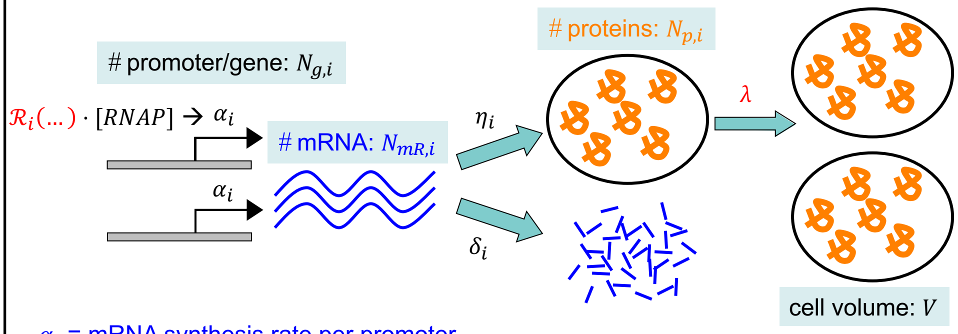
Topic 5: Global effects on gene expression



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

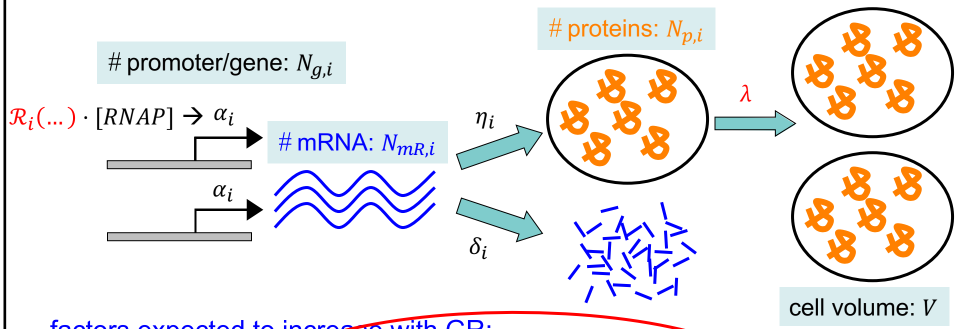
$$[P_i]^* = [g_i]\frac{\alpha_i\eta_i}{\delta_i\lambda} \propto \cancel{\frac{1}{\lambda}}$$

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

conc of genes and gene products
$[g_i] = N_{g,i}/V$
$[mR_i] = N_{mR,i}/V$
$[P_i] = N_{p,i}/V$

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

Topic 5: Global effects on gene expression



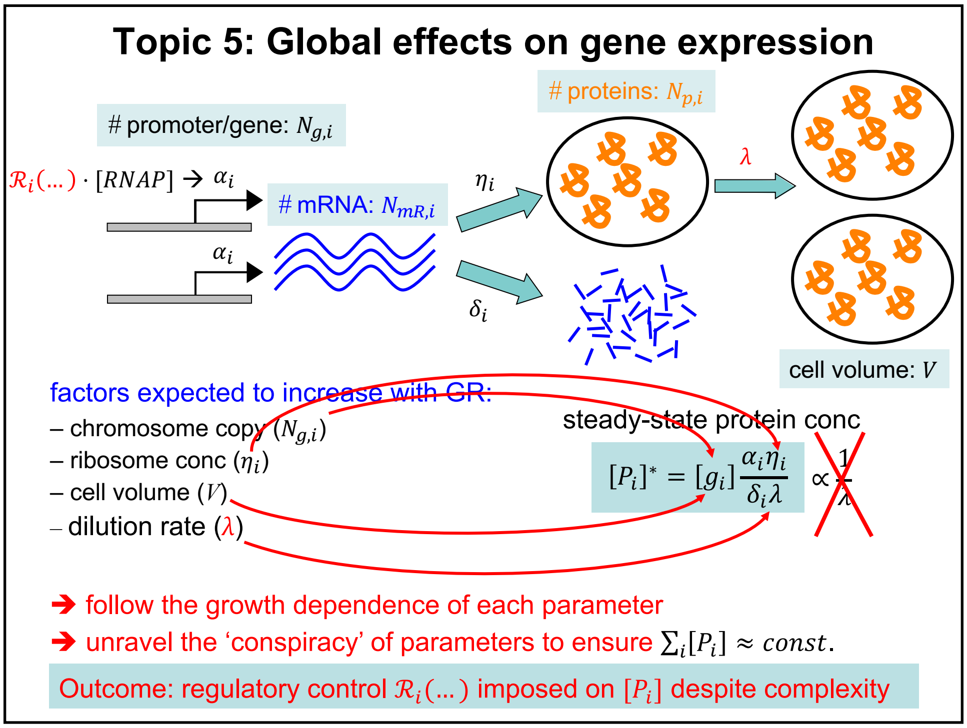
factors expected to increase with GR:
– chromosome copy ($N_{g,i}$)
– ribosome conc (η_i)
– cell volume (V)
– dilution rate (λ)

steady-state protein conc

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

- growth dependence of gene expression complex
- growth dependence of genetic circuits even more complex

➔ robust endogenous circuits have taken such effects into account
➔ must understand these effects in order to design robust synthetic circuits



Topic 5: Global effects on gene expression

promoter/gene: $N_{g,i}$

$\mathcal{R}_i(\ldots)\cdot[RNAP] \rightarrow \alpha_i$

α_i

mRNA: $N_{mR,i}$

η_i

δ_i

proteins: $N_{p,i}$

λ

cell volume: V

factors expected to increase with GR:

– chromosome copy ($N_{g,i}$)
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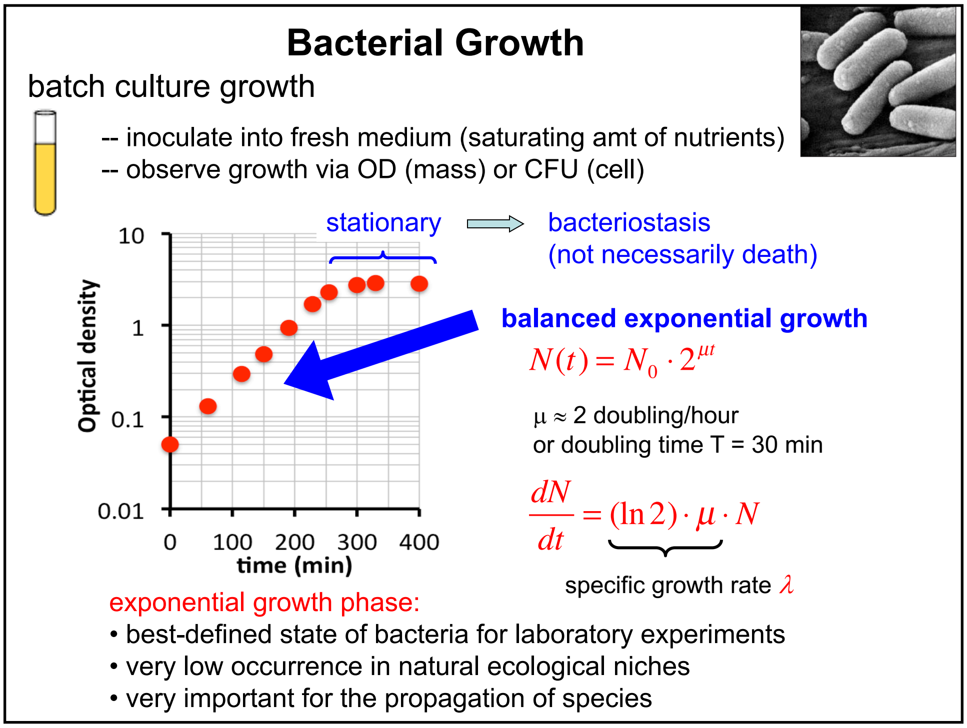
steady-state protein conc

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

➔ follow the growth dependence of each parameter
➔ unravel the 'conspiracy' of parameters to ensure $\sum_i [P_i] \approx const.$

Outcome: regulatory control $\mathcal{R}_i(\ldots)$ imposed on $[P_i]$ despite complexity



Bacterial Growth

batch culture growth

-- inoculate into fresh medium (saturating amt of nutrients)
-- observe growth via OD (mass) or CFU (cell)

stationary ⇒ bacteriostasis (not necessarily death)

balanced exponential growth

$$N(t) = N_0 \cdot 2^{\mu t}$$

μ ≈ 2 doubling/hour
or doubling time T = 30 min

$$\frac{dN}{dt} = \underbrace{(\ln 2)\cdot\mu}_{\text{specific growth rate } \lambda}\cdot N$$

exponential growth phase:

- best-defined state of bacteria for laboratory experiments
- very low occurrence in natural ecological niches
- very important for the propagation of species

Bacterial Growth

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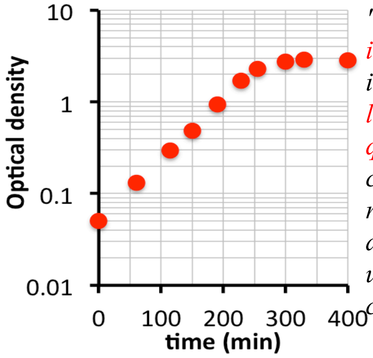

"...the growth of bacterial cultures, despite the immense complexity of the phenomena to which it testifies, generally obeys relatively simple laws, which make it possible to define certain quantitative characteristics of the growth cycle...The accuracy, the ease, the reproducibility of bacterial growth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

-- J. Monod (1949)

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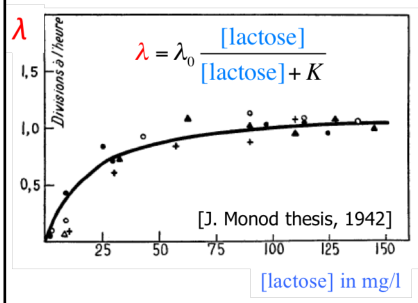

[J. Monod thesis, 1942]

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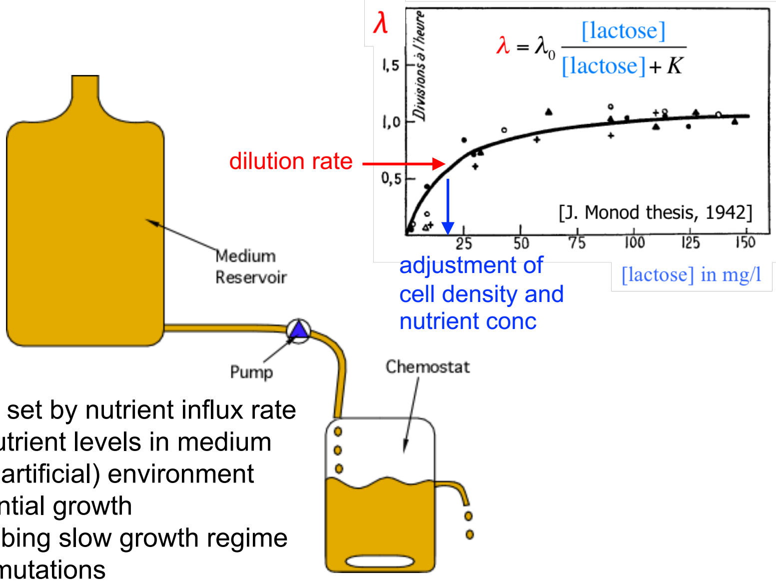
exponential growth phase:

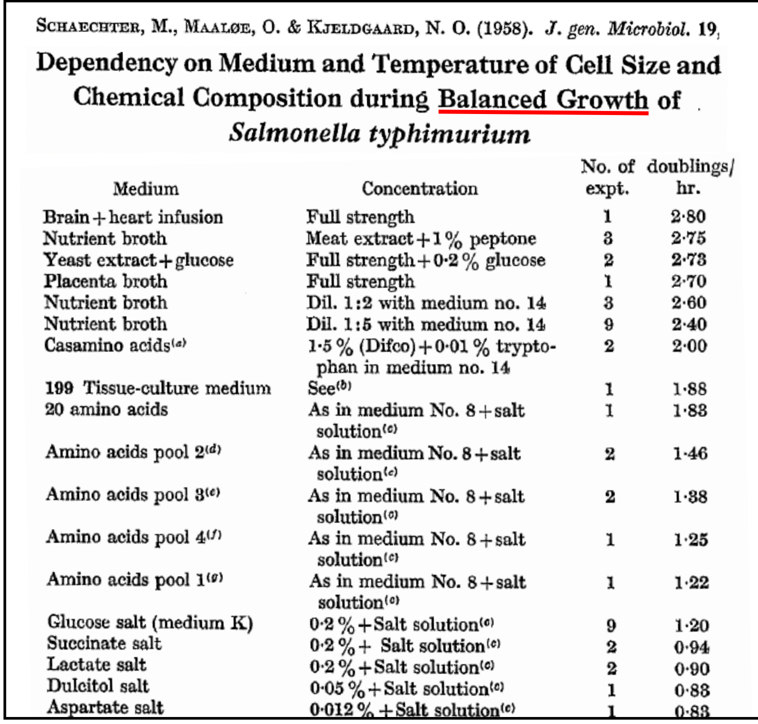
- best-defined state of bacteria for laboratory experiments
- very low occurrence in natural ecological niches
- very important for the propagation of species

alternative: continuous culture



[J. Monod thesis, 1942]

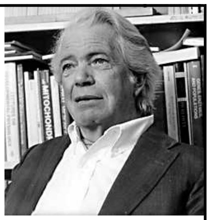
-- growth rate set by nutrient influx rate
-- measure nutrient levels in medium
-- "ideal" (but artificial) environment for exponential growth
-- best for probing slow growth regime
-- beware of mutations



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*

Medium	Concentration	No. of expt.	doublings/ hr.
Brain+heart infusion	Full strength	1	2·80
Nutrient broth	Meat extract+1 % peptone	3	2·75
Yeast extract+glucose	Full strength+0·2 % glucose	2	2·73
Placenta broth	Full strength	1	2·70
Nutrient broth	Dil. 1:2 with medium no. 14	3	2·60
Nutrient broth	Dil. 1:5 with medium no. 14	9	2·40
Casamino acids[a]	1·5 % (Difco)+0·01 % tryptophan in medium no. 14	2	2·00
199 Tissue-culture medium	See[b]	1	1·88
20 amino acids	As in medium No. 8+salt solution[c]	1	1·83
Amino acids pool 2[d]	As in medium No. 8+salt solution[c]	2	1·46
Amino acids pool 3[e]	As in medium No. 8+salt solution[c]	2	1·38
Amino acids pool 4[f]	As in medium No. 8+salt solution[c]	1	1·25
Amino acids pool 1[g]	As in medium No. 8+salt solution[c]	1	1·22
Glucose salt (medium K)	0·2 %+Salt solution[c]	9	1·20
Succinate salt	0·2 %+ Salt solution[c]	2	0·94
Lactate salt	0·2 %+Salt solution[c]	2	0·90
Dulcitol salt	0·05 %+Salt solution[c]	1	0·88
Aspartate salt	0·012 % +Salt solution[c]	1	0·83



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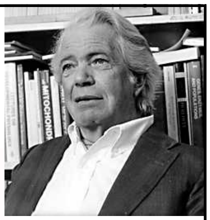

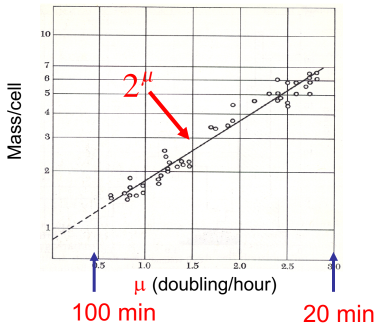

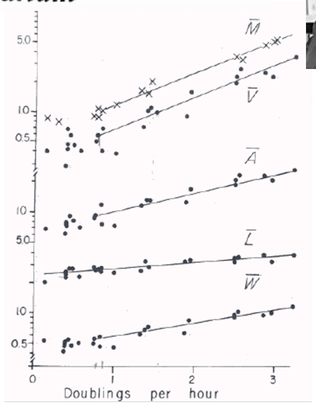

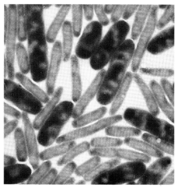

- dependence on the medium through growth rate
- cell mass can change several folds
- exponential dependence on μ (in doubling/hr): $m \propto 2^\mu$
- cell size also depends exponentially on μ : $V \propto 2^\mu$

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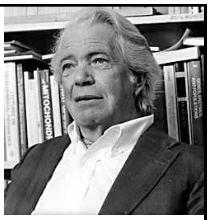
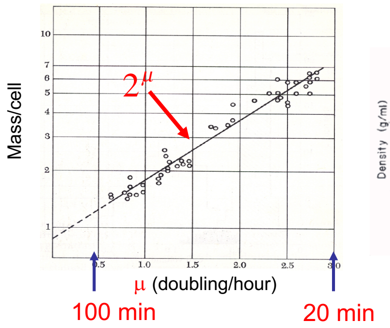
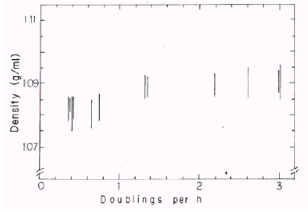
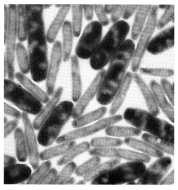


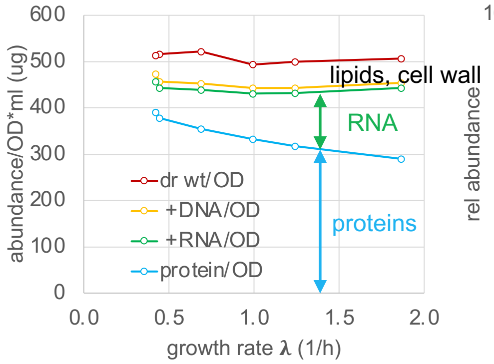


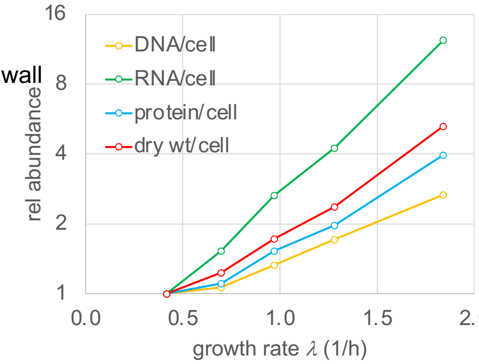




- dependence on the medium through growth rate
- cell mass can change several folds
- exponential dependence on μ (in doubling/hr): $m \propto 2^\mu$
- cell size also depends exponentially on μ : $V \propto 2^\mu$
- cell density ~ constant ➔ biomass/water ~ constant

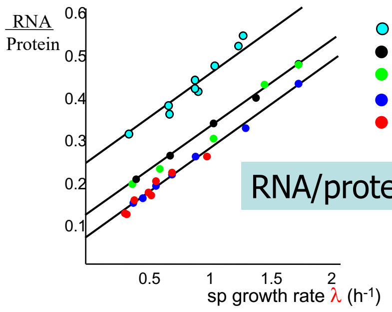
growth-rate dependence of macromolecular composition





- total cell dry wt/OD*ml ≈ const across growth conditions
- xxx/OD ∝ xxx/cytoplasmic water volume ∝ conc of xxx
- weak GR-dependence of protein/OD ≈ $\sum_i [P_i]$
- cellular abundances have strong GR dependence
  (largely from GR dependence of dr wt/cell ≈ 'cell size')
- relative abundances also have significant GR-dependences

growth-rate dependence of macromolecular composition



RNA/protein = $a \cdot \lambda + b$

RNA ≈ rRNA
RNA/protein ≈ ribosome conc (later)

- total cell dry wt/OD*ml ≈ const across growth conditions
- xxx/OD ∝ xxx/cytoplasmic water volume ∝ conc of xxx
- weak GR-dependence of protein/OD ≈ $\sum_i [P_i]$
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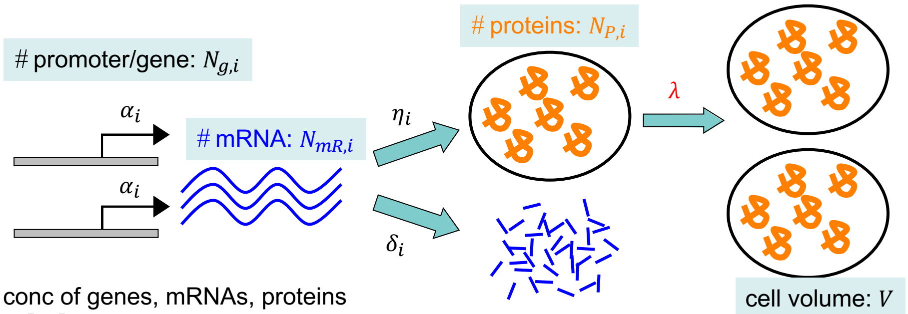
Growth-rate dependence of gene expression



conc of genes, mRNAs, proteins

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

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

steady-state protein conc

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

➔ follow the growth dependence of each parameter

➔ unravel the 'conspiracy' of global control to ensure $\sum_i [P_i] \approx const.$

Mass-GR relation from control of DNA replication initiation

- doubling time of *E. coli* can vary over 10x
  [from ~20 min to > 200 min]
- ~40 min required to replicate chromosome
  = "C-period"
- fixed time of ~20 min between completion of one round of replication and cell division
  = "D-period"

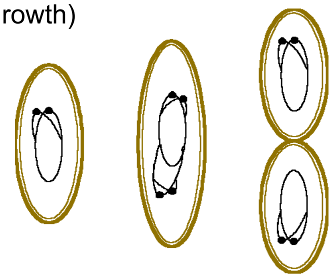
[Cooper & Helsmstetter, 1968]

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

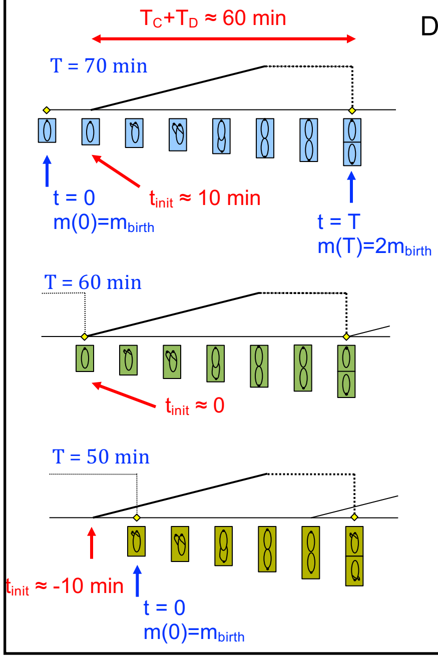
➔ doublint time < 60 min: multiple replication forks
(hence increased "gene dosage" at fast growth)

➔ initiation of new round of DNA replication
occurs ~60 min before cell division

➔ gene copy number/vol depends on GR



Quantitative relation between cell growth and DNA replication



Donachie's hypothesis [Donachie, 68]

- assume exponential growth of cell mass

$$m_{cell}(t) = m_{birth} \cdot 2^{\mu t}$$

- use Schaecter et al's empirical finding

$$\bar{m}_{cell} \propto m_{birth} = m^\star \cdot 2^{\mu(T_C+T_D)}$$

➔ obtain $m_{cell}(t) = m^\star \cdot 2^{\mu(t+T_C+T_D)}$

➔ initiation mass $T-(T_C+T_D)$

$$m_{init} \equiv m_{cell}(t_{init}) = m^\star \cdot \underbrace{2^{\mu T}}_{2}$$

constant init mass for different GR μ

➔ known as "Donachie's mass"

(≈ 1.7um per replication ori for E. coli)

➔ in terms of init mass, cell mass at division is

$$m_{div} \equiv m_{cell}(T) = m_{init} \cdot 2^{\mu\cdot(T_C+T_D)}$$

➔ simplest "explanation" of Schaetcter's exponential Mass-GR relation

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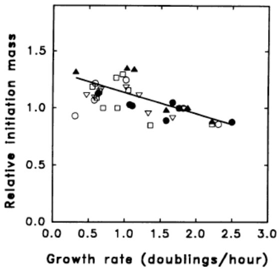
Quantitative relation between cell growth and DNA replication

early studies found moderate growth-rate dependence



confirmation of Donachie hypothesis:

-- single-cell study (Wallden et al, 2016)
-- mutants that changed C,D but not μ (Zheng et al, 2016)
-- extensive perturbative study (Si et al, 2017)

$$\bar{m}_{cell} = m_0 \cdot 2^{\mu(T_C+T_D)}$$

"unit cell mass"

Donachie's hypothesis [Donachie, 68]

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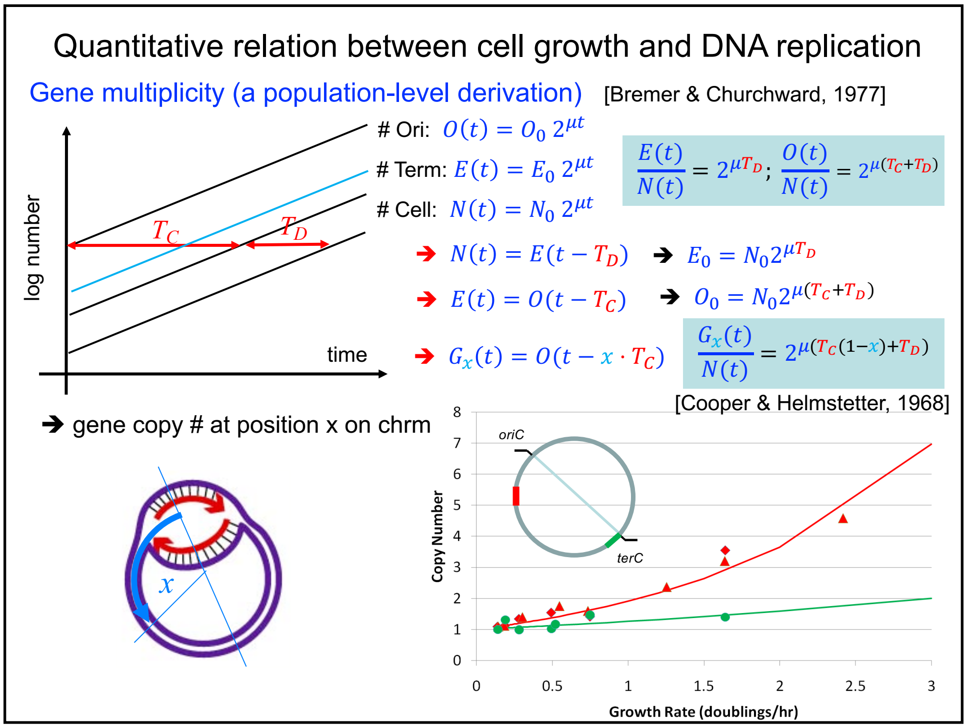


Quantitative relation between cell growth and DNA replication

Gene multiplicity (a population-level derivation) [Bremer & Churchward, 1977]

Ori: $O(t) = O_0\, 2^{\mu t}$

Term: $E(t) = E_0\, 2^{\mu t}$

Cell: $N(t) = N_0\, 2^{\mu t}$

$$\frac{E(t)}{N(t)} = 2^{\mu T_D};\ \frac{O(t)}{N(t)} = 2^{\mu(T_C+T_D)}$$

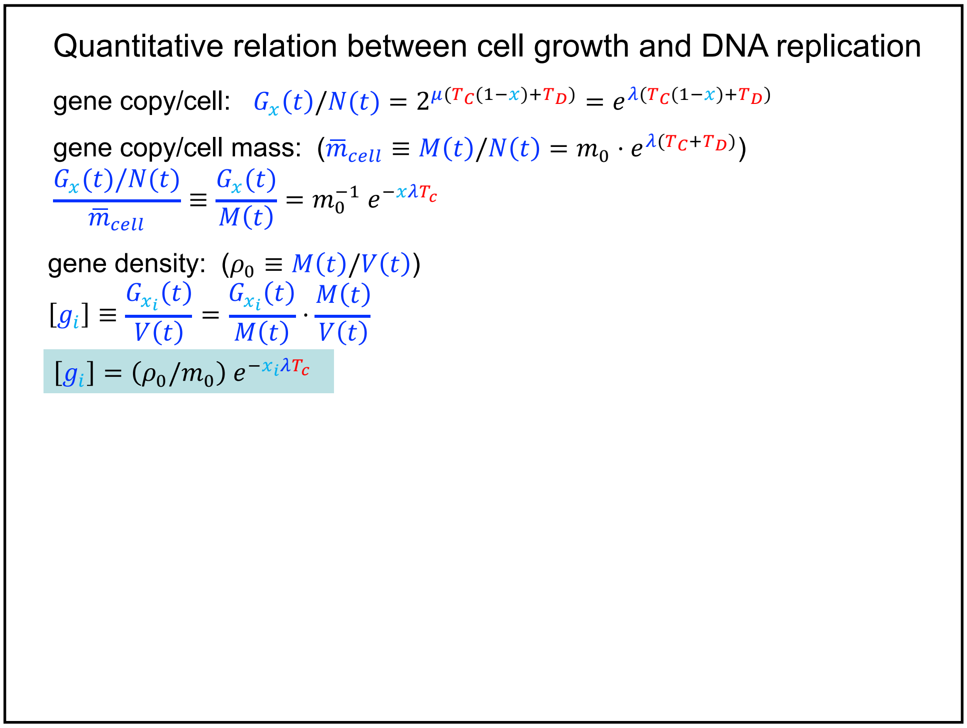
➔ $N(t) = E(t - T_D)$ ➔ $E_0 = N_0 2^{\mu T_D}$

➔ $E(t) = O(t - T_C)$ ➔ $O_0 = N_0 2^{\mu(T_C+T_D)}$

➔ $G_x(t) = O(t - x \cdot T_C)$ $\frac{G_x(t)}{N(t)} = 2^{\mu(T_C(1-x)+T_D)}$

[Cooper & Helmstetter, 1968]

➔ gene copy # at position x on chrm



Quantitative relation between cell growth and DNA replication

gene copy/cell: $G_x(t)/N(t) = 2^{\mu(T_C(1-x)+T_D)} = e^{\lambda(T_C(1-x)+T_D)}$

gene copy/cell mass: $(\bar{m}_{cell} \equiv M(t)/N(t) = m_0 \cdot e^{\lambda(T_C+T_D)})$

$$\frac{G_x(t)/N(t)}{\bar{m}_{cell}} \equiv \frac{G_x(t)}{M(t)} = m_0^{-1}\, e^{-x\lambda T_c}$$

gene density: $(\rho_0 \equiv M(t)/V(t))$

$$[g_i] \equiv \frac{G_{x_i}(t)}{V(t)} = \frac{G_{x_i}(t)}{M(t)} \cdot \frac{M(t)}{V(t)}$$

$$[g_i] = (\rho_0/m_0)\, e^{-x_i \lambda T_c}$$

Quantitative relation between cell growth and DNA replication

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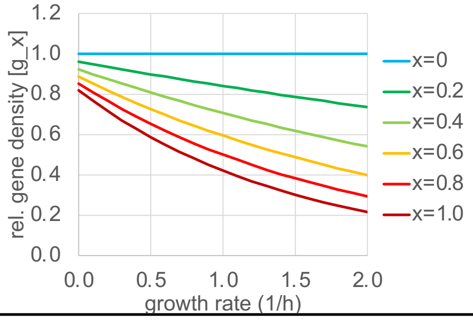
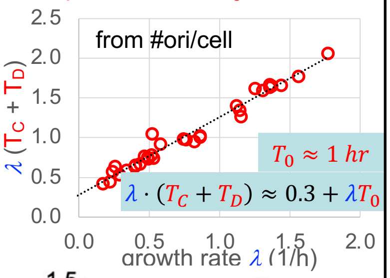
$$[g_i] = (\rho_0/m_0)\, e^{-x_i\lambda T_c} \quad \propto e^{-x_i(2/3)(0.3+\lambda T_0)}$$

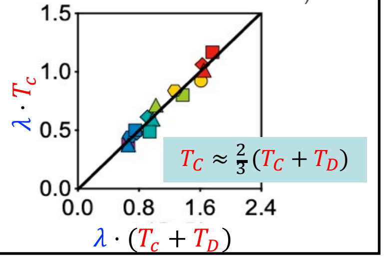


dependence of T_c on GR



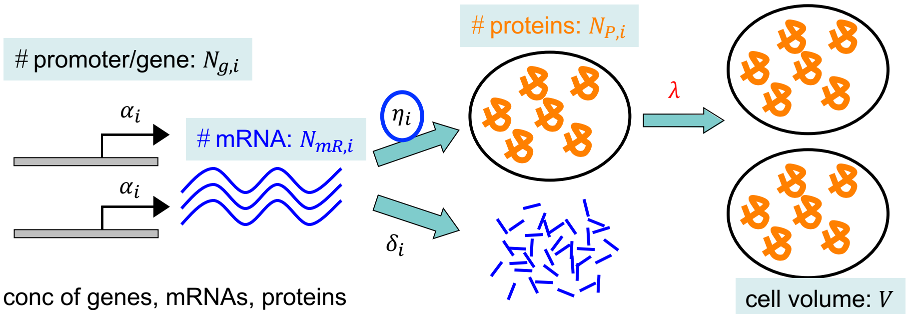
$T_0 \approx 1\ hr$

$\lambda \cdot (T_C + T_D) \approx 0.3 + \lambda T_0$



$T_C \approx \frac{2}{3}(T_C + T_D)$

Growth-rate dependence of gene expression



conc of genes, mRNAs, proteins

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$[P_i] = N_{p,i}/V$

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

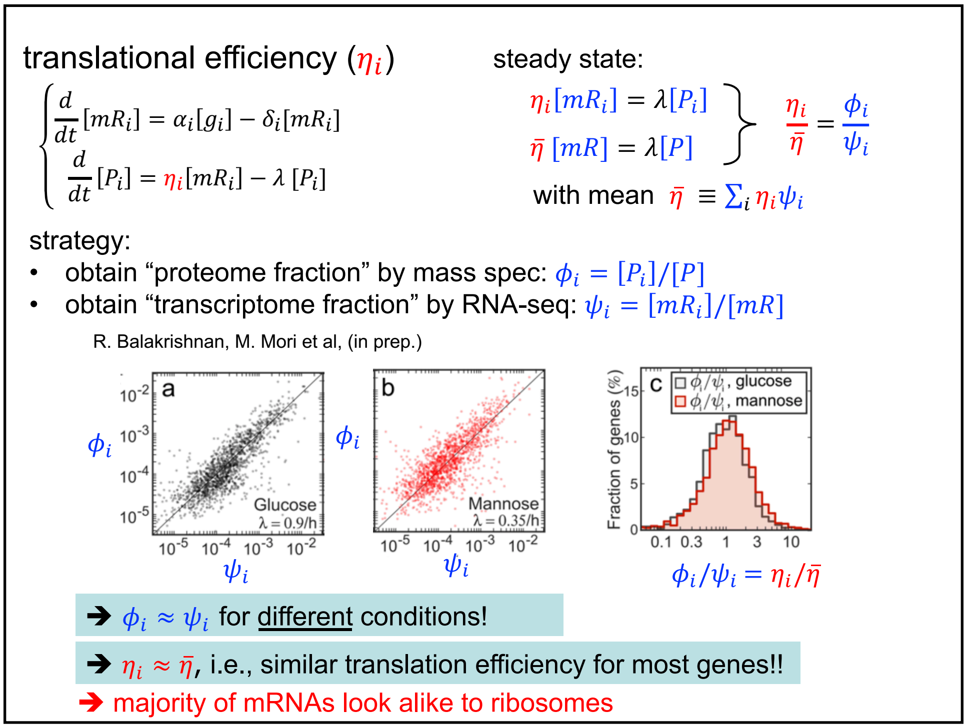
steady-state protein conc

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

➔ follow the growth dependence of each parameter

➔ unravel the ‘conspiracy’ of global control to ensure $\sum_i [P_i] \approx const.$

$$[g_i] = (\rho_0/m_0)\, e^{-x_i\lambda T_c} \quad \propto e^{-x_i(2/3)(0.3+\lambda T_0)}$$



translational efficiency (η_i)

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

steady state:

$$\left.\begin{aligned} \eta_i[mR_i] &= \lambda[P_i] \\ \bar{\eta}\,[mR] &= \lambda[P] \end{aligned}\right\} \frac{\eta_i}{\bar{\eta}} = \frac{\phi_i}{\psi_i}$$

with mean $\bar{\eta} \equiv \sum_i \eta_i \psi_i$

strategy:

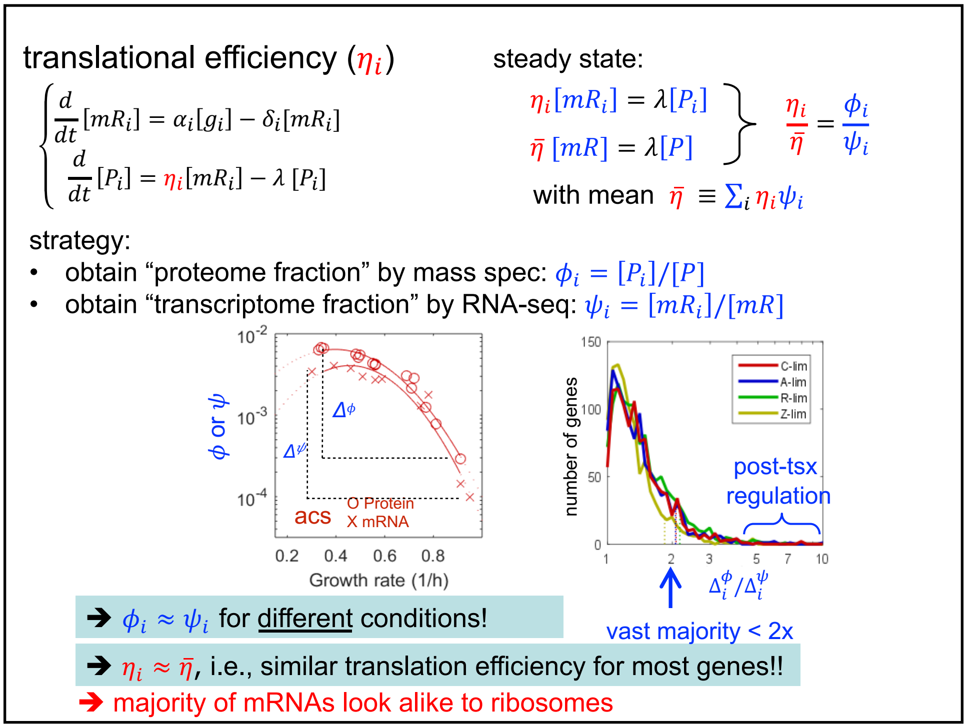
- obtain “proteome fraction” by mass spec: $\phi_i = [P_i]/[P]$
- obtain “transcriptome fraction” by RNA-seq: $\psi_i = [mR_i]/[mR]$

R. Balakrishnan, M. Mori et al, (in prep.)

➔ $\phi_i \approx \psi_i$ for <u>different</u> conditions!

➔ $\eta_i \approx \bar{\eta}$, i.e., similar translation efficiency for most genes!!

➔ majority of mRNAs look alike to ribosomes



translational efficiency (η_i)

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

steady state:

$$\left.\begin{aligned} \eta_i[mR_i] &= \lambda[P_i] \\ \bar{\eta}\,[mR] &= \lambda[P] \end{aligned}\right\} \frac{\eta_i}{\bar{\eta}} = \frac{\phi_i}{\psi_i}$$

with mean $\bar{\eta} \equiv \sum_i \eta_i \psi_i$

strategy:

- obtain “proteome fraction” by mass spec: $\phi_i = [P_i]/[P]$
- obtain “transcriptome fraction” by RNA-seq: $\psi_i = [mR_i]/[mR]$

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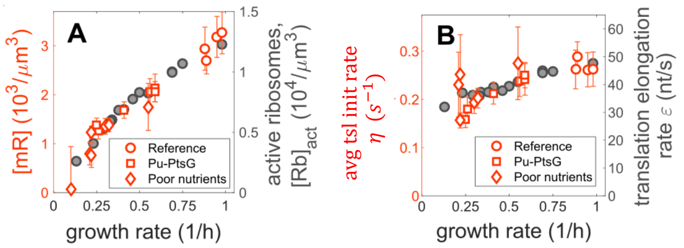
with mean $\bar{\eta} \equiv \sum_i \eta_i \psi_i$

Q: source of <u>constraint</u> on the total flux of protein synthesis $\bar{\eta}[mR]$?

➔ mRNA abundance nearly matched to the growth rate

➔ translational initiation rate η nearly growth independent

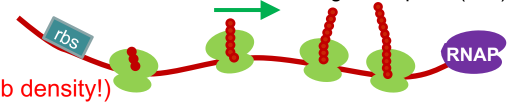


total flux of protein synthesis:

$$\lambda[P]\ell = \varepsilon \cdot [Rb]_{act}$$

➔ $[\mathrm{mR}] \approx 0.22\,[Rb]_{act}$ (const Rb density!)

ε: Rb elongation speed (nt/s)



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translational efficiency (η_i)

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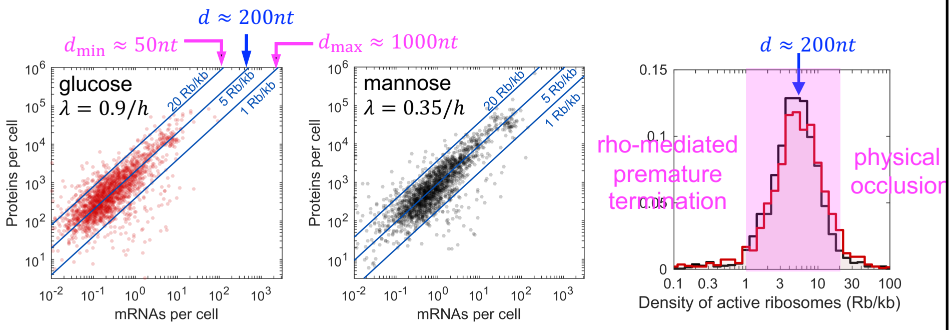
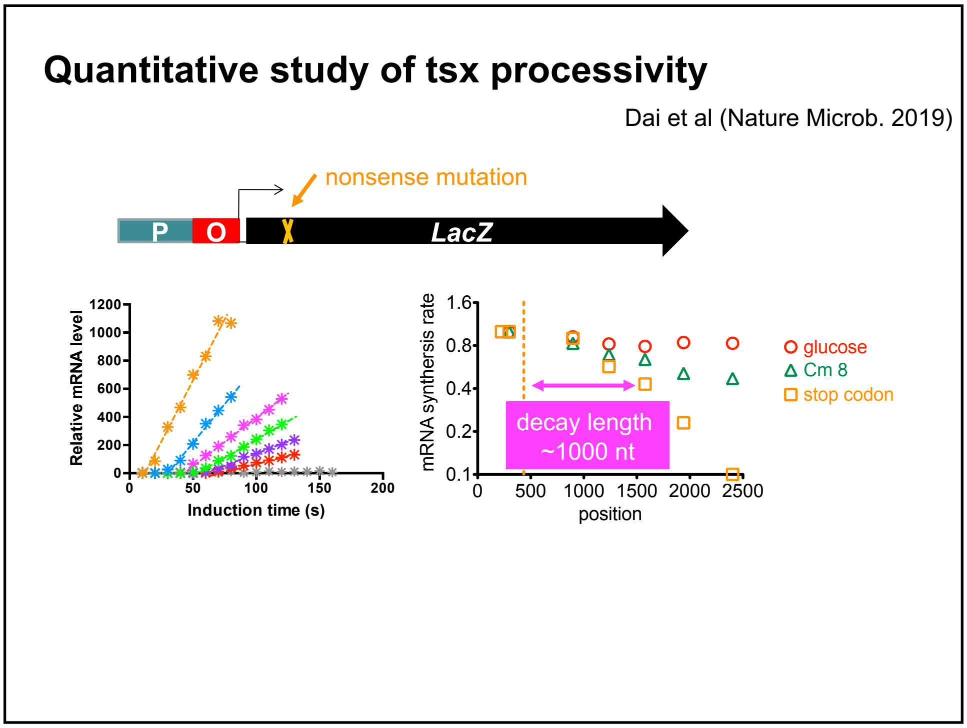
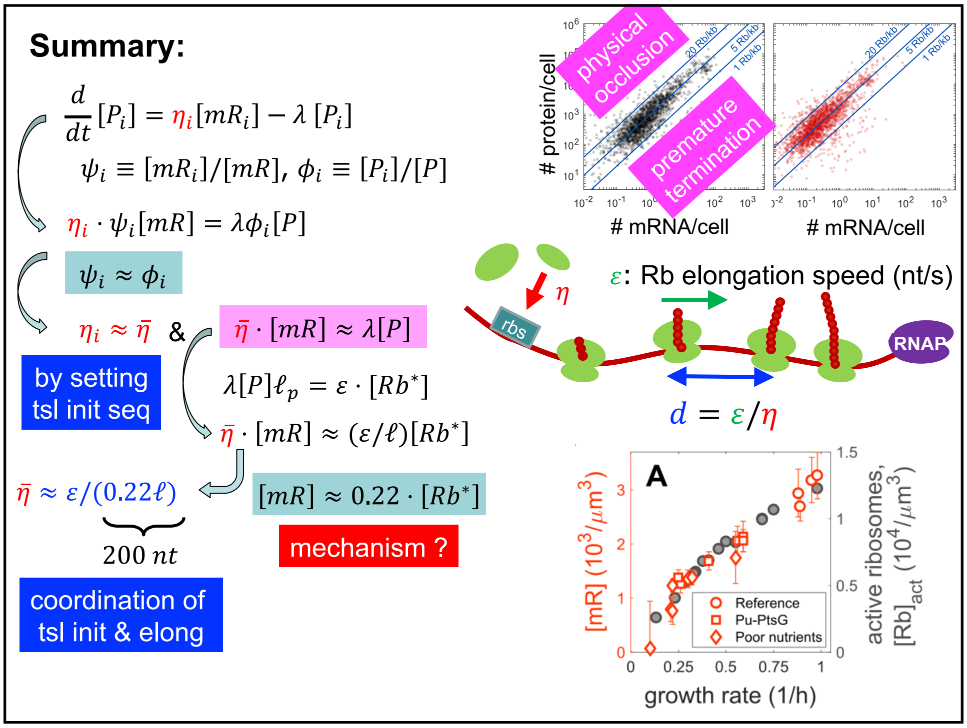


➔ $\bar{d} \approx 200\ nt$ (with $\varepsilon \approx 50nt/s$ → $\bar{\eta} \approx 1/(4s)$)



Quantitative study of tsx processivity

Dai et al (Nature Microb. 2019)



Summary:

$$\frac{d}{dt}[P_i] = \eta_i[mR_i] - \lambda\,[P_i]$$

$$\psi_i \equiv [mR_i]/[mR],\ \phi_i \equiv [P_i]/[P]$$

$$\eta_i \cdot \psi_i[mR] = \lambda\phi_i[P]$$

$$\psi_i \approx \phi_i$$

$\eta_i \approx \bar{\eta}$ & $\bar{\eta} \cdot [mR] \approx \lambda[P]$

by setting tsl init seq

$$\lambda[P]\ell_p = \varepsilon \cdot [Rb^*]$$

$$\bar{\eta} \cdot [mR] \approx (\varepsilon/\ell)[Rb^*]$$

$$\bar{\eta} \approx \underbrace{\varepsilon/(0.22\ell)}_{200\,nt}$$

$$[mR] \approx 0.22 \cdot [Rb^*]$$

mechanism ?

coordination of tsl init & elong

ε: Rb elongation speed (nt/s)

$$d = \varepsilon/\eta$$