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Supplementary Information for

A unifying autocatalytic network based framework for bacterial growth laws

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Supporting Information Text

Time evolution equation for protein abundances

Let P_i be the abundance of protein molecules of type i, R be the abundance of ribosomes, F be the abundance of substrate molecules, and m_i be the abundance of mRNA transcripts of the corresponding protein. Then the time evolution of the protein abundance can be written down in the following general form, in the simple approximation of an ordinary differential equation (ODE)

$$\frac{dP_i}{dt} = \frac{\min\left(\alpha_{P_i}R, \alpha_{P_i}F, \frac{\tau_{P_i}}{\tau_{aa}s_R}m_i, ...\right)}{\tau_{P_i}} \quad [1]$$

Here τ_{P_i} is the minimum time duration required to translate the protein. α_{P_i} is the relative allocation of ribosomes/substrate to the synthesis of the protein—the fraction of time ribosomes spend translating that protein, or equivalently, the fraction of ribosomes/substrates that are translating that protein at any given time. τ_{aa} is the minimum time duration required to elongate a polypeptide chain by one amino acid, i.e., τ_{aa}^{-1} is the translation elongation rate. s_R is the minimum footprint of the ribosome, i.e., the length of mRNA transcript, in units of 3 codons, equal to the size of ribosome binding domain including the minimum space that must exist between ribosomes. The term $\frac{\tau_{P_i}}{\tau_{aa}s_R}$, therefore, corresponds to the maximum number of ribosomes that can simultaneously bind to the corresponding mRNA and translate it. In this way, this term forms one part of the relative allocation of ribosomes towards synthesizing the corresponding protein. The other part comes from the mRNA copy number, m_i; i.e., the allocation parameter towards a protein is determined by its mRNA transcript's copy number and the length of the transcript. The allocation parameter α_{P_i} and the quantity $\frac{\tau_{P_i}}{\tau_{aa}s_R}$ can also be modulated in the cell by translation initiation factors.

If we want to account for protein degradation, we can write the above equation as

$$\frac{dP_i}{dt} = \frac{\min\left(\alpha_{P_i}R, \alpha_{P_i}F, \frac{\tau_{P_i}}{\tau_{aa}s_R}m_i, ...\right)}{\tau_{P_i}} - \frac{P_i}{\tau_{life(P_i)}} \quad [2]$$

where $\tau_{life(P_i)}$ is the lifetime of the protein. Depending on which of the terms inside the bracket $\left(\alpha_{P_i}R, \alpha_{P_i}F, \frac{\tau_{P_i}}{\tau_{aa}s_R}m_i, ...\right)$ is limiting, different limitation regimes are defined, as explained in the Methods section of main paper. However, independent of which of the component on the right hand side is limiting, the above equation for protein synthesis can be written in terms of any of the components; if we want to write the time evolution equation for protein synthesis in terms of ribosomes then it can be written as

$$\frac{dP_i}{dt} = \frac{\alpha_{P_i}R}{\tau_{P_i}}\eta_R - \frac{P_i}{\tau_{life(P_i)}} \quad [3]$$

with η_R being the fraction of time that ribosomes may be waiting for any other limiting component, if the ribosomes themselves are not the limiting one,

$$\eta_R = \min\left(1, F/R, \frac{\frac{\tau_{P_i}}{\tau_{aa}s_R}m_i}{\alpha_{P_i}R}, ...\right) \quad [4]$$

We may choose to identify $\tau_{Pi} \equiv \tau_{P_i}/\eta_R$ as the effective time duration for translation, taking resource limitation into account. In normal to fast growth conditions, cells tend to employ control mechanisms, through micro-molecules such as ppGpp, DksA, etc., to avoid resource mismatch and the resulting translation slow down. Therefore, in normal-to-fast growth conditions, $\eta_R = 1$ is maintained.

In balanced exponential growth we can write (existence of exponential growth is discussed in the Methods section),

$$\left(\mu + \frac{1}{\tau_{life(P_i)}}\right) = \frac{\alpha_{P_i}}{\tau_{P_i}}\left(\frac{R}{P_i}\right) \quad [5]$$

where μ is the growth rate of the exponentially growing cell. This is one of the two type of growth laws discussed in the main text, which we called the relative abundance growth law.

Time evolution equation for mRNA abundances

Protein synthesis also requires transcription of mRNAs. Therefore, we need to write the time evolution equation for the transcription machinery of the cell. If m_i is the number of copies of mRNA transcripts of protein i, then

$$\frac{dm_i}{dt} = \frac{\min(\alpha_{m_i}Rpol, \alpha_{m_i}N, \frac{\tau_{m_i}}{\tau_{nt}s_{Rpol}}G_i, ...)}{\tau_{m_i}} - \frac{m_i}{\tau_{life(m_i)}} \quad [6]$$

where α_{m_i}, analogous to eq. 1, is the relative allocation of resources like RNA polymerase (whose abundance is represented by Rpol) and nucleotides (whose abundance is represented by N), to the transcription of the mRNA of protein i. G_i is the gene

copy number. τ_{m_i} is the minimum time duration to transcribe the mRNA, τ_{nt} is the minimum time duration to elongate a mRNA transcript by one nucleotide, and s_{Rpol} is the minimum footprint of the RNAP, i,e., the length of the gene equal to the size of RNAP binding domain, including the minimum space that must exist between RNAPs. Therefore, the term $\frac{\tau_{m_i}}{\tau_{nt} s_{Rpol}}$ corresponds to the maximum number of RNA polymerases that can bind simultaneously to the corresponding gene. As before, these allocation parameters also include modulation of binding affinities to the genes through Transcription Factors.

The black box of protein synthesis

Protein synthesis, in general, is a multi-step process. We can therefore ask how good is the back box, 1 step process, approximation that is generally employed. We can open the black box of protein synthesis to incorporate various intermediate states like spending time in resting pools, assembly, etc. Let us consider a protein complex, like say RNA polymerase or ribosome, which is made up of multiple protein subunits. If τ_{a_j} is the effective time duration to translate j-th subunit, $\tau_{pool(aj)}$ is the time it spends diffusing in the cytoplasm before being incorporated into the assembly, $\tau_{rest(P)}$ is the time duration that the protein complex spends before becoming actively functioning, and $\tau_{busy(P)}$ is the time duration the active protein takes to perform one round of its function before resting, then the time evolution of the abundances in the different states can be written in terms of the following set of coupled ODEs

$$\frac{da_{j(pool)}}{dt} = \frac{\alpha_{a_j} R_{busy}}{\tau_{a_j}} - \frac{a_{j(pool)}}{\tau_{pool(a_j)}} \quad [7]$$

$$\frac{da_{j(act)}}{dt} = \frac{a_{j(pool)}}{\tau_{pool(a_j)}} - \frac{a_{j(act)}}{\tau_{SA(P)}} \quad [8]$$

$$\frac{dP_{rest}}{dt} = \frac{a_{j(act)}}{\tau_{SA(P)}} - \frac{P_{rest}}{\tau_{rest(P)}} + \frac{P_{busy}}{\tau_{busy(P)}} - \frac{P_{rest}}{\tau_{life(P)}} \quad [9]$$

$$\frac{dP_{busy}}{dt} = \frac{P_{rest}}{\tau_{rest(P)}} - \frac{P_{busy}}{\tau_{busy(P)}} - \frac{P_{busy}}{\tau_{life(P)}} \quad [10]$$

where $\tau_{life(P)}$ is the lifetime of the protein and $\tau^{SA(P)}$ is the assembly duration of the protein complex from its subunit. Note that even though the actual assembly process is quite complex, here we are considering a simplistic Tetris like model where the protein complex is ready whenever the limiting protein is incorporated. Other quantities are as defined before. The terms $\frac{P_{busy}}{\tau_{busy(P)}}$ and $\frac{P_{rest}}{\tau_{rest(P)}}$ are interconversion terms between active and resting states.

Putting in exponential solution, we get the following growth laws involving relative abundances

$$(\mu + \frac{1}{\tau_{pool(a_j)}}) = \frac{\alpha_{a_j} R_{busy}}{\tau_{a_j} a_{j(pool)}} \quad [11]$$

$$(\mu + \frac{1}{\tau_{SA(P)}}) = \frac{a_{j(pool)}}{\tau_{pool(a_j)} a_{j(act)}} \quad [12]$$

$$\mu(P_{rest} + P_{busy}) + \frac{(P_{rest} + P_{busy})}{\tau_{life(P)}} = \frac{a_{j(act)}}{\tau_{SA(P)}} \quad [13]$$

Multiplying the four equations leads to the following growth law

$$(\mu + \frac{1}{\tau_{life(P)}})(\mu\tau_{SA(P)} + 1)(\mu\tau_{pool(a_j)} + 1) = \frac{\alpha_{a_j} R_{busy}}{\tau_{a_j} P_{tot}} \quad [14]$$

Under normal conditions $\tau_{pool(a_j)}, \tau_{SA(P)} \approx$ secs. and $\mu \approx 1/(30 * 60) \approx 1/2000$ sec^{-1}. This means the terms $(\mu\tau + 1) \ll 1$. Also, lifetimes of proteins are in hours, and therefore protein degradation can be neglected under normal conditions. Therefore, under normal growth conditions, the above equation can be approximated as a one step process

$$\mu = \frac{\alpha_{a_j} R_{busy}}{\tau_{a_j} P_{tot}} \quad [15]$$

The coupled transcription-translation autocatalytic cycles and the resulting growth laws

The cellular transcription-translation machinery, which is responsible for all the protein synthesis, also needs to reproduce itself to sustain exponential growth. In this section, we focus on the coupled autocatalytic cycles of the ribosomes and RNA polymerases, and show how they reduce to various known and new growth laws. The coupled ODEs of the core

transcription-translation autocatalytic cycles can be written as

$$\frac{d\ rRNA_j}{dt} = \frac{\alpha_{rRNA_j} Rpol_b}{\tau_{rRNA_j}} - \frac{\min(RP_{j(act)}, rRNA_j)}{\tau_{SA(R)}} \quad [16]$$

$$\frac{d\ m_{RP_j}}{dt} = \frac{\alpha_{rp_j} Rpol_b}{\tau_{rp_j}} - \frac{m_{RP_j}}{\tau_{life(m(RP_j))}} \quad [17]$$

$$\frac{d\ m_{Rpoj}}{dt} = \frac{\alpha_{rpo_j} Rpol_b}{\tau_{rpoj}} - \frac{m_{Rpo_j}}{\tau_{life(m(Rpo_j))}} \quad [18]$$

$$\frac{dRpo_{j(pool)}}{dt} = \frac{\min(\alpha_{Rpo_j} R_b, R_{m(Rpo_j)} m_{Rpo_j})}{\tau_{Rpo_j}} - \frac{Rpo_{j(pool)}}{\tau_{pool(Rpo_j)}} \quad [19]$$

$$\frac{dRpo_{j(act)}}{dt} = \frac{Rpo_{j(pool)}}{\tau_{pool(Rpo_j)}} - \frac{Rpo_{j(act)}}{\tau_{SA(Rpol)}} \quad [20]$$

$$\frac{dRpol_{rest}}{dt} = \frac{Rpo_{j(act)}}{\tau_{SA(Rpol)}} - \frac{Rpol_{rest}}{\tau_{rest(R)}} + \sum_i \frac{\alpha_{m_i}}{\tau_{m_i}} Rpol_b - \frac{Rpol_{rest}}{\tau_{life(Rpol)}} \quad [21]$$

$$\frac{dRpol_b}{dt} = \frac{Rpol_{rest}}{\tau_{rest(R)}} - \sum_i \frac{\alpha_{m_i}}{\tau_{m_i}} Rpol_b - \frac{Rpol_b}{\tau_{life(Rpol)}} \quad [22]$$

$$\frac{dRP_{j(pool)}}{dt} = \frac{\min(\alpha_{RP_j} R_b, R_{m(RP_j)} m_{RP_j})}{\tau_{RP_j}} - \frac{RP_{j(pool)}}{\tau_{pool(RP_j)}} \quad [23]$$

$$\frac{dRP_{j(act)}}{dt} = \frac{RP_{j(pool)}}{\tau_{pool(RPj)}} - \frac{\min(RP_{j(act)}, rRNA_j)}{\tau_{SA(R)}} \quad [24]$$

$$\frac{dR_{rest}}{dt} = \frac{\min(RP_{j(act)}, rRNA_j)}{\tau_{SA(R)}} - \frac{R_{rest}}{\tau_{rest(R)}} + \sum_i \frac{\alpha_{P_i}}{\tau_{P_i}} R_b - \frac{R_{rest}}{\tau_{life(R)}} \quad [25]$$

$$\frac{dR_b}{dt} = \frac{R_{rest}}{\tau_{rest(R)}} - \sum_i \frac{\alpha_{P_i}}{\tau_{P_i}} R_b - \frac{R_b}{\tau_{life(R)}} \quad [26]$$

where $rRNA_j$ is the abundance of ribosomal RNA (rRNA) subunit of type j, m_{RP_j} is the abundance of the mRNA of j-th ribosomal protein subunit, and m_{Rpo_j} is the abundance of the mRNA of RNA polymerase's j-th protein subunit. $Rpo_{j(pool)}$ is the abundance of RNA polymerase's j-th protein subunit in its newly synthesized, freely floating state, $Rpo_{j(act)}$ is the abundance of RNA polymerase's j-th protein subunit that are entering active assembly step, $Rpol_{rest}$ is the abundance of the newly formed, resting RNA polymerases, and $Rpol_b$ is the abundance of actively transcribing RNA polymerases; $RP_{j(pool)}, RP_{j(act)}, R_{rest}$, and R_b are similar quantities, but for ribosomes and its subunits. τ_{rRNA_j} is the time duration needed to transcribe rRNA, τ_{rp_j} is the transcription duration of the mRNA of j-th ribosomal protein subunit, τ_{rpo_j} is the transcription duration of the mRNA of RNA polymerase's j-th protein subunit, τ_{Rpo_j} is the translation duration of RNA polymerase's j-th protein subunit, and τ_{RP_j} is the translation duration of j-th ribosomal protein subunit. $\tau_{SA(Rpol)}, \tau_{SA(R)}$ are the assembly durations of RNA polymerases and ribosomes from their respective protein subunits. τ_{pool}, τ_{rest}, and τ_{life} are the resting durations and lifetimes of the corresponding quantities. α_{rRNA_j} is the allocation parameter of RNA polymerases towards transcribing rRNA, α_{rp_j} is the allocation parameter towards transcribing the mRNA of j-th ribosomal protein subunit, and α_{rpo_j} is the allocation parameter towards transcribing the mRNA of RNA polymerase's j-th protein subunit. α_{Rpo_j} and α_{RP_j} are the allocation parameters of ribosomes towards translating RNA polymerase's and ribosomal protein's subunits respectively. $R_{m(Rpo_j)}$ and $R_{m(RP_j)}$ are the number of ribosomes bound to the corresponding mRNA transcripts. The term $\sum_i \frac{\alpha_{m_i}}{\tau_{m_i}} Rpol_b$ refers to all the transcription activity RNA polymerases perform in the cell, after which they rest, before going back to their active state. Similarly, $\sum_i \frac{\alpha_{P_i}}{\tau_{P_i}} R_b$ refers to all the translation activities of active ribosomes.

The ribosomal protein cycle: To study the ribosomal protein autocatalytic cycle we write the above coupled set of ODEs, for the abundances of ribosomes which synthesizes ribosomal proteins, and for the abundances of ribosomal proteins which constitute the ribosomes, in their various activity states, as

$$\frac{dRP_{j(pool)}}{dt} = \frac{\alpha_{RP_j} R_b}{\tau_{RP_j}} - \frac{RP_{j(pool)}}{\tau_{pool(RP_j)}} \quad [27]$$

$$\frac{dRP_{j(act)}}{dt} = \frac{RP_{j(pool)}}{\tau_{pool(RP_j)}} - \frac{RP_{j(act)}}{\tau_{SA(R)}} \quad [28]$$

$$\frac{dR_{rest}}{dt} = \frac{RP_{j(act)}}{\tau_{SA(R)}} - \frac{R_{rest}}{\tau_{rest(R)}} + \sum_i \frac{\alpha_{P_i}}{\tau_{Pi}} R_b - \frac{R_{rest}}{\tau_{life(R)}} \quad [29]$$

$$\frac{dR_b}{dt} = \frac{R_{rest}}{\tau_{rest(R)}} - \sum_i \frac{\alpha_{P_i}}{\tau_{P_i}} R_b - \frac{R_b}{\tau_{life(R)}} \quad [30]$$

Putting in the exponential solution, we get

$$(\mu + \frac{1}{\tau_{pool(RP_j)}}) = \frac{\alpha_{RP_j} R_b}{\tau_{RP_j} RP_{j(pool)}} \quad [31]$$

$$(\mu + \frac{1}{\tau_{SA(R)}}) = \frac{RP_{j(pool)}}{\tau_{pool(RP_j)} RP_{j(act)}} \quad [32]$$

$$\mu(R_{rest} + R_b) + \frac{(R_{rest} + R_b)}{\tau_{life(R)}} = \frac{RP_{j(act)}}{\tau_{SA(R)}} \quad [33]$$

Each of the above three equations is already a growth law, involving relative abundances of various quantities. Eq.33 is akin to Little's law of self assembly in Factory physics. Now, if we multiply the three equations, we get

$$(\mu\tau_{SA(R)} + 1)(\mu\tau_{pool(RP_j)} + 1)\left(\mu R_{tot} + \frac{R_{tot}}{\tau_{life(R)}}\right) = \frac{\alpha_{RP_j}}{\tau_{RP_j}} R_b \quad [34]$$

Note that this is akin to diagonalizing the matrix of the above linear set of coupled ODEs, and obtaining the characteristic function.

Relation to existing results: In equation 34, if we assume $\mu \ll 1/\tau_{SA(R)}$ and $\mu \ll 1/\tau_{pool(RP_j)}$, we get

$$\mu R_{tot} + \frac{R_{tot}}{\tau_{life(R)}} = \frac{\alpha_{RP_j}}{\tau_{RP_j}} R_b \quad [35]$$

or,

$$\mu\tau_{RP_j} + \frac{\tau_{RP_j}}{\tau_{life(R)}} = \alpha_{RP_j} \frac{R_b}{R_{tot}} \quad [36]$$

The term on the right hand side of the above equation reads — the fraction of active ribosomes that are allocated to translate more ribosomes. The second term on the left hand side, therefore, stands for this allocation of ribosomes towards new ribosomes at zero growth ($\mu = 0$). This is equivalent to the well known growth law of Terry et. al. (1).

$$\frac{\mu}{\gamma} + \phi_0 = \phi_R \quad [37]$$

with γ being the translation rate of the ribosome, ϕ_R being the ribosome mass fraction and ϕ_0 the ribosome mass fraction at zero growth. Below we show when this correspondence between mass fraction and allocation parameter is accurate.

Summing equation 34 over all the subunits gives

$$\sum_{j \in Ribosomes} \left((\mu\tau_{SA(R)} + 1)(\mu\tau_{pool(RP_j)} + 1)\left(\mu + \frac{1}{\tau_{life(R)}}\right) R_{tot}\right) = \sum_{j \in Ribosomes} \left(\frac{\alpha_{RP_j}}{\tau_{RP_j}} R_b\right) \quad [38]$$

Equation 34, when written for any general protein i, appears as

$$(\mu\tau_{SA(P_i)} + 1)(\mu\tau_{pool(p_j)} + 1)\left(\mu + \frac{1}{\tau_{life(P_i)}}\right) P_{i(tot)} = \frac{\alpha_{P_i}}{\tau_{P_i}} R_b \quad [39]$$

with $\tau_{SA(P_i)} = 0, \tau_{pool(p_j)} = 0$ if it is a single component protein. Summing over all the proteins in the proteome, we get

$$\sum_{i \in Proteome} \left((\mu\tau_{SA(P_i)} + 1)(\mu\tau_{pool(p_j)} + 1)\left(\mu + \frac{1}{\tau_{life(P_i)}}\right) P_{i(tot)}\right) = \sum_{i \in Proteome} \left(\frac{\alpha_{P_i}}{\tau_{P_i}} R_b\right) \quad [40]$$

Dividing equations 38 and 40 we get

$$\frac{\sum_{j \in Ribosomes} \left((\mu\tau_{SA(R)} + 1)(\mu\tau_{pool(RP_j)} + 1)\left(\mu + \frac{1}{\tau_{life(R)}}\right) R_{tot}\right)}{\sum_{i \in Proteome} \left((\mu\tau_{SA(P_i)} + 1)(\mu\tau_{pool(p_j)} + 1)\left(\mu + \frac{1}{\tau_{life(P_i)}}\right) P_{i(tot)}\right)} = \frac{\sum_{j \in Ribosomes} \left(\frac{\alpha_{RP_j}}{\tau_{RP_j}} R_b\right)}{\sum_{i \in Proteome} \left(\frac{\alpha_{P_i}}{\tau_{P_i}} R_b\right)} \quad [41]$$

Under the approximation $\mu \ll 1/\tau_{SA}$, $\mu \ll 1/\tau_{pool}$, and long lifetime for the proteins, can we write the above equation as

$$\frac{\sum_{j \in Ribosomes} \mu R_{tot}}{\sum_{i \in Proteome} \mu P_{i(tot)}} = \frac{\sum_{j \in Ribosomes} \left(\frac{\alpha_{RP_j}}{\tau_{RP_j}} R_b\right)}{\sum_{i \in Proteome} \left(\frac{\alpha_{P_i}}{\tau_{P_i}} R_b\right)} \quad [42]$$

or,

$$\frac{\sum_{j \in Ribosomes} \tau_{RP_j} R_{tot}}{\sum_{i \in Proteome} \tau_{P_i} P_{i(tot)}} = \frac{\sum_{j \in Ribosomes} \left(\alpha_{RP_j} R_b\right)}{\sum_{i \in Proteome} \left(\alpha_{P_i} R_b\right)} \quad [43]$$

or,

$$\frac{\tau_R R_{tot}}{\sum_{i\in Proteome}\tau_{Pi}P_{i(tot)}} = \frac{\alpha_R R_b}{1.\ R_b} \quad [44]$$

or,

$$\frac{\tau_{aa}L_{aa(R)}R_{tot}}{\sum_{i\in Proteome}\tau_{aa}L_{aa(P_i)}P_{i(tot)}} = \alpha_R \quad [45]$$

as $\tau_R = L_{aa(R)}\tau_{aa}$, $L_{aa(R)}$ being the length of the protein in the units of number of amino acids, and τ_{aa} being the elongation time per amino acid. The above equation then becomes

$$\frac{\tau_{aa}M_{R(tot)}}{\sum_{i\in Proteome}\tau_{aa}M_{Pi(tot)}} = \alpha_R \quad [46]$$

where $M_{R(tot)}(= L_{aa(R)}R_{tot}m_{aa})$ is the mass of the ribosome; $m_{aa}$is the average mass of an amino acid. Therefore,

$$\frac{M_{R(tot)}}{M_{Proteome(tot)}} \equiv \phi_R = \alpha_R \quad [47]$$

We see that the relative allocation of ribosomes towards making more ribosomes is equal to the steady state ribosome mass fraction under the approximations that $\mu \ll 1/\tau_{SA}$, $\mu \ll 1/\tau_{pool}$, and that the proteins are long lived. These conditions are satisfied under most normal bacterial growth conditions and, therefore, so is equation 37.

Equation 36, under the assumption of long lifetime of ribosomes, gives

$$\mu\tau_{RP_j} = \alpha_{RP_j}\frac{R_b}{R_{tot}} \quad [48]$$

This is equivalent to the closed cycle ribosomal protein growth law presented in (2).

Note that we can also obtain the ratio R_b/R_{tot} from eq.30, with long lifetime limit, as

$$(\mu + \sum_i \frac{\alpha_{P_i}}{\tau_{P_i}})\tau_{rest(R)} = \frac{R_{rest}}{R_b} \quad [49]$$

$$\implies \quad \frac{1}{1 + (\mu + \sum_i \frac{\alpha_{P_i}}{\tau_{P_i}})\tau_{rest(R)}} = \frac{R_b}{R_{tot}} \quad [50]$$

which can be called the resting growth laws.

The ribosomal RNA cycle: To study the ribosomal RNA (rRNA) autocatalytic cycle, we need to write the coupled set of ODEs for the abundances of rRNAs, RNA polymerases that transcribe them, RNA polymerase's protein subunits that constitute the RNA polymerases, and ribosomes that translate those protein subunits, in their various activity states:

$$\frac{d\ rRNA_j}{dt} = \frac{\alpha_{rRNA_j}Rpol_b}{\tau_{rRNA_j}} - \frac{rRNA_j}{\tau_{SA(R)}} \quad [51]$$

$$\frac{dRpo_{j(pool)}}{dt} = \frac{\alpha_{Rpo_j}R_b}{\tau_{Rpo_j}} - \frac{Rpo_{j(pool)}}{\tau_{pool(Rpo_j)}} \quad [52]$$

$$\frac{dRpo_{j(act)}}{dt} = \frac{Rpo_{j(pool)}}{\tau_{pool(Rpo_j)}} - \frac{Rpo_{j(act)}}{\tau_{SA(Rpol)}} \quad [53]$$

$$\frac{dRpol_{rest}}{dt} = \frac{Rpo_{j(act)}}{\tau_{SA(Rpol)}} - \frac{Rpol_{rest}}{\tau_{rest(R)}} + \sum_i \frac{\alpha_{m_i}}{\tau_{m_i}}Rpol_b - \frac{Rpol_{rest}}{\tau_{life(Rpol)}} \quad [54]$$

$$\frac{dRpol_b}{dt} = \frac{Rpol_{rest}}{\tau_{rest(R)}} - \sum_i \frac{\alpha_{m_i}}{\tau_{m_i}}Rpol_b - \frac{Rpol_b}{\tau_{life(Rpol)}} \quad [55]$$

$$\frac{dR_{rest}}{dt} = \frac{rRNA_j}{\tau_{SA(R)}} - \frac{R_{rest}}{\tau_{rest(R)}} + \sum_i \frac{\alpha_{P_i}}{\tau_{P_i}}R_b - \frac{R_{rest}}{\tau_{life(R)}} \quad [56]$$

$$\frac{dR_b}{dt} = \frac{R_{rest}}{\tau_{rest(R)}} - \sum_i \frac{\alpha_{P_i}}{\tau_{P_i}}R_b - \frac{R_b}{\tau^{life(R)}} \quad [57]$$

Putting in the exponential solution, we get

$$(\mu + \frac{1}{\tau_{SA(R)}}) = \frac{\alpha_{rRNA_j} Rpol_b}{\tau_{rRNA_j} rRNA_j} \quad [58]$$

$$(\mu + \frac{1}{\tau_{pool(Rpo_j)}}) = \frac{\alpha_{Rpo_j} R_b}{\tau_{Rpo_j} Rpo_{j(pool)}} \quad [59]$$

$$(\mu + \frac{1}{\tau_{SA(Rpol)}}) = \frac{Rpo_{j(pool)}}{\tau_{pool(Rpo_j)} Rpo_{j(act)}} \quad [60]$$

$$\mu(Rpol_{rest} + Rpol_b) + \frac{(Rpol_{rest} + Rpol_b)}{\tau_{life(Rpol)}} = \frac{Rpo_{j(act)}}{\tau_{SA(Rpol)}} \quad [61]$$

$$\mu(R_{rest} + R_b) + \frac{(R_{rest} + R_b)}{\tau_{life(R)}} = \frac{rRNA_j}{\tau_{SA(R)}} \quad [62]$$

Again, each of these equations are a growth law, relating relative abundances to cellular parameters and the growth rate. We can also use the above equations to get relative abundance growth law between RNA polymerase and ribosomes.

$$(\mu\tau_{SA(Rpol)} + 1)(\mu\tau_{pool(Rpoj)} + 1)\left(\mu Rpol_{tot} + \frac{Rpol_{tot}}{\tau^{life(Rpol)}}\right) = \frac{\alpha_{Rpoj}}{\tau_{Rpoj}} R_b \quad [63]$$

which under the assumption $\mu \ll 1/\tau_{SA(Rpol)}$, $\mu \ll 1/\tau_{pool(Rpoj)}$, and long life-time for RNA polymerase simplifies to

$$\frac{\mu\tau_{Rpo_j}}{\alpha_{Rpo_j}} = \frac{R_b}{Rpol_{tot}} \quad [64]$$

Multiplying equations 58 - 62, we get

$$(\mu\tau_{SA(Rpol)} + 1)(\mu\tau_{SA(R)} + 1)(\mu\tau_{pool(Rpo_j)} + 1)\left(\mu R_{tot} + \frac{R_{tot}}{\tau_{life(R)}}\right)\left(\mu Rpol_{tot} + \frac{Rpol_{tot}}{\tau_{life(Rpol)}}\right) = \frac{\alpha_{rRNA_j}\alpha_{Rpo_j}}{\tau_{rRNA_j}\tau_{Rpo_j}} R_b Rpol_b \quad [65]$$

Now, if we assume $\mu \ll 1/\tau_{SA(R)}$, $\mu \ll 1/\tau_{SA(Rpol)}$ and $\mu \ll 1/\tau_{pool(Rpo_j)}$, we get

$$\left(\mu R_{tot} + \frac{R_{tot}}{\tau_{life(R)}}\right)\left(\mu Rpol_{tot} + \frac{Rpol_{tot}}{\tau_{life(Rpol)}}\right) = \frac{\alpha_{rRNA_j}\alpha_{Rpo_j}}{\tau_{rRNA_j}\tau_{Rpo_j}} R_b Rpol_b \quad [66]$$

Under further assumption of long lifetime of ribosomes and RNA polymerases, we get

$$\mu^2 = \frac{\alpha_{rRNA_j}\alpha_{Rpo_j}}{\tau_{rRNA_j}\tau_{Rpo_j}} \frac{R_b}{R_{tot}} \frac{Rpol_b}{Rpol_{tot}} \quad [67]$$

This is equivalent to the closed cycle ribosomal RNA growth law presented in (2).

The RNA polymerase cycle: RNA polymerase (Rpol) autocatalytic cycle can also be seen as the mRNA autocatalytic cycle. To study the RNA polymerase autocatalytic cycle, we need to write the coupled set of ODEs for the abundances of mRNAs of RNA polymerase's protein subunits, RNA polymerases that transcribe them, and RNA polymerase's protein subunits that constitute the RNA polymerases, in their various activity states. We do not need ODEs for the abundances of ribosomes as we will write the translation of the proteins in terms of their mRNAs,

$$\frac{d\ m_{Rpo_j}}{dt} = \frac{\alpha_{rpo_j} Rpol_b}{\tau_{rpo_j}} - \frac{m_{Rpo_j}}{\tau_{life(m(Rpo_j))}} \quad [68]$$

$$\frac{dRpo_{j(pool)}}{dt} = \frac{R_{m(Rpo_j)} m_{Rpo_j}}{\tau_{Rpo_j}} - \frac{Rpo_{j(pool)}}{\tau_{pool(Rpo_j)}} \quad [69]$$

$$\frac{dRpo_{j(act)}}{dt} = \frac{Rpo_{j(pool)}}{\tau_{pool(Rpo_j)}} - \frac{Rpo_{j(act)}}{\tau_{SA(Rpol)}} \quad [70]$$

$$\frac{dRpol_{rest}}{dt} = \frac{Rpo_{j(act)}}{\tau_{SA(Rpol)}} - \frac{Rpol_{rest}}{\tau_{rest(R)}} + \sum_i \frac{\alpha_{mi}}{\tau_{mi}} Rpol_b - \frac{Rpol_{rest}}{\tau_{life(Rpol)}} \quad [71]$$

$$\frac{dRpol_b}{dt} = \frac{Rpol_{rest}}{\tau_{rest(R)}} - \sum_i \frac{\alpha_{mi}}{\tau_{mi}} Rpol_b - \frac{Rpol_b}{\tau_{life(Rpol)}} \quad [72]$$

The form of the above equation is analogous to that of ribosome's assembly from its constituent protein subunits, with similar definition of the terms, except that we are working with transcription level description. Putting in the exponential solution, we

get the following growth laws

$$(\mu + \frac{1}{\tau_{life(m(Rpo_j))}}) = \frac{\alpha_{rpo_j} Rpol_b}{\tau_{rpo_j} m_{Rpo_j}} \quad [73]$$

$$(\mu + \frac{1}{\tau_{pool(Rpo_j)}}) = \frac{R_{m(Rpo_j)} m_{Rpo_j}}{Rpo_{j(pool)} \tau_{Rpo_j}} \quad [74]$$

$$(\mu + \frac{1}{\tau_{SA(Rpol)}}) = \frac{Rpo_{j(pool)}}{\tau_{pool(Rpo_j)} Rpo_{j(act)}} \quad [75]$$

$$\mu(Rpol_{rest} + Rpol_b) + \frac{(Rpol_{rest} + Rpol_b)}{\tau_{life(Rpol)}} = \frac{Rpo_j}{\tau_{SA(Rpol)}} \quad [76]$$

Multiplying the four equations, we get

$$(\mu\tau_{SA(Rpol)} + 1)(\mu\tau_{life(m(Rpo_j))} + 1)(\mu\tau_{pool(Rpo_j)} + 1)\left(\mu + \frac{1}{\tau_{life(Rpol)}}\right) = \frac{\alpha_{rpo_j} R_{m(Rpo_j)} \tau_{life(m(Rpo_j))}}{\tau_{Rpo_j} \tau_{rpo_j}} \frac{Rpol_b}{Rpol_{tot}} \quad [77]$$

Now, assuming $\mu \ll 1/\tau_{pool(Rpo_j)}$ and that the lifetime of RNA polymerases are long, we get

$$\mu(\mu\tau_{SA(Rpol)} + 1)(\mu\tau_{life(m(Rpo_j))} + 1) = \frac{\alpha_{rpo_j} R_{m(Rpo_j)} \tau_{life(m(Rpo_j))} Rpol_b}{\tau_{Rpo_j} \tau_{rpo_j} Rpol_{tot}} \quad [78]$$

which is the equation that we use to analyse RNA polymerase growth cycle experiments.

The constancy of ribosome allocation (RNA/Protein ratio) under perturbation of RNAP's transcription activity: Under mRNA limitation, the ribosomal protein autocatalytic cycle is written as

$$\frac{dRP_{j(pool)}}{dt} = \frac{R_{m(RP_j)} m_{RP_j}}{\tau_{RP_j}} - \frac{RP_{j(pool)}}{\tau_{pool(RP_j)}} \quad [79]$$

$$\frac{dRP_{j(act)}}{dt} = \frac{RP_{j(pool)}}{\tau_{pool(RP_j)}} - \frac{RP_{j(act)}}{\tau_{SA(R)}} \quad [80]$$

$$\frac{dR_{rest}}{dt} = \frac{RP_{j(act)}}{\tau_{SA(R)}} - \frac{R_{rest}}{\tau_{rest(R)}} + \sum_i \frac{\alpha_{P_i}}{\tau_{P_i}} R_b - \frac{R_{rest}}{\tau_{life(R)}} \quad [81]$$

$$\frac{dR_b}{dt} = \frac{R_{rest}}{\tau_{rest(R)}} - \sum_i \frac{\alpha_{P_i}}{\tau_{P_i}} R_b - \frac{R_b}{\tau_{life(R)}} \quad [82]$$

Putting in the exponential solution, we get the following growth laws

$$(\mu + \frac{1}{\tau_{pool(RP_j)}}) = \frac{R_{m(RP_j)} m_{RP_j}}{\tau_{RP_j} RP_{j(pool)}} \quad [83]$$

$$(\mu + \frac{1}{\tau_{SA(R)}}) = \frac{RP_{j(pool)}}{\tau_{pool(RP_j)} RP_{j(act)}} \quad [84]$$

$$\mu(R_{rest} + R_b) + \frac{(R_{rest} + R_b)}{\tau_{life(R)}} = \frac{RP_{j(act)}}{\tau_{SA(R)}} \quad [85]$$

Now, if we multiply the three equations, we get

$$(\mu\tau_{SA(R)} + 1)(\mu\tau_{pool(RP_j)} + 1)\left(\mu R_{tot} + \frac{R_{tot}}{\tau_{life(R)}}\right) = \frac{R_{m(RP_j)}}{\tau_{RPj}} m_{RP_j} \quad [86]$$

Assuming $\mu \ll 1/\tau_{pool(RP_j)}$, $\mu \ll 1/\tau_{SA(R)}$, and that the lifetime of ribosomes are long, we get

$$\mu R_{tot} = \frac{R_{m(RP_j)}}{\tau_{RP_j}} m_{RP_j} \quad [87]$$

or,

$$\mu R_{tot} L_{aa(RP_j)} \tau_{aa} = R_{m(RP_j)} m_{RP_j} \quad [88]$$

as $\tau_{RP_j} = L_{aa(RP_j)}\tau_{aa}$, $L_{aa(RP_j)}$ being the length of the protein in the units of number of amino acids, and τ_{aa} being the elongation time per amino acid. Summing over all protein subunits of ribosome

$$\mu R_{tot} \tau_{aa} \sum_{j \in ribosomes} L_{aa(RP_j)} = \sum_{j \in ribosomes} R_{m(RP_j)} m_{RP_j} \quad [89]$$

Therefore,

$$\mu \frac{M_R}{M_{aa}} \tau_{aa} = \sum_{j \in ribosomes} R_{m(RP_j)} m_{RP_j} \quad [90]$$

where M_{aa} is the average mass of one amino acid. Similarly, for any protein i we can write

$$\mu P_{i(tot)} = \frac{R_{m(P_i)}}{\tau_{P_i}} m_{P_i} \quad [91]$$

or,

$$\mu \frac{M_{P_i}}{M_{aa}} \tau_{aa} = R_{m(P_i)} m_{P_i} \quad [92]$$

Summing over the entire proteome

$$\mu \frac{M_{Proteome}}{M_{aa}} \tau_{aa} = \sum_i R_{m(P_i)} m_{P_i} \quad [93]$$

Taking the ratio of the two,

$$\frac{M_R}{M_{Proteome}} = \frac{\sum_{j \in ribosomes} R_{m(RP_j)} m_{RP_j}}{\sum_{i \in \text{Proteome}} R_{m(P_i)} m_{P_i}} \quad [94]$$

Since $M_R \propto M_{rRNA} \propto M_{RNA}$, we see how global reduction in mRNA availability will keep RNA/Protein ratio fixed.

Note that when protein production was ribosome limited then we had (47)

$$\frac{M_R}{M_{Proteome}} = \alpha_R. \quad [95]$$

Also note that in the mRNA limited regime, the mass fraction of all proteins, not just ribosomes, will remain unchanged. For example, in similar way to above, we can show that in the mRNA limited regime, mass fraction of RNAP will be

$$\frac{M_{Rpol}}{M_{Proteome}} = \frac{\sum_{j \in RNAP} R_{m(Rpo_j)} m_{Rpo_j}}{\sum_{i \in \text{Proteome}} R_{m(P_i)} m_{P_i}} \quad [96]$$

General growth law for a metabolic protein

We can obtain growth laws corresponding to a metabolic protein by studying the substrate synthesis cycle involving that protein (red cycle in Fig. 1 of main text). Let P be the abundance of the metabolic protein that synthesize the substrate, and F be the abundance of the substrate, say amino acid or ATP. Then in the simple limit of involvement of only one metabolic protein, and assuming long lifetime of the metabolic protein, we get

$$\frac{dP}{dt} = \alpha_P \frac{R}{\tau_P} \quad [97]$$

$$\frac{dF}{dt} = \frac{P}{\tau_{metab.(F)}} - \frac{R}{\tau_F} \quad [98]$$

where τ_P is the time duration needed to translate P, $\tau_{metab.(F)}$ is the timescale for synthesis of the substrate, and τ_F is the average time duration in which one unit of the substrate is utilized. α_P is the allocation parameter towards synthesizing P. Putting in the exponential solution, and writing $R \equiv F\eta_F$, with $\eta_F = R/F$, we get

$$\mu P = \alpha_P \frac{F\eta_F}{\tau_P} \quad [99]$$

$$(\mu + \frac{\eta_F}{\tau_F})F = \frac{P}{\tau_{metab.(F)}} \quad [100]$$

Multiplying the two equations, we get the closed cycle growth law

$$\mu(\frac{\mu\tau_F}{\eta_F} + 1) = \frac{\alpha_P \tau_F}{\tau_P \tau_{metab.(F)}} \quad [101]$$

or,

$$\mu \approx \frac{\alpha_P \tau_F}{\tau_P \tau_{metab.(F)}} \quad [102]$$

as $\mu\tau_F \ll 1$ and $\eta_F \sim 1$.

Growth law for tRNA synthetase

The transport of amino acid substrates to the ribosome's translation site is facilitated by dedicated tRNAs and corresponding tRNA charging enzyme aminoacyl-tRNA synthetase. Let R be the abundance of ribosomes, F_i be the abundance of amino acid substrate of type i, P_i be the abundance of the metabolic protein synthesizing the amino acid of type i, T_i be the abundance of the tRNA synthetase responsible for charging amino acid of type i to its corresponding tRNA, and F_i' be the abundance of amino acid of type i made available at the translation site via tRNAs. Assuming long lifetimes for the constituents, and that all are active, the ODEs for the time evolution of this system can be written

$$\frac{dP_i}{dt} = \alpha_{P_i}\frac{R}{\tau_{P_i}} \quad [103]$$

$$\frac{dT_i}{dt} = \alpha_{T_i}\frac{R}{\tau_{T_i}} \quad [104]$$

$$\frac{dF_i}{dt} = \frac{P_i}{\tau_{metab.(i)}} - \frac{T_i}{\tau_{charging(i)}} \quad [105]$$

$$\frac{dF_i'}{dt} = \frac{T_i}{\tau_{charging(i)}} - \frac{f_{usage(i)}R}{\tau_{aa}} \quad [106]$$

where $\tau_{charging(i)}$ is the time duration tRNA synthetase of type i takes to charge a tRNA with its corresponding amino acid, and τ_{aa} is the average time duration in which one unit of the amino acid is utilized in the cell. $f_{usage(i)}$ is the fraction of time the amino acid of type i appears in the amino acid sequence of the cell's expressed proteome; therefore, $f_{usage(i)}$ is the fraction of ribosomes that will demand the amino acid of type i at every elongation step. Other terms have similar definitions as in previous cases. To obtain the growth law for tRNA synthetase, we focus on the cycle involving T_i and F_i'. Putting in the exponential solution and writing $R \equiv F_i'\eta_{F_i'}$, with $\eta_{F_i'} = R/F_i'$, we get the following closed loop growth law for tRNA synthetase

$$\mu T_i = \frac{\alpha_{T_i}}{\tau_{T_i}}F_i'\eta_{F_i'} \quad [107]$$

$$(\mu + \frac{f_{usage(i)}\eta_{F_i'}}{\tau_{aa}})F_i' = \frac{T_i}{\tau_{charging(i)}} \quad [108]$$

Each of these equations are a growth law, relating relative abundances to cellular parameters and the growth rate. Multiplying the above two equations, we get the following closed cycle growth law

$$\mu(\mu\frac{\tau_{aa}}{f_{usage(i)}\eta_{F_i'}} + 1) = \frac{\alpha_{T_i}\tau_{aa}}{\tau_{T_i}\tau_{charging(i)}f_{usage(i)}} \quad [109]$$

or,

$$\mu \approx \frac{\alpha_{T_i}\tau_{aa}}{\tau_{T_i}\tau_{charging(i)}f_{usage(i)}} \quad [110]$$

as $\mu\frac{\tau_{aa}}{f_{usage(i)}\eta_{F_i'}} \ll 1$.

Growth law for tRNA

The growth law for tRNA is similar to that of the tRNA synthetase, but now involving RNA polymerase's transcription of the tRNA. Let $tRNA_i$ be the abundance of tRNA responsible for carrying the amino acid of type i, α_{tRNA_i} be its allocation parameter, τ_{tRNA_i} be the time duration needed to transcribe the tRNA, and $\tau_{transfer(i)}$ be the time to transfer the amino acid to the translation site. Assuming long lifetimes for the constituents, the coupled set of ODEs for the tRNA autocatalytic cycle can be then written as

$$\frac{d\ tRNA_i}{dt} = \frac{\alpha_{tRNA_i}Rpol_b}{\tau_{tRNA_i}} \quad [111]$$

$$\frac{dF_i'}{dt} = \frac{tRNA_i}{\tau_{transfer(i)}} - \frac{f_{usage(i)}R}{\tau_{aa}} \quad [112]$$

$$\frac{dRpo_j}{dt} = \frac{\alpha_{Rpo_j}R}{\tau_{Rpo_j}} - \frac{Rpo_j}{\tau_{SA(Rpol)}} \quad [113]$$

$$\frac{dRpol_{rest}}{dt} = \frac{Rpo_j}{\tau_{SA(Rpol)}} - \frac{Rpol_{rest}}{\tau_{rest(Rpol)}} + \sum_i \frac{\alpha_{mi}}{\tau_{mi}}Rpol_b \quad [114]$$

$$\frac{dRpol_b}{dt} = \frac{Rpol_{rest}}{\tau_{rest(Rpol)}} - \sum_i \frac{\alpha_{mi}}{\tau_{mi}}Rpol_b \quad [115]$$

[116]

where all other terms are as defined earlier. Putting in the exponential solution, and writing $R \equiv \eta_{F'i} F'_i$, we get

$$\mu\ tRNA_i = \frac{\alpha_{tRNA_i}}{\tau_{tRNA_i}} Rpol_b \quad [117]$$

$$(\mu + \frac{f_{usage(i)}\eta_{F'_i}}{\tau_{aa}})F'_i = \frac{tRNA_i}{\tau_{transfer(i)}} \quad [118]$$

$$(\mu + \frac{1}{\tau_{SA(Rpol)}})Rpo_j = \frac{\alpha_{Rpo_j}\eta_{F'_i}F'_i}{\tau_{Rpo_j}} \quad [119]$$

$$\mu(Rpol_{rest} + Rpol_b) = \frac{Rpo_j}{\tau_{SA(Rpol)}} \quad [120]$$

$$[121]$$

Again, each of these equations are a growth law, relating relative abundances to cellular parameters and the growth rate. Multiplying the above equations, we get

$$\mu(\mu\tau_{SA(Rpol)} + 1)\left(\mu\frac{\tau_{aa}}{f_{usage(i)}\eta_{F'_i}} + 1\right)\mu Rpol_{tot} = \frac{\alpha_{tRNA_i}\alpha_{Rpo_j}\tau_{aa}}{f_{usage(i)}\tau_{tRNA_i}\tau_{Rpo_j}\tau_{transfer(i)}} Rpol_b \quad [122]$$

or,

$$\mu^2 \approx \frac{\alpha_{tRNA_i}\alpha_{Rpo_j}\tau_{aa}}{f_{usage(i)}\tau_{tRNA_i}\tau_{Rpo_j}\tau_{transfer(i)}} \frac{Rpol_b}{Rpol_{tot}} \quad [123]$$

as $\mu\frac{\tau_{aa}}{f_{usage(i)}\eta_{F'_i}} \ll 1$ and $\mu\tau_{SA(Rpol)} \ll 1$.

Growth law involving membrane synthesis

Consider the autocatalytic cycle of the ribosomes, a membrane synthesis associated stitcher protein, like say MreB, the cluster of stitcher proteins which make the incision, and the membrane which provides the environment for ribosomes to work efficiently. The time evolution of their abundances will be given as

$$\frac{dP}{dt} = \alpha_P\frac{R}{\tau_P} - \frac{P}{T_P\tau_{SA(C)}} \quad [124]$$

$$\frac{dC}{dt} = \frac{P}{T_P\tau_{SA(C)}} \quad [125]$$

$$\frac{dS}{dt} = \frac{CW}{\tau_m} \quad [126]$$

where R, P, and C are the abundances of ribosomes, stitcher proteins and the cluster of stitcher proteins respectively; S is the surface area of the membrane. τ_P is the time required to translate the stitcher protein, $\tau_{SA(C)}$ is the time to assemble the cluster and τ_m is the linear speed of insertion of membrane material by the cluster (in units of length/time). T_P is the threshold number of stitcher proteins needed to form the cluster which can make incision in the membrane, and W is the width of the inserted membrane material (Peptidoglycan). Since in the steady state all the components in the cycle grow exponentially at the same rate, we not only can see why the length will grow exponentially (width of the cell typically stays constant in E.Coli growing in a given medium), but also obtain growth laws corresponding to its components:

$$(\mu + \frac{1}{T_P\tau_{SA(C)}}) = \frac{\alpha_P R}{\tau_P P} \quad [127]$$

$$\mu(\mu\tau_{SA(C)}T_P + 1) = \frac{\alpha_P R}{\tau_P C} \quad [128]$$

$$\mu = \frac{CW}{\tau_m S} \quad [129]$$

Each of the above equation is a relative abundance growth law. In the main text, we use Eq. 129 to analyze the growth rate reduction due to the application of Triclosan.

References

1. Scott M et al., (2010) Interdependence of Cell Growth and Gene Expression: Origins and Consequences. *Science* 330:1099-1102.
2. Kostinski S, Reuveni S (2020) Ribosome Composition Maximizes Cellular Growth Rates in E. coli. *Phys. Rev. Lett.* 125:028103.