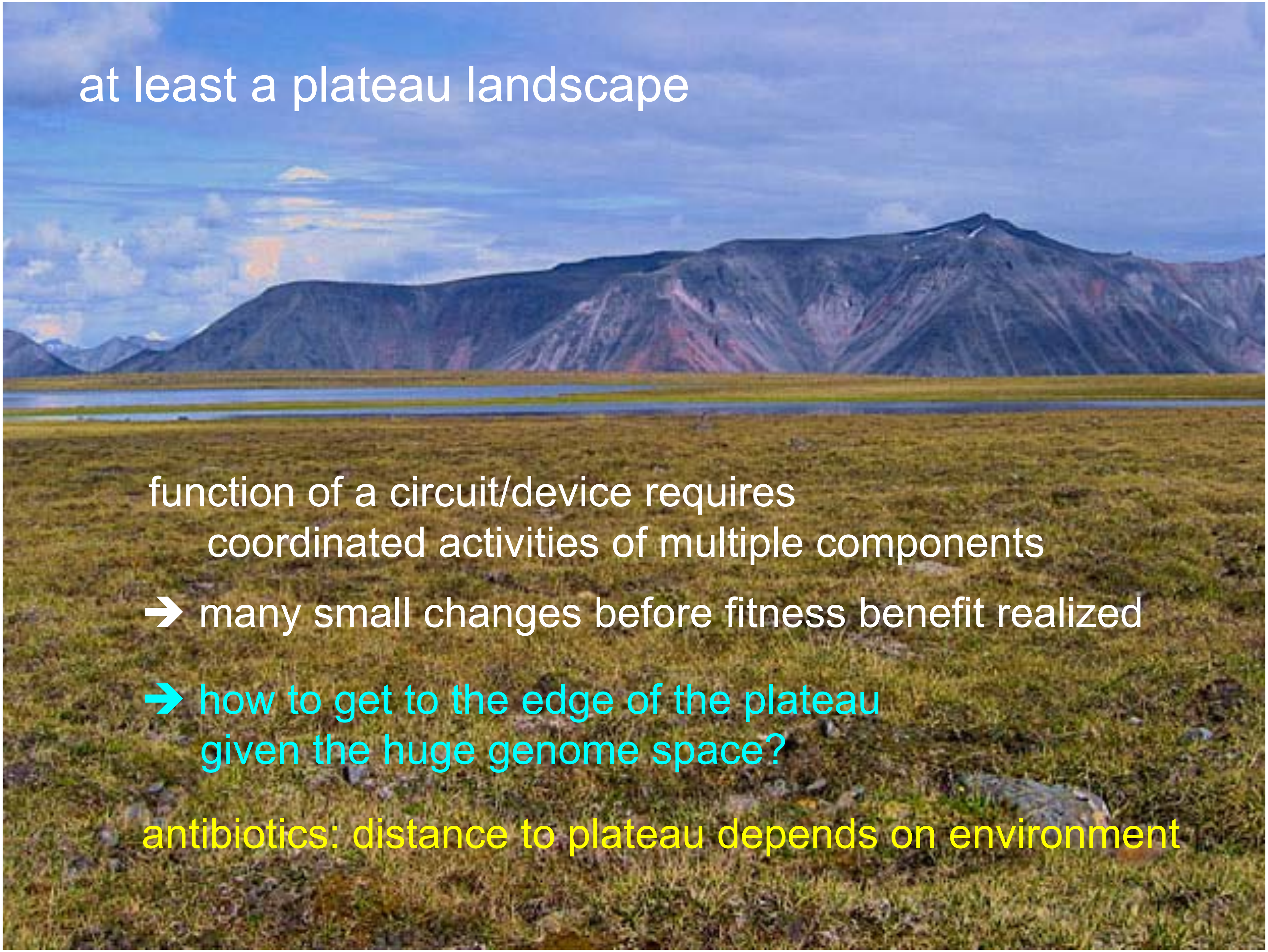


Another perspective: fixed ext Cm level







at least a plateau landscape

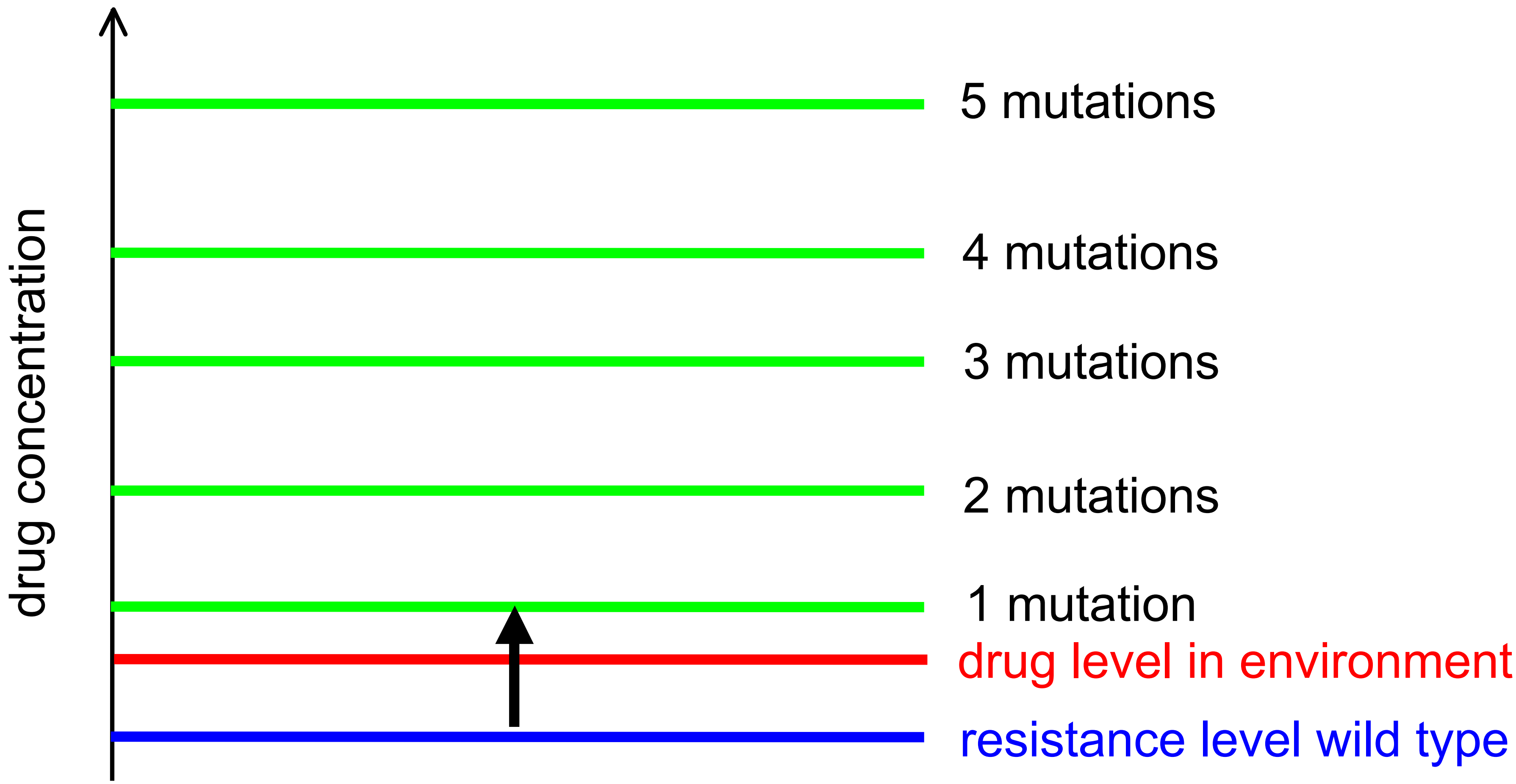
function of a circuit/device requires
coordinated activities of multiple components

➔ many small changes before fitness benefit realized

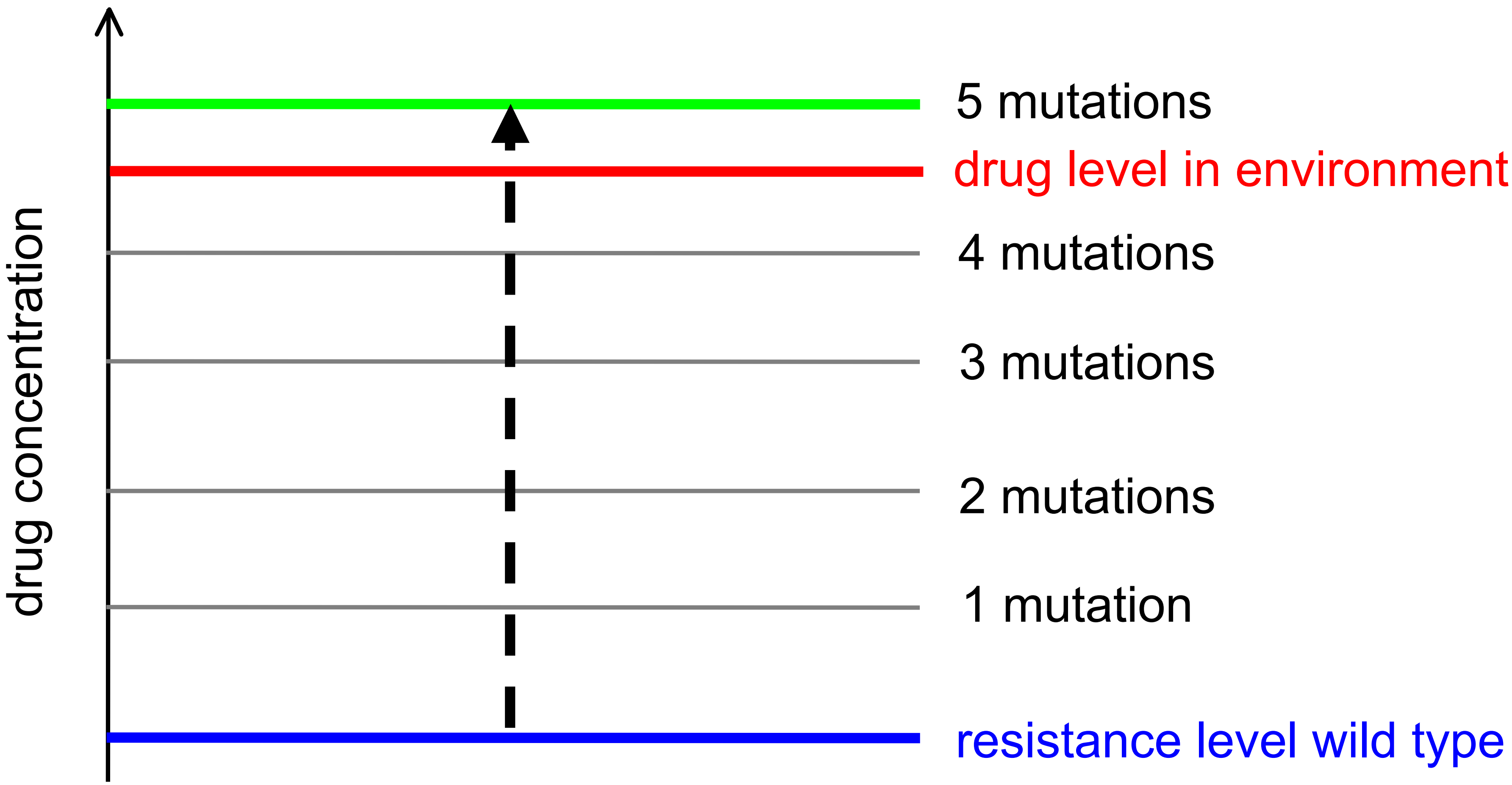
➔ how to get to the edge of the plateau
given the huge genome space?

antibiotics: distance to plateau depends on environment

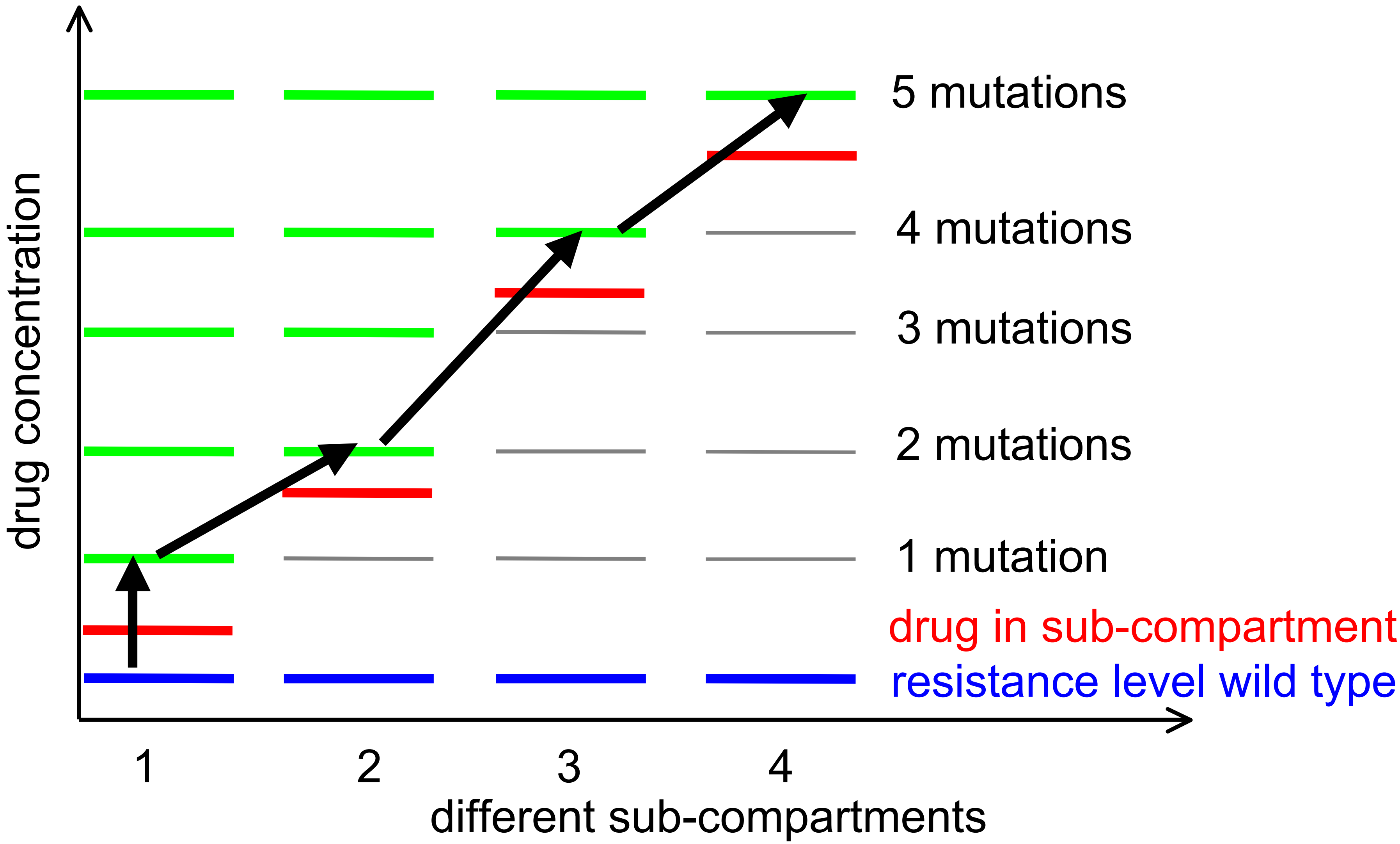
Adaptation easy if plateau close by



Adaptation hard if plateau far away

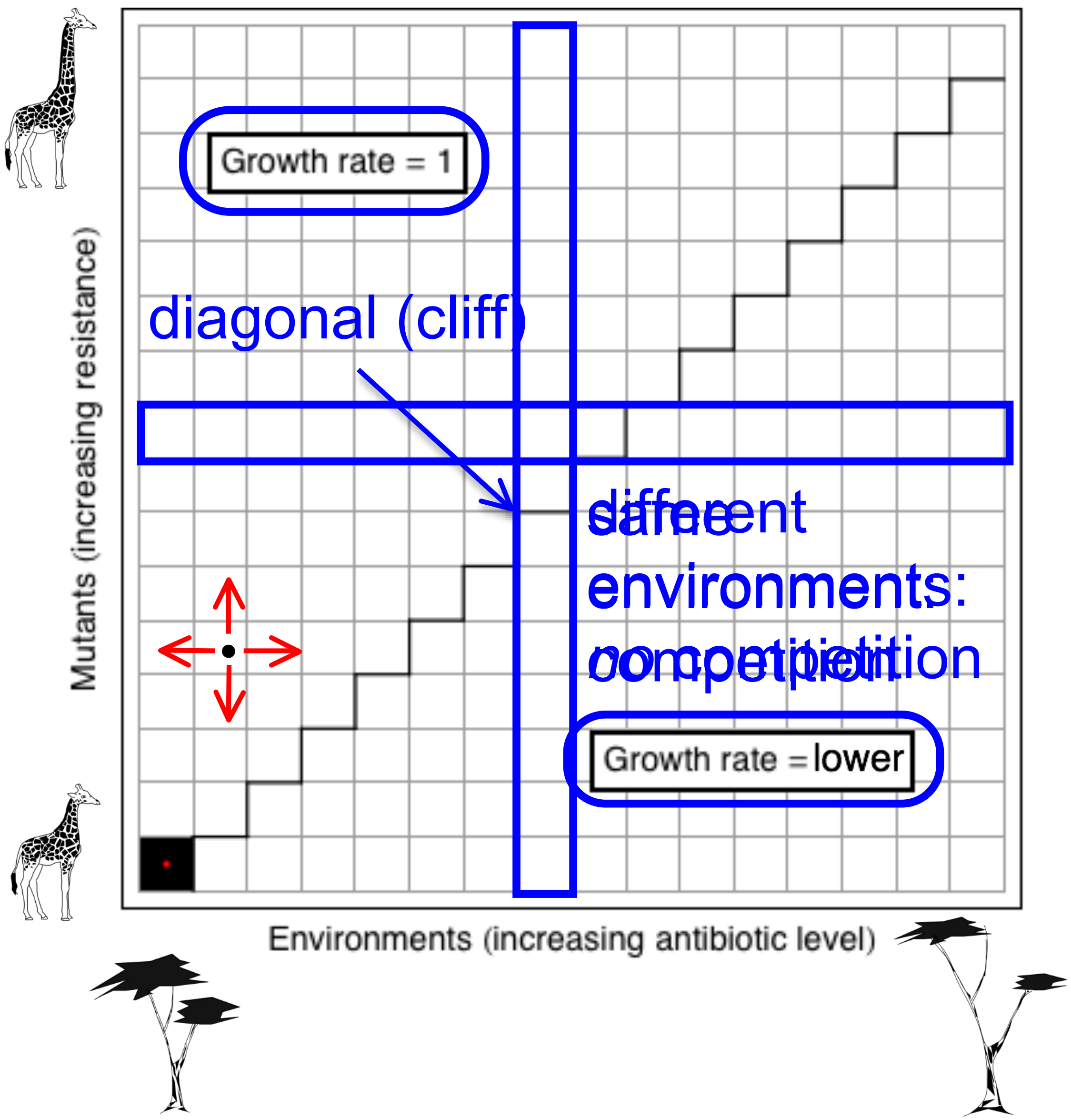


Spatial heterogeneities provide "staircase"



The "staircase model"

Landscape:

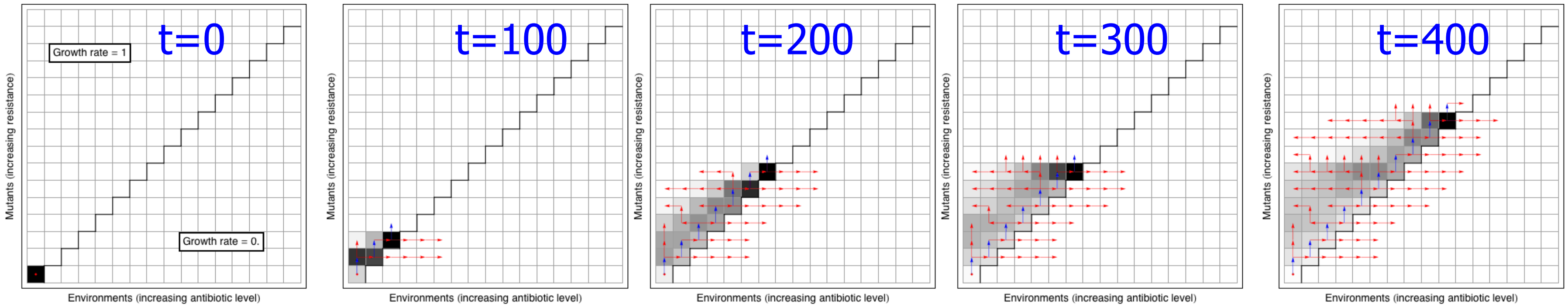


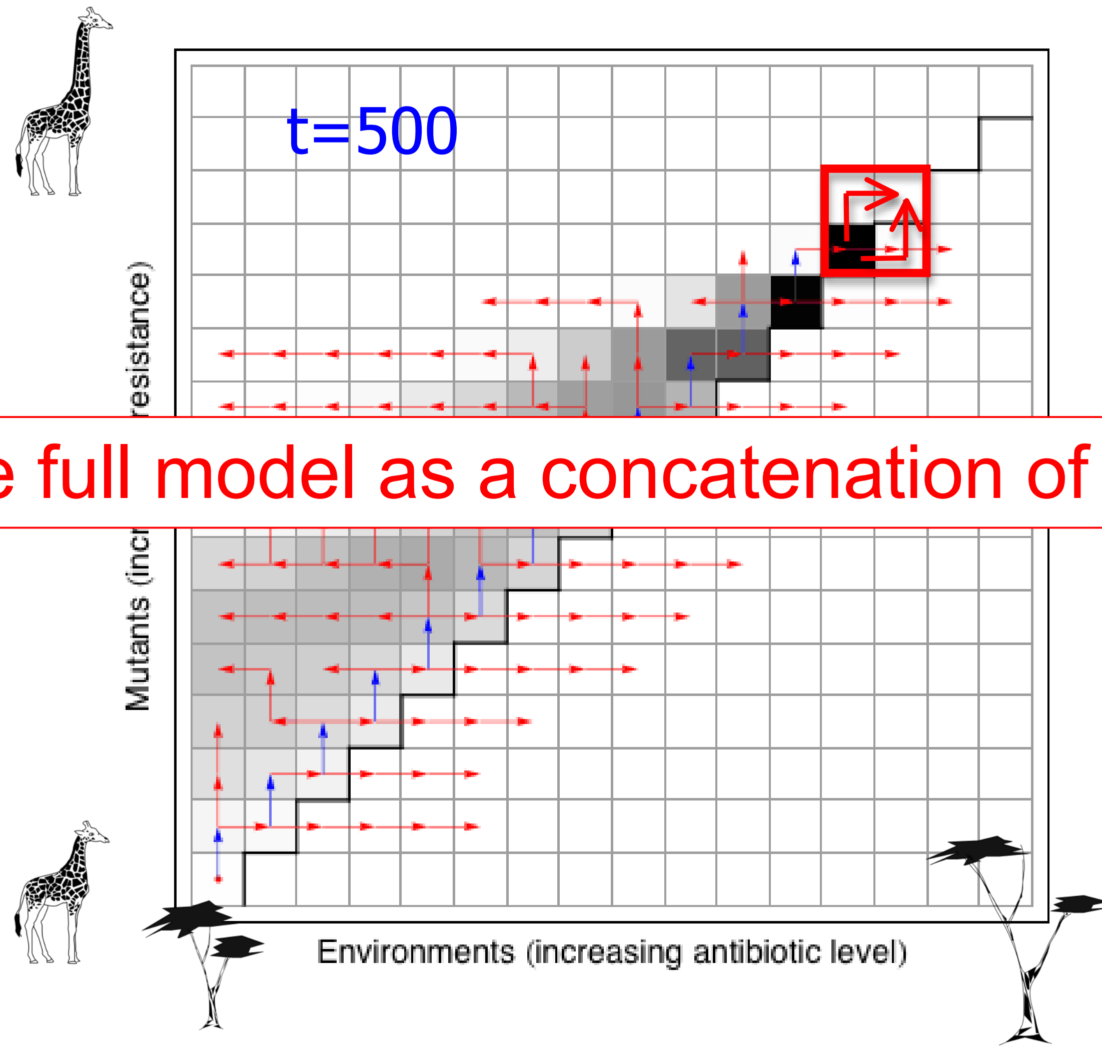
Processes and rates:

Mutation	μ_f
	μ_b
Migration	ν
	ν
Death	δ
Growth	$\gamma_{ij}(\rho_{ij})$

$$\gamma_{ij}(\rho_{ij}) = \lambda_{ij} \cdot \left(1 - \rho_{ij} / K\right)$$

("logistic")



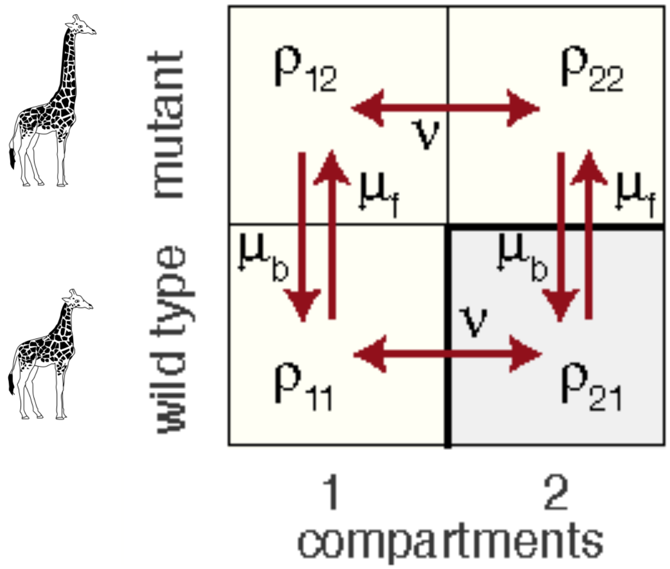


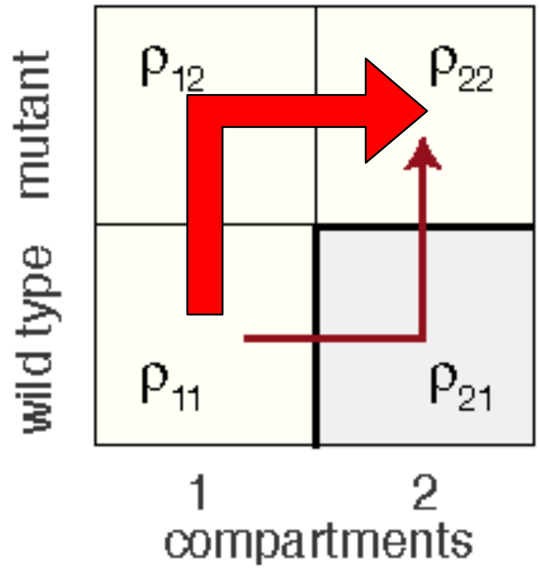
The full model as a concatenation of 2x2 models

Quantitative descrip. of the evolution-migration process?

2x2 case

- how long to go from (1,1) to (2,2)?
- by the upper or lower pathway?





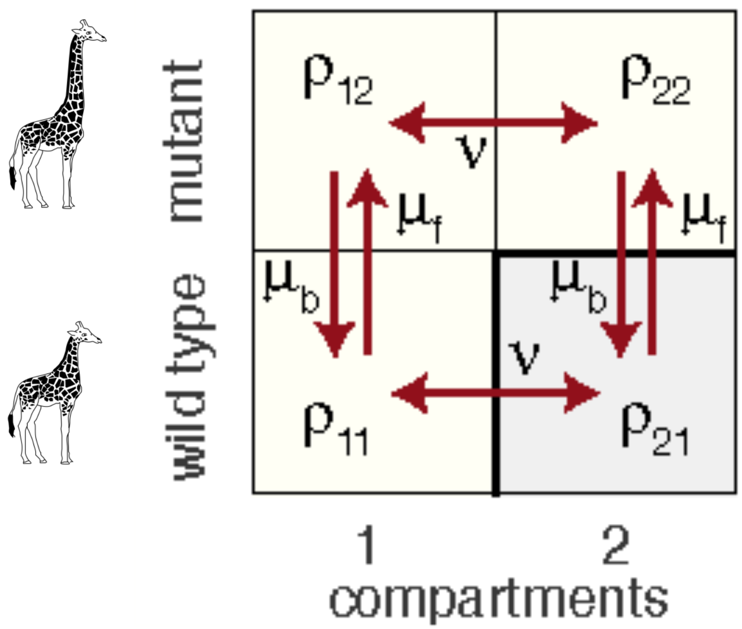
Analytic soln: path ↱ dominates

[Hermsen & TH, PRL, 2010]

$$\text{mean 1st arival time: } \tau \approx \frac{1}{\sqrt{\nu\cdot(\delta+\nu)}} f\left(\mu_f K/(\delta+\nu)\right) \quad \text{for } \mu_f, \mu_b \ll \nu < \delta$$

$$\text{with} \quad f(x) = \begin{cases} x^{-1} & \text{for } x \ll 1 \text{ (mutation-limited)} \\ x^{-1/2} & \text{for } x \gg 1 \text{ (migration-limited)} \end{cases}$$

Dependence on the fitness landscape (λ_{ij})



death rate: δ

growth rate: $\lambda_{ij} \cdot (1 - n_i / K)$

• landscape used so far:

1	1
1	0

• cost of adaptation (c):

1-c	1
1	0

• fitness of lower plateau (λ_0)

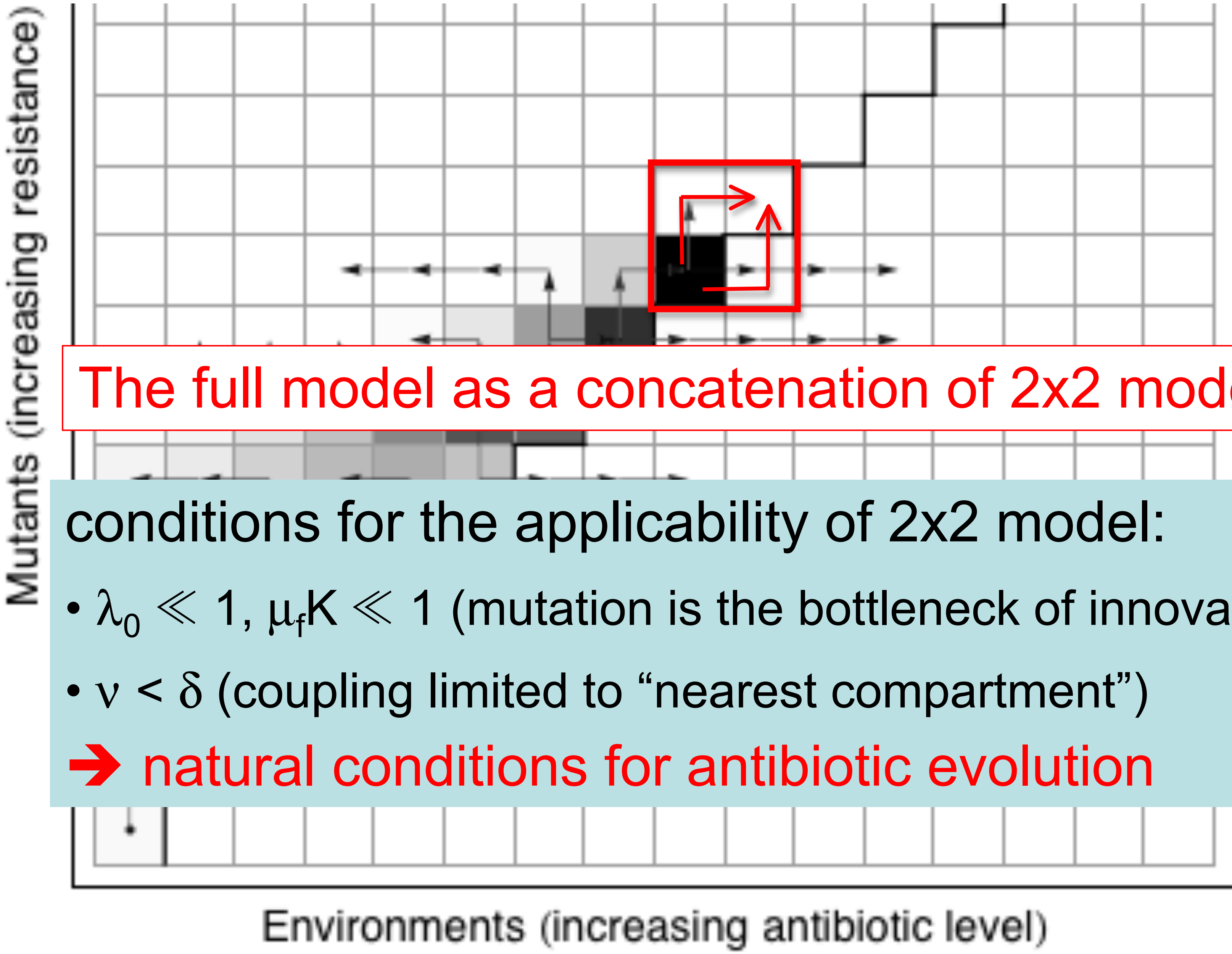
1	1
1	λ_0

$\Rightarrow$ cost of adaptation not important unless $c > \sqrt{\nu / \delta}$

$\Rightarrow$ abrupt drop in fitness ($\lambda_0 < \nu + \delta$) crucial

➔ plateau landscape eliminates competitors

Quantitative descrip. of the evolution-migration process?



The full model as a concatenation of 2x2 models

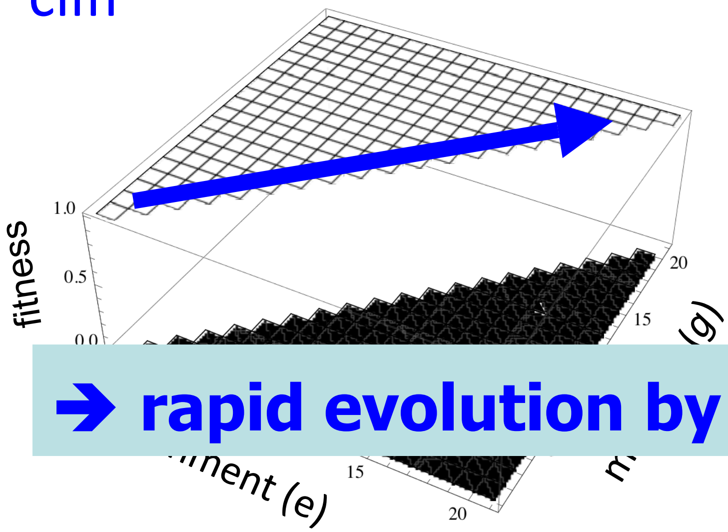
conditions for the applicability of 2x2 model:

- $\lambda_0 \ll 1$, $\mu_f K \ll 1$ (mutation is the bottleneck of innovation)
- $\nu < \delta$ (coupling limited to "nearest compartment")

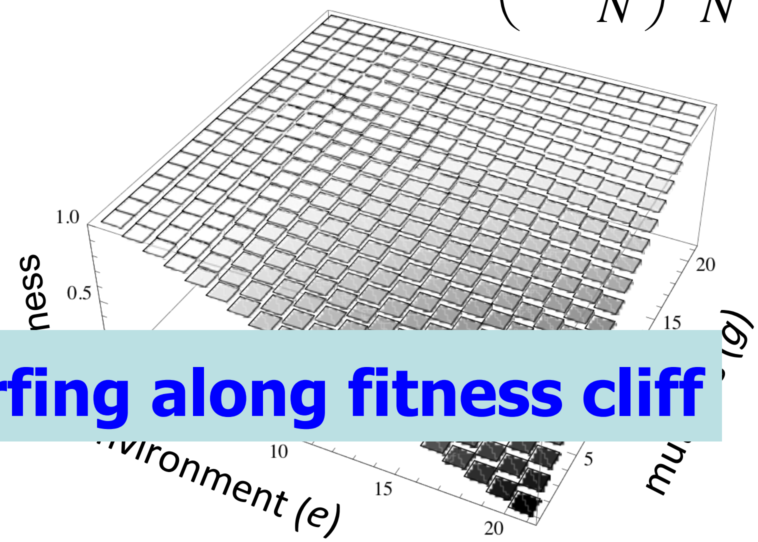
➔ natural conditions for antibiotic evolution

Plateau vs Fuji landscape

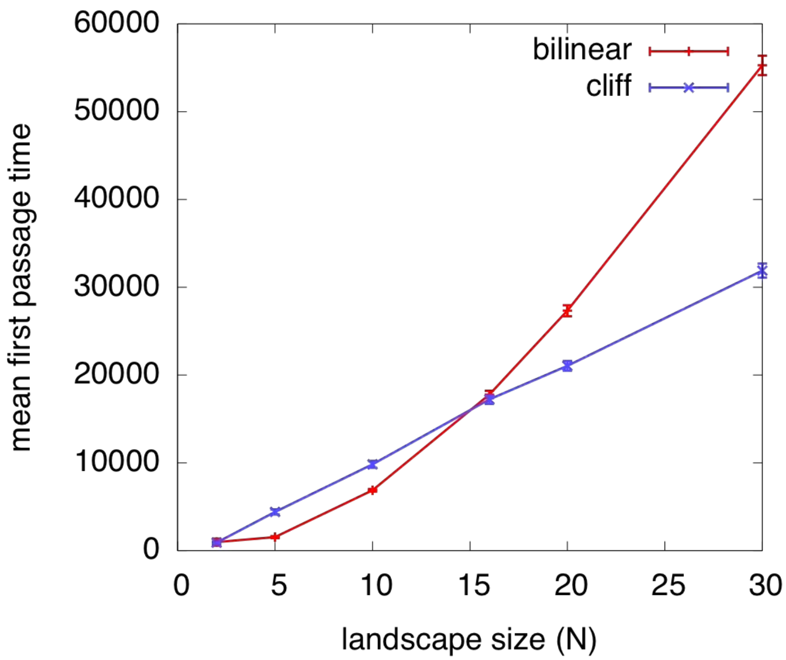
cliff



bilinear: $\lambda = 1 - \left(1 - \frac{g}{N}\right) \cdot \frac{e}{N}$

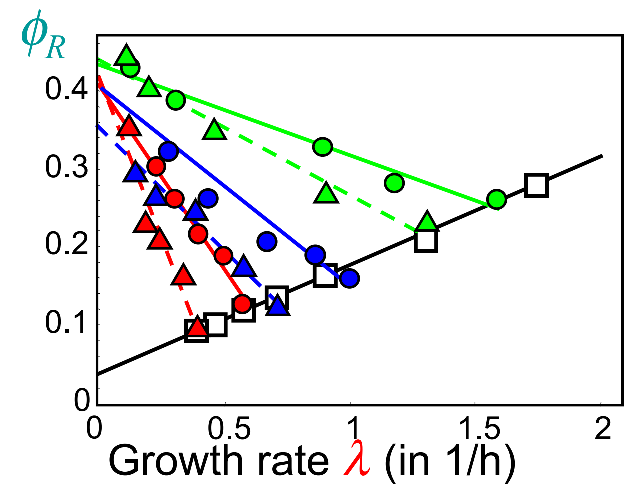


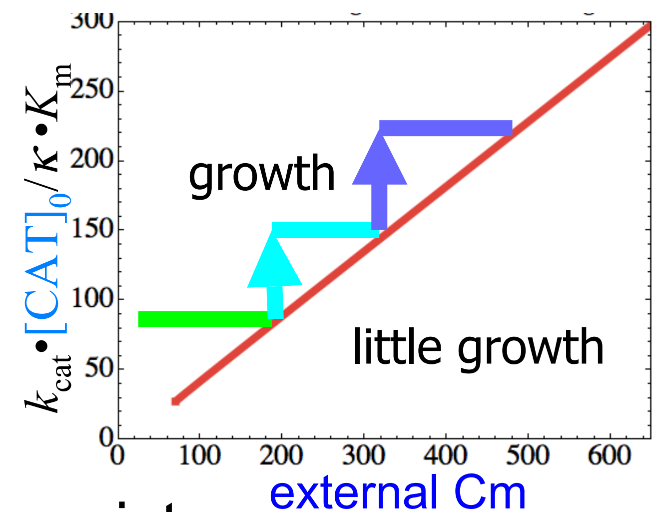
➔ rapid evolution by surfing along fitness cliff



Summary:

- strong coupling between physiology and genetic circuits
- describable through simple growth laws (few parameters!)
- laws ➔ growth theory
  ➔ genotype-fitness relation
- abrupt fitness landscape:
  survive or perish (cf "survival of fittest")

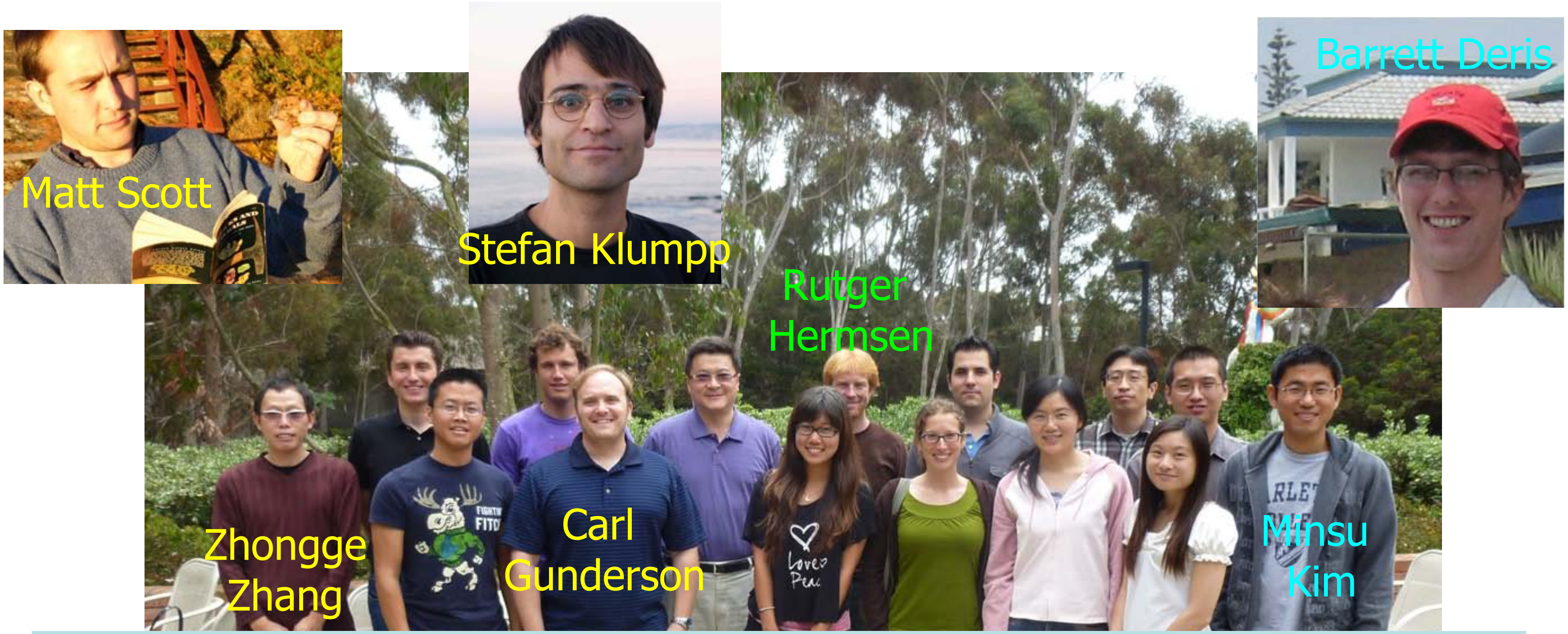




➔ built-in recipe for rapid evolution of drug resistance for bacteria exposed to a continuum of drug levels
(via mutation, invasion, and colonization of new niches)

➔ **evolution effectively "directed" by the fitness cliff**

➔ expect to be generic for translational inhibiting drugs



"Now in the further development of science, we want more than just a formula. First we have an observation, then we have numbers that we measure, then we have a law which summarizes all the numbers. But the real glory of science is that we can find a way of thinking such that the law is evident."

from *The Feynman Lectures on Physics*

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