

Slowing translation to avoid cellular depletion of active ribosomes at slow growth rates

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(Received 29 April 2025; accepted 23 December 2025; published 2 March 2026)

To sustain bacterial growth, a fraction of active ribosomes must be allocated to synthesize ribosomal proteins. Two control parameters modulate the growth rate: the fraction of ribosomes allocated to ribosomal protein synthesis and the translation speed. At longer doubling times, which approach the active ribosome lifetime, the limited number of ribosomes poses a significant constraint in allocating ribosomes to ribosomal protein synthesis. We show that, to maintain slow growth while reducing the risk of depletion of the active cellular ribosome population, some bacteria must significantly reduce translation speed below its maximum, as previously observed. This change in the controls is due to a universal shot noise limitation that applies at slow growth rates in all kingdoms of life.

DOI: [10.1103/3nwf-b259](https://doi.org/10.1103/3nwf-b259)

I. INTRODUCTION

All bacterial cells share a universal set of molecular machines that cooperatively self-replicate the cellular content, themselves included. This universal set of molecular machines is organized in a unique autocatalytic network structure called the von Neumann architecture [1–4], not to be confused with the von Neumann architecture for a stored-program computer [5]. A central part of this von Neumann autocatalytic network architecture for self-replication is the universal constructor (UC)—“a machine that makes machines”—by translating instructions into actions that result in synthesizing other machines, given raw material and energy. The instructions can also instruct the machine to synthesize a copy of itself.

In the cell, the universal constructor comprises a subnetwork of essential molecular machines, commonly called the transcription-translation machinery. This machinery can synthesize all proteins in the cell—the central dogma—and self-replicate its components [4]. We have previously demonstrated that for any essential molecular machine in the cell, one can formulate a “growth law” that links the overall cellular doubling time with the physiological parameters that characterize the machine: (1) the machine’s processing rate, (2) the fraction of machines (or the fraction of allocated time) dedicated to replicating copies of themselves, and (3) the machine’s active lifetime. Notably, we have established that the well-known ribosome growth law [6–9] is a specific instance of a growth law derived from the von Neumann architecture

model. The derivation of growth laws is based on applying the principle of conservation of matter to autocatalytic networks [4] (see Sec. III).

At steady growth conditions, the doubling rate of ribosomes, RNA polymerases, and essential proteins, e.g., in metabolism, all share the same value, which is a Perron-like eigenvalue of the entire autocatalytic network irreducible coupling matrix, as we have previously shown [4].

In Refs. [10,11], it was experimentally demonstrated that ribosomes slow their translation speed at slow growth rates. In the same experiments, the RNA-to-protein ratio decreased approximately linearly with growth rate, but approached a finite value at zero growth rate. To theoretically account for both these experimental observations, we demonstrate that it is essential to consider the duration of two additional physiological processes: the ribosome assembly duration, previously assumed to be negligible because it is much smaller compared to the doubling time, and the ribosome active lifetime, previously neglected because it was much longer than the doubling times considered.

Accounting for these two processes allows us to achieve a good fit for all the measured data and, in particular, for the observed slowing down of the ribosomes’ translation speed as a function of growth rate, while providing falsifiable predictions for the ribosome assembly duration, the ribosome active lifetime, and the fraction of ribosomes in assembly, which we define explicitly in what follows.

Because a given observed growth rate can be attained with multiple ribosome allocation fractions and translation speed combinations, the question arises: Why and under what circumstances is a specific choice of allocation fraction and translation speed combination that yields the same observed growth rate evolutionarily beneficial? In particular, we address the question of why the reduction of translation speed with decreasing growth rate was selected in *Escherichia coli*.

In the autocatalytic cycle of ribosomes—“ribosomes make more ribosomes,” a fraction $0 < \alpha < 1$ of the ribosomes is allocated to synthesize ribosomal proteins. These ribosomal

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proteins subsequently enter the ribosome self-assembly process, which synthesizes new ribosomes. The same allocation fraction α of the newly synthesized ribosomes is allocated to synthesize more ribosomal proteins, thus completing the cycle. If the translation speed, the active ribosome lifetime, and the duration of the ribosome assembly process are fixed, the only remaining method to control the growth rate is the allocation fraction α .

Under slow growth conditions, the total number of ribosomes decreases approximately quadratically with growth rate [12]. In *E. coli*, growing at a doubling time of 100 min, the number of ribosomes decreases 12-fold compared to a doubling time of 20 min. In the limit of long doubling times, spanning a few hundred minutes, the total number of ribosomes in *E. coli* approaches 5400 ribosomes (see Appendix B). At such extended doubling times, it becomes increasingly challenging to fine-tune the ribosome allocation fraction. For example, if the allocation parameter is 1%, 54 out of 5400 ribosomes on average are allocated to synthesize ribosomal proteins. In other words, on average, one ribosome is assigned to synthesize each ribosomal protein.

Therefore, a fluctuation in the allocation of a single ribosome can result in significant fluctuations in the synthesis rate of that ribosomal protein, subsequently leading to fluctuations in the ribosome synthesis rate itself. As the ribosome synthesis rate approaches the ribosome inactivation rate Γ_0 from above, fluctuations in the actual ribosome synthesis rate can result in underproduction and, as a result, lead to the depletion of the active cellular ribosome population and hence to growth arrest.

Hence, the finite active lifetime of ribosomes sets a lower bound on the number of ribosomes that must be allocated to synthesize ribosomal proteins. Going below this number leads to risky fluctuations that can deplete the active ribosome population.

Recently, Ref. [13] demonstrated that fluctuations in the number of essential proteins can lead to growth arrest when the mean number of proteins is above the minimal number required for growth but close enough that fluctuations can bring it below the threshold. To mitigate this risk, the authors suggest that proteins be produced in excess, i.e., overabundant, and that the criterion for how overabundant they should be depends on the natural variance in their numbers, which results from the noisy nature of their synthesis via the central dogma transcription-translation machinery. Furthermore, a minimal number of messenger RNAs (mRNAs) per essential protein of 1 per cell cycle was suggested. In the context of our current work, this minimum also implies a minimum on the ribosome allocation parameter for essential proteins. The fact that one cannot decrease the allocation parameter indefinitely forces cells to adopt strategies beyond controlling the ribosome allocation parameter to sustain slow growth in harsh environments. Here, we suggest that controlling the translation rate will allow the cell to sustain slow growth and mitigate the risk of growth arrest due to fluctuations in ribosome number, given their finite lifetimes.

Here, we study this challenge, which bacterial cells face at slow growth rates but not under fast growth conditions. A previous study in Ref. [14] showed that protein degradation cannot be overlooked at slow growth rates, as it

requires an increasing number of ribosomes to be allocated to maintain the proteome as the growth rate slows. Here, we show that ribosome active lifetime imposes another strong constraint on how cells can exert control over their growth rate in harsh environments, where the growth rate that can be sustained is significantly lower than in more permissive environments.

We show that temporal fluctuations in ribosome synthesis rate near the ribosome inactivation rate increase the risk of depleting the active ribosomal population in the cell leading to growth arrest. To mitigate this risk, we suggest that the cells in this study (*E. coli* and *Corynebacterium glutamicum*) utilize translation speed as an additional control parameter.

II. RESULTS

A. Ribosome growth laws with active lifetime and assembly durations

Using our model, as presented in Sec. III, we derived two ribosome growth laws that take into account the ribosome allocation fraction, the ribosomal protein synthesis rate, and two additional durations: ribosome assembly and ribosome active lifetime, Γ_0^{-1} , which is the duration the ribosome can be active, i.e., translate mRNA to synthesize proteins:

$$\mu = \frac{\phi_i}{\tau_{SA_i}} - \Gamma_0, \quad (1)$$

where μ is the growth rate and τ_{SA_i} is the residual assembly time of ribosomal protein i —the average duration from its insertion into the assembly process until the completion of the ribosome assembly process and the birth of a new functional ribosome that contains this protein. ϕ_i is the ratio between the number of ribosomal proteins of type i in the assembly process and the total number of ribosomes, which we also call the work-in-process fraction (WIP) of ribosomal proteins of type i .

Another growth law we derived is

$$\mu = \left(\frac{\alpha_i}{\tau_i} - \Gamma_0 \right) \frac{1}{1 + \phi_i}, \quad (2)$$

where α_i is the fraction of ribosomes synthesizing ribosomal protein i and τ_i^{-1} is the synthesis rate of ribosomal protein i .

Assuming ϕ_i equals zero and noting that $v_{aa} = \frac{L_i}{\tau_i}$, we obtain

$$\mu = \frac{v_{aa}}{L_i} \left(\alpha_i - \frac{\Gamma_0 L_i}{v_{aa}} \right) = \frac{v_{aa}}{f L_i} \left(f \alpha_i - \frac{f \Gamma_0 L_i}{v_{aa}} \right). \quad (3)$$

Identifying $\kappa = \frac{v_{aa}}{f L_i}$, $\Phi = f \alpha_i$, and $\Phi_0 = \frac{f \Gamma_0 L_i}{v_{aa}}$, where f is a factor that relates the ribosomal protein mass fraction (Φ) to the allocation parameter, $f \approx 1/2 \times 0.9$; see Appendix B. In this way, we recover the known ribosome growth law [6–9].

Note that $\frac{v_{aa}}{L_i \Gamma_0}$ is the number of proteins of type i a single ribosome can make in its lifetime.

A simple yet important insight from this equation is the observation that to sustain growth, i.e., to have $\mu > 0$, either every single ribosome should synthesize ribosomal protein i a fraction α_i of its active lifetime, or, a fraction α_i of the ribosomes should synthesize ribosomal protein i at any given moment.

So far, we have presented ribosome growth laws derived for a single essential ribosomal protein. This is by no means a constraint, and we can also obtain, after some algebra, growth laws that depend on sets of ribosomal proteins, including all of them in one set:

$$\mu(L + \hat{L}) = v_{aa} \left(\alpha - \frac{\Gamma_0}{v_{aa}} L \right), \quad (4)$$

where L is the total ribosomal protein length in amino acids and $\hat{L}$ is the sum over all the ribosomal protein lengths weighted by their relative part in the WIP (see Appendix C).

Using our generalized growth law, we extract the ribosomal active lifetime and the fraction of ribosomes in assembly using the measured growth rate as a function of the RNA-to-protein ratio and the measured translation speed (see Sec. III). Based on the measured translation speed as a function of growth rate, the known mRNA length of all ribosomal proteins, and the measured RNA-to-protein ratio, we used our theoretical model to infer the ribosome active lifetime to be $\Gamma_0^{-1} = 500 \pm 60$ (1 σ) min (see Sec. III).

The fraction of ribosomes in assembly— ϕ , predicted from the model, is 10%—based on a calculation from one of the longest ribosomal protein operons (rplN-rplX-rplE-rpsN-rpsH-rplF-rplR-rpsE-rpmD-rplO-secY-rpmJ). This estimate changes up to 21% when it is based on another long ribosomal protein operon (rpsJ-rplC-rplD-rplW-rplB-rpsS-rplV-rpsC-rplP-rpmC-rpsQ); see Sec. III. Basing the calculation on all the large subunit ribosomal proteins also yields $\phi_i = 20\%$.

In contrast, a calculation based on the rps ribosomal proteins (small ribosomal subunit proteins) or the rpm ribosomal proteins (a separate group of small ribosomal proteins that belong to the large ribosomal subunit) yields a higher estimate of the relative work in process (relative WIP) ϕ_i ($\phi_i = 30\%$ for rps proteins and $\phi_i = 60\%$ for rpm proteins). Note that if the ratio between the number of intermediates—a subset of ribosomal proteins and partially assembled ribosomes containing them—to complete ribosomes is higher than another ratio, this is an indication that this subset is in excess and thus does not limit the rate of assembly of ribosomes.

Utilizing these two parameters, namely, $\phi_i = 0.1$, $\Gamma_0^{-1} = 500$ min enables us to create a theoretical curve for the ribosome translation speed (measured in amino acids per second) as a function of the growth rate; this curve nicely fits the measured data from Ref. [11], as seen in Fig. 1.

The two fitted parameters of our model—the ribosome active lifetime and the ribosome fraction in assembly—can be measured and contrasted with our predictions. Using these parameters, we also obtained a good fit for the measured dependence of the RNA-to-protein ratio as a function of the growth rate as seen in Fig. 1.

We performed a similar analysis for another bacterium, *C. glutamicum*, a gram-positive bacterium belonging to the class Actinomycetes [10]. The results, which are consistent with our model, are presented in Fig. 1 and in inset (A) of Fig. 1 (data depicted by squares, theory in solid green). Since we did not have data on α_i , we plotted αv_{aa} as a function of the growth rate μ , where v_{aa} is the ribosome translation speed measured in amino acids per second. We found that αv_{aa} is a linear function of the growth rate

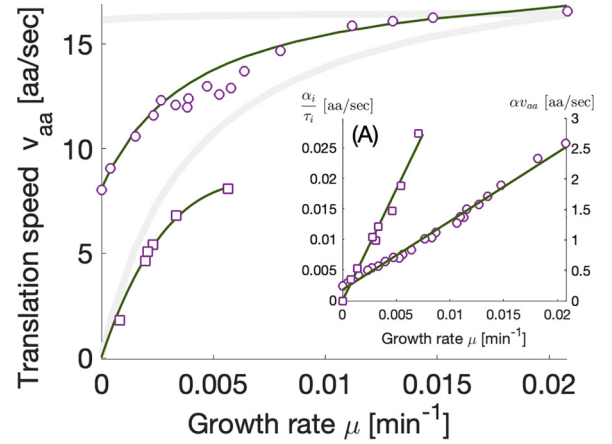


FIG. 1. Ribosome translation speed v_{aa} in amino acids per second vs growth rate in (min^{-1}) for *E. coli* from Ref. [11] (purple circles) and *C. glutamicum* (purple squares) from Ref. [10]. Theoretical curves are in green solid lines. The lower light-gray solid line represents the limit of an infinite ribosome lifetime. In this limit, we adjusted the ribosome assembly time to obtain a good fit for the translation speed at fast growth rates. The upper solid gray line represents the zero ribosome assembly time limit, where we adjusted the ribosome lifetime to obtain a good fit for the translation speed at fast growth rates. Both parameters are required to obtain a good fit for the data while accounting for the translation speed and the RNA-to-protein ratio as a function of the growth rate. Specifically, a finite ribosome lifetime is essential for explaining the observed nonzero RNA-to-protein ratio at zero growth rate. Accounting for both timescales, we obtain a prediction for the ribosome lifetime of *E. coli* $\Gamma_0^{-1} = 500 \pm 60$ min. Our model predicts a ribosome assembly time that varies linearly with the doubling time and approaches 150 min at the zero growth rate limit, in accordance with Ref. [15]. In inset (A), we show the data and the fit from which we extracted the ribosome assembly time and lifetime. The left y axis and the corresponding purple circles depict data for *E. coli* taken from Ref. [11], and the right y axis and the corresponding purple squares depict data for *C. glutamicum* taken from Ref. [10].

μ , in accordance with the prediction of our model, suggesting that the cell is actively maintaining ϕ_i constant (constant WIP [16]).

Our extended ribosome growth law, Eq. (10), accounts for the relationship between the ribosome translation speed, the RNA-to-protein ratio, and the growth rate by using the ribosome active lifetime and the ribosome assembly duration as additional fitting parameters to the standard ribosome growth law [6–9].

However, we can also ask why ribosomal translation speed slows at slow growth rates, since this is not the only possibility consistent with the observed growth rate. For example, the same growth rate could have been obtained by reducing the allocation parameter while keeping the translation speed at its maximum. This is what happens in other bacteria and even in *E. coli* at fast growth rates.

B. Effect of bacteriostatic antibiotics targeting the ribosome on active ribosome lifetime

Active ribosome lifetime (Γ_0^{-1})—the overall duration during which the ribosome can actively translate—is one of two

parameters that we added to the standard ribosome growth law. Previously, we tested our model using data from an experiment that modulated ribosome assembly duration [4]. Here, we further test our model using experimental data in which the ribosome's active lifetime was manipulated. For that purpose, we used data from Ref. [17] on the dependence of growth rate on the concentration of chloramphenicol and erythromycin, both bacteriostatic antibiotics that inhibit ribosome translation. Chloramphenicol inhibits the transfer of amino acids to the growing peptide chain in the ribosome. Erythromycin disrupts transfer RNA (tRNA) translocation from the A site to the P site within the ribosome. Because the A site stays occupied, it prevents new charged tRNA from entering the ribosome, rendering it inactive.

In Ref. [17], bacteria were grown in different media with varying concentrations of these antibiotics. We use our version of the growth law, Eq. (20), and assume that the concentration of antibiotics adversely modulates the active lifetime of ribosomes via a Michaelis-Menten-like dependence: $\bar{\Gamma}_0(c) = \Gamma_0(1 + \frac{Ac}{k+c})$, where $\bar{\Gamma}_0^{-1}(c)$ is the ribosome active lifetime as a function of the concentration of antibiotics c , $\Gamma_0^{-1} = 500$ is the ribosome active lifetime without antibiotics, which we found before, $A > 1$ is the amplitude, a dimensionless parameter that gives the maximal effect of the antibiotics in the limit of very high concentrations, and k is the fitted Michaelis-Menten saturation parameter.

To obtain the fit, we used the formula $\mu = \frac{\Phi(c)}{2\tau_U} - \bar{\Gamma}_0(c)$. The good fits obtained in Fig. 2 further consolidate our model.

C. Mitigating ribosome population depletion risk

If the only way to control the growth rate was via ribosome allocation, the translation speed would have been kept at its maximal value by keeping the charged tRNA and elongation factor G (EF-G) pools fully charged. In such a scenario, the fraction of ribosomes allocated to make ribosomal proteins would decrease as the growth rate decreases. However, at some point, the number of ribosomes dedicated to synthesizing each essential ribosomal protein becomes of the order of 1, i.e., just a few ribosomes per mRNA.

At this point, any random fluctuation in the allocation can lead to large fluctuations in the synthesis of new ribosomes. If these fluctuations cause the ribosome production rate to fall below the ribosome inactivation rate, the ribosome population will decrease, and there is a risk of depletion of the active ribosome fraction. We thus see that, perhaps unexpectedly, there is a control challenge in sustaining slow growth rates (see Ref. [13]).

A well-known result from branching processes [20] states that if the doubling time of a population of N self-replicating agents is shorter than their lifetime, the depletion probability tends to zero at the large N limit ($N \gg 1$). However, as we show, if the number N is small, the depletion probability can become non-negligible even though the doubling rate is shorter than the lifetime, if they are close.

In *E. coli*, the absolute number of ribosomes decreases approximately quadratically as a function of the growth rate (Appendix A, data taken from Ref. [12]). When the number of ribosomes allocated to synthesize a specific essential ribosomal protein in the cell is on the order of 1, we enter a regime

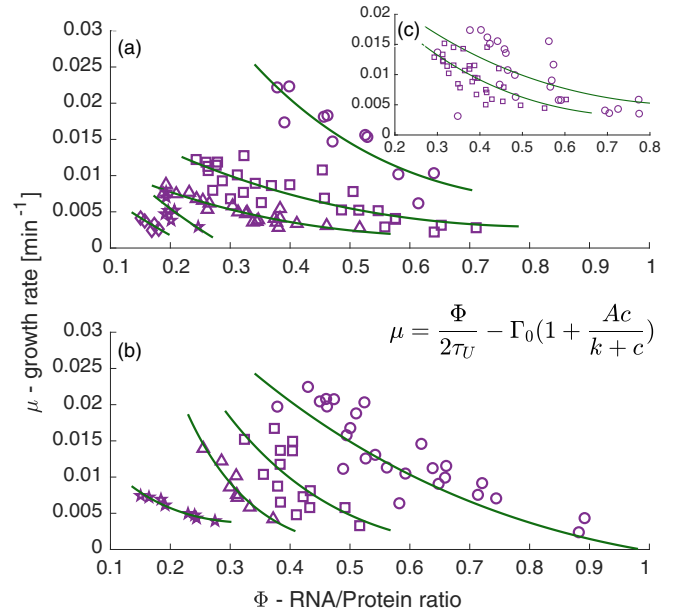


FIG. 2. Growth rate vs RNA-to-protein ratio. Depicted by circles—data from *E. coli* grown mostly in 3-morpholinopropanesulfonic acid (MOPS) buffer environments with different Carbon and Nitrogen sources, taken from Ref. [17]. In these experiments, varying concentrations of chloramphenicol (in nine different environments) or erythromycin [in two different environments—two left-most (and lowest) lines in inset (b)] were administered, and both the RNA-to-protein ratio and the growth rate were measured per concentration. To obtain the fit depicted by the green solid lines, we modulated the ribosome active lifetime according to an MM term with two fitting parameters, the amplitude, $A > 1$ —the maximal multiplicative factor the ribosome inactivation rate Γ_0 can gain in the limit of very high concentration of antibiotics, and k —the concentration of antibiotics that leads to a $\frac{1}{2}A$ factor. Notably, we obtained the same k value per antibiotic type, regardless of the nutrients present in the environment. In particular, we obtained $k = 37.0 \pm 2.6$ (1σ) μM for Chloramphenicol and $k = 460 \pm 40$ μM for Erythromycin (see Appendix D for all the fits and additional information), which is consistent with the measured minimum inhibitory concentration (MICs) [18,19]. A solid green line depicts our theory. It aligns well with the data, utilizing three fitting parameters as described in the main text: τ_U , which is related to the ribosomal protein synthesis duration load via the growth law [Eq. (20)], A and k , which are, respectively, the saturation and concentration at half saturation constants of the Michaelis-Menten (MM) term; see equation in Fig. 2. We used $\Gamma_0^{-1} = 500$ min, which is our estimate for the ribosome active lifetime duration.

known as the shot noise limit. In this regime, the instantaneous synthesis rate of new ribosomes, which directly depends on manufacturing critical components, i.e., synthesizing essential ribosomal proteins, significantly increases. These fluctuations can cause the synthesis rate of new ribosomes to drop below the ribosome inactivation rate, consequently leading to depletion of the active ribosome population.

This risk can be mitigated by reallocating more ribosomes, as observed when antibiotics that target ribosomes are administered [7,8]. Yet, when the total number of ribosomes is small (on the order of a few thousands), this strategy is no

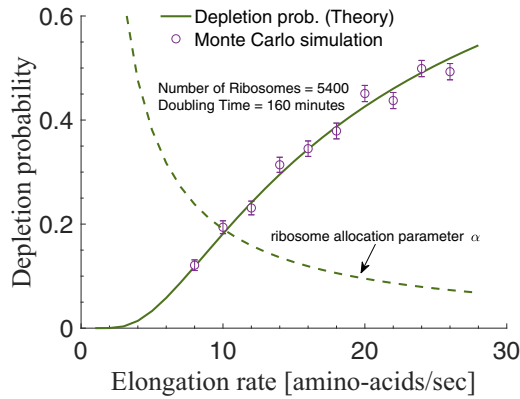


FIG. 3. Depletion probability of the active fraction of cellular ribosomes for a fixed average doubling time of 160 ± 25 (1σ) min, with a total of 5400 ribosomes. The green solid curve represents the theoretical calculation of the depletion rate based on Eq. (19), and the circles are the result of a Monte Carlo simulation of the ribosome autocatalytic cycle, with a random allocation, and random assembly time whose average is ≈ 30 min [15]. The dashed line in the figure is the ribosome allocation parameter—the fraction of ribosomes allocated to the synthesis of ribosomal proteins. The theoretical exact calculation of the depletion probability fits the simulation results well. The fact that a simple, mathematically exact formula for the depletion probability of a small population of self-replicating agents can capture a much more detailed simulation of noisy ribosome allocation, self-assembly, and inactivation supports the assertion that our formula can also describe the complexity of the actual biological process. As shown in the figure, reducing translation speed while increasing the total number of ribosomes and allocating them more toward ribosomal protein synthesis allows the cell to maintain the same growth rate while significantly reducing the risk of active ribosome depletion. In particular, slowing the ribosome speed from its maximum rate (22 amino acids/s) to a rate of 10 amino acids/s decreases the probability of active ribosome depletion by approximately a factor of 3.

longer helpful, and it is necessary to either keep the number of ribosomes high while reducing their translation speed or use temporal allocation (to be explained below).

To demonstrate the effect of the ribosome slowdown strategy on the active ribosome population depletion probability, we calculated the probability of depletion with a given fixed growth rate and a fixed initial number of ribosomes as a function of the ribosome translation speed. We present our results in Fig. 3.

Using an event-driven Monte Carlo simulation (the code of the simulation is available in Ref. [21]) of a population of ribosomes engaged in the subcycle that self-assemble more ribosomes, specifically focusing on the large subunit, which is thought to be the bottleneck for ribogenesis, we estimated the depletion probability from the simulation by directly counting depletion events in it. Mean values are plotted as circles in Fig. 3, while error bars depict the standard deviation. We also plot the theoretical active ribosome depletion probability (solid line); see Sec. III for the analytic derivation.

As the figure shows, the depletion probability of the active ribosome population increases monotonically with the translation speed for a given doubling time of 160 min. For example, slowing the ribosome translation speed from its maximum (22 amino acids/s) to 10 amino acids/s decreases the probability of depletion by approximately a factor of 3.

The Monte Carlo simulation is an event-driven code that simulates a continuous-time multispecies Crump-Mode-Jagers (CMJ)-type branching process [20]. In short, free ribosomes are randomly allocated for the synthesis of ribosomal proteins according to the allocation parameter $0 < \alpha_i < 1$, $\sum_i \alpha_i \leq \alpha < 1$. The synthesis durations are also random, with a mean synthesis duration $\frac{L_i}{v_{aa}}$, L_i being the number of amino acids in ribosomal protein i , and v_{aa} is the translation speed in units of amino acids per second (aa/s). Each ribosome is active for a random duration with a mean active lifetime of Γ_0^{-1} .

Whenever a complete set of ribosomal proteins is formed for the first time randomly, it is taken to the assembly process, whose duration is a random variable with mean τ_{SA} . Upon completion of the assembly process, a new ribosome joins the free ribosome pool, and a random active lifetime is assigned to it. All the distributions in the simulation were assumed to be independent.

All processes in the simulation occur concurrently, so ribosomes are allocated to synthesize a new generation of ribosomal proteins before the previous generation finishes being assembled to form new ribosomes. This overlapping of generations is a hallmark of CMJ branching processes. We assume that ribosomal RNA (rRNA) is abundant and therefore immediately available during assembly. A more accurate modeling approach for ribogenesis is possible but falls outside the scope of this work.

We also analytically calculated the depletion probability of a small population of N self-replicating agents, replicating at an average doubling time $\frac{\ln 2}{\mu}$, and with random active lifetime whose average is Γ_0^{-1} . We obtained under simplified assumptions that the depletion probability is given by $P_{\text{depletion}}(N) = (\min(1, \frac{\Gamma_0}{\mu}))^N$.

We note that the analytical calculation of the probability of depletion of a finite population of N replicating agents is not present in the standard literature that we are aware of in branching processes, e.g., ref. [20], perhaps because typically, in the theory of branching processes, results are often derived in the limit $N \gg 1$. See, however, Ref. [22] for a calculation of the dynamics toward depletion (with probability one), albeit with correlated depletion of both mother and daughter.

The agreement between the exact analytical calculation for the depletion probability based on a simple model and the detailed simulation that includes random ribosome allocation, ribosomal protein synthesis, self-assembly, and inactivation strengthens our assertion that the calculation of the depletion probability using a simple model is capable of capturing the complexity of a realistic biological cell.

We conclude that translation slowdown is an evolutionarily selected control logic: reducing translation speed in harsh environments allows the cell to maintain slow growth while

minimizing the risk of depleting its active ribosome fraction and avoiding growth arrest.

D. Temporal allocation of ribosomes

Our model reveals that if the total number of ribosomes in the cell is very small—hundreds to several thousands—as is the case for small cells, allocating a fraction of them to ribosomal protein synthesis significantly increases the risk of active ribosome depletion.

At these low numbers, slowing down translation is no longer a good risk mitigation strategy because there is a minimal translation speed below which either the ribosome's active lifetime or the ribosomal protein's lifetime [14] is shorter than the time required to synthesize and replace them. Thus, for these small cells, controlling their (small) growth rate via controlling ribosome allocation or via slowing down of translation, or both, ceases to be a viable control strategy to reduce the depletion risk, and so we predict that evolution had to resort to a different solution altogether, in this case.

From a general systems engineering perspective [16], the solution is to allocate temporally, i.e., schedule, most, if not all, of the ribosomes in the cell to specific tasks, e.g., ribosomal protein synthesis. Equation (10) can now be interpreted as a time average, and α_i as the average percentage of its active lifetime each ribosome is allocated to synthesize ribosomal protein of type i .

Qualitatively, this method is used by the tiny, obligatory photosynthetic bacterium *Prochlorococcus marinus* [23]. It is known that most of its ribosomes are allocated to translate ribosomal protein at dawn by the circadian regulator. Further research is required to test our model more quantitatively.

We predict that the temporal allocation strategy is general and expect it to occur in different slow-growing tiny bacteria, e.g., the SAR11 bacterium (with fewer than 1000 ribosomes). We expect ribosomes in these bacteria to be allocated to specific tasks in an all-or-none manner in time, governed by a temporal scheduler/modulator, circadian, or other.

1. Circadian ribosome growth law

Consider a cell that divides the day into two different time intervals that comprise a periodic circadian cell cycle. In the first interval (e.g., near dawn), it employs most of the active ribosomes to synthesize ribosomal proteins; in the other interval, the ribosomes synthesize other nonribosomal proteins or rest. The total cycle time, $T = 24$ h, is divided into $T_{\text{on}} = \alpha T$ —the ribosomal protein synthesis duration and $(1 - \alpha)T$ —the nonribosomal synthesis duration.

To calculate the average growth rate over one cycle time T , we look at the relation between the number of ribosomes at the beginning of the cycle and the number of ribosomes at the end of the cycle. Assuming that at the beginning of the cycle there where $U(t)$ ribosomes and during the cycle all the ribosomes synthesize new ribosomes for a period of T_{on} , the number of ribosomes at the end of the cycle is given by $U(t + T) = U(t)e^{\frac{T_{\text{on}}}{\tau_i}}$, where τ_i as before is the ribosomal protein synthesis time. The average growth law is thus given by $\mu = \frac{1}{T} \log\left(\frac{U(t+T)}{U(t)}\right) = \frac{T_{\text{on}}}{T} \frac{1}{\tau_i} = \frac{\alpha}{\tau_i}$.

III. METHODS

Consider a simplified model for the autocatalytic ribosome subcycle [4], in which ribosomes randomly create ribosomal proteins from ribosomal protein mRNA. The ribosomal proteins join the ribosome assembly process and bind directly to their target site on the rRNA or to the partially assembled ribosome, eventually forming new ribosomes and thus completing the cycle.

It is often implicitly assumed that the ribosome's inactivation rate is negligible, i.e., the ribosome's active lifetime is significantly larger than the doubling time. Similarly, the ribosome assembly duration is also typically neglected, i.e., assumed to be very small compared to the doubling time. Instead, we take both of these timescales to be finite.

We define ϕ_i —relative work in process as the relative number of ribosomal proteins of type i that are free or part of a partially assembled ribosome relative to the total number of active ribosomes in the cell. For example, at a doubling time of 24 min, it is estimated that, on average, an *E. coli* will have 72 000 ribosomes [12]. Thus, if the relative WIP of a specific ribosomal protein is 10%, there are 7200 such proteins in different stages of the ribosome assembly process.

Consider a deterministic process with a ribosome active lifetime of $\tau_A = \Gamma_0^{-1}$, translation duration τ_i , and ribosome self-assembly duration τ_{SA} . Let $U_0(t)$ be the number of ribosomes just about to be free. Then,

$$U_0(t) = U_0(t - \tau_i) + \alpha_i U_0(t - \tau_i - \tau_{SA_i}) - \alpha_i U_0(t - \tau_i - \tau_{SA_i} - \tau_A), \quad (5)$$

i.e., the number of ribosomes that are just freed is equal to the number of ribosomes that started translating $t - \tau_i$ min ago: $U_0(t - \tau_i)$, plus the number of ribosomes just born from the assembly process whose duration is τ_{SA_i} min: $\alpha_i U_0(t - \tau_i - \tau_{SA_i})$, minus the number of ribosomes that were born exactly a lifetime τ_A ago and are hence are becoming inactive just now: $\alpha_i U_0(t - \tau_i - \tau_{SA_i} - \tau_A)$.

Consider the ribosome self-assembly process, where ribosomal proteins enter the assembly process to bind with rRNA or with a partially assembled ribosome. Assume a ribosomal protein a_i to be limiting. Then, it is taken to the assembly process as soon as it is synthesized. Let τ_{SA_i} be the residual assembly duration measured from when a_i enters the ribosome assembly process to when the ribosome assembly is complete, with a_i embedded in a ribosome. Finally, let the fraction of ribosomes allocated to translate protein a_i be α_i . Only free ribosomes can be allocated to do so, hence the number of newly formed ribosomes that are just born is $\alpha_i U_0(t - \tau_i - \tau_{SA_i})$. Similarly, one can identify the number of ribosomes that die right now as those born $\tau_{SA_i} - \tau_A$ min ago.

Consider next the total number of ribosomes at time t : $U(t)$ [note that $U(t) \neq U_0(t)$]. Then,

$$U(t) = U(t - \tau_{SA_i}) + a_i(t - \tau_{SA_i}) - a_i(t - \tau_{SA_i} - \tau_A), \quad (6)$$

i.e., the total number of ribosomes right now equals the number of ribosomes that were present $t - \tau_{SA_i}$ min ago, plus the newly born ribosomes right now, $a_i(t - \tau_{SA_i})$ minus the ribosomes that were born a lifetime τ_A min ago, and hence are now about to die: $a_i(t - \tau_{SA_i} - \tau_A)$.

Next, we insert an exponential ansatz: $\bar{v}(t) = \bar{v}_0 e^{\mu t}$, in Eq. (5), where $\bar{v}(t) = (U(t), a(t))$, which holds at steady growth conditions (see e.g. [24]), due to the autocatalytic nature of the process. Assuming all timescales are distributed randomly and independently from known distributions, we integrate over all times to obtain a simple growth law:

$$1 = P_{\tau_i}(\mu)(1 + \alpha P_{\tau_{SA_i}}(\mu)(1 - P_A(\mu))), \quad (7)$$

where $P_{\tau_i}(s = \mu)$ is the Laplace transform of the distribution of protein synthesis durations, $P_{\tau_{SA_i}}(s = \mu)$ is the Laplace transform of the (residual) distribution of ribosome assembly duration, and $P_A(s = \mu)$ is the Laplace transform of the distribution of ribosome lifetimes, all evaluated at growth rate $s = \mu$.

The protein translation duration τ_i , the ribosome self-assembly duration τ_{sa} , and the active ribosome lifetime duration τ_A represent distinct and concurrent biological processes, thus taken to be independent in our model.

Next, we insert the same exponential ansatz: $\bar{v}(t) = \bar{v}_0 e^{\mu t}$ in Eq. (6) and obtain a second growth law for the growth rate μ as a function of the Laplace transform of the distribution of residual assembly times of protein a_i and its relative WIP $\phi_i = \frac{a_i}{U}$:

$$1 = P_{\tau_{SA_i}}(\mu)(1 + \phi_i(1 - P_A(\mu))). \quad (8)$$

Suppose that the distribution of residual assembly duration is exponential. In that case, Eq. (8) reduces to the following growth law of ribosome assembly:

$$\mu = \frac{\phi_i}{\tau_{SA_i}} - \Gamma_0, \quad (9)$$

i.e., the growth rate equals the rate of production of new ribosomes minus their depletion rate $\Gamma_0 = \frac{1}{\tau_A}$.

Similarly, if we further assume exponential distributions and use Eq. (7) we obtain

$$\mu = \left(\frac{\alpha_i}{\tau_i} - \Gamma_0 \right) \frac{1}{1 + \phi_i}. \quad (10)$$

So far, we have presented ribosome growth laws derived for a single essential ribosomal protein. This is by no means a constraint, and we can also obtain, after some algebra, growth laws that depend on sets of ribosomal proteins, including all of them in one set:

$$\mu(L + \hat{L}) = v_{aa} \left(\alpha - \frac{\Gamma_0}{v_{aa}} L \right), \quad (11)$$

where L is the total ribosomal protein length in amino acids and $\hat{L}$ is the sum over all the ribosomal protein lengths weighted by their relative part in the WIP (see Appendix C for a more detailed example).

To obtain the theoretical curve depicted in Fig. 1, we first used Eq. (7), namely, $(\frac{\alpha_i}{\tau_i}) = \mu \times (1 + \phi_i) + \Gamma_0$ and used both the measured RNA-to-protein ratio and the translation speed v_{aa} as a function of growth rate.

First, we calculated the allocation parameter α from the RNA-to-protein ratio measurements ($\frac{R}{P}$) and knowledge of the ribosome rRNA content—which is 2/3 of the ribosome's mass [25]. We equate $\alpha = 0.9 \times \frac{1}{2} \frac{R}{P}$, where 0.9 approximately accounts for the rRNA fraction from the total RNA measured [26]; see Appendix A for further details. To obtain

the allocation parameter per ribosomal protein α_i , we assume optimal allocation, i.e., that there is a product feedback inhibition control that makes sure no ribosomal protein is overly synthesized relative to the others [27,28,36]. This assumption leads to the following relation, $\frac{\alpha_i}{\alpha_j} = \frac{L_i}{L_j}$, where L_i is the number of amino acids in ribosomal protein i . Thus, $\alpha_i = \alpha \frac{L_i}{\sum_j L_j}$.

We assume that the translation speed v_{aa} is roughly the same across the mRNA of all ribosomal proteins. Thus, $\tau_i = \frac{L_i}{v_{aa}}$. Using these two relations, we plotted the allocation parameter times v_{aa} as a function of the growth rate μ to obtain both the ribosome active lifetime and the WIP: ϕ_i , which are the slope (minus one) and the y intercept of this graph [see data in inset (A) of Fig. 1, depicted with open circles].

Knowing ϕ_i and Γ_0 , we calculated the translation speed v_{aa} per growth rate using our theory [Eq. (7)] and compared it with the measured values, as shown in Fig. 1.

A. Calculating the probability of depletion

To calculate the depletion probability of a population of self-replicating agents in general, we first calculated the probability of depletion of a single replicator. Let q^* be the probability a single replicator is depleted before replicating, i.e.,

$$q^* = \text{prob}(\tilde{\tau} > \tilde{L} - \tilde{A}), \quad (12)$$

where $\tilde{\tau}$ is the random doubling time, $\tilde{L}$ random lifetime, and $\tilde{A}$ random age, so $\tilde{L} - \tilde{A}$ is the random variable that represents the residual lifetime of the replicating agent. Let q be the probability of depletion of a newborn ($\tilde{A} = 0$) before replicating. Let P^* be the probability of depletion of a population that started with a single replicating agent:

$$P^* = q^* + (1 - q^*)P^2, \\ P^2 = q^2 + 2q(1 - q)P^2 + (1 - q)^2P^4. \quad (13)$$

In the first line of Eq. (13), the first term stands for the probability of dying before doubling. The second term is the probability that the replicating agent completes one round of doubling and that the two populations that branched from the two copies subsequently die.

In the second line of Eq. (13), the first term represents the probability that both replicating agents die before completing their doubling rounds. The second term is the probability that one of the replicating agents will die before completing its doubling round. At the same time, the other will be able to complete its round of doubling but subsequently die alongside its progenitor. The third and last term is the probability that both replicating agents complete their doubling rounds and that all four agents then die. The probability that the replicating agent will die (P) is the same across all generations, yielding a self-consistent equation.

Solving for P in Eq. (13), we find two solutions, the trivial one $P = 1$, i.e., certain depletion, and the nontrivial solution that yields $P = \frac{q}{1 - q}$. Inserting the nontrivial P in the first line of Eq. (13), we obtain the depletion probability as a function of q and q^* :

$$P^* = q^* + (1 - q^*) \frac{q^2}{(1 - q)^2}. \quad (14)$$

The total depletion probability of N agents, each with a stochastic independent active lifetime duration, drawn from the same distribution— P_N^* , is thus given by

$$P_N^* = \Pi_j^N \left(q_j^* + (1 - q_j^*) \frac{q^2}{(1 - q)^2} \right), \quad (15)$$

where q_j^* is the probability of depletion of the j th replicating agent. For the sake of completeness, note that if correlations in the active lifetime duration exist, they can be taken into account by adding a positive renormalization parameter $r > 0$ such that the depletion probability will be given by $P_{[rN]}^*$, where $r > 1$ if the correlations are negative, and $r < 1$ if they are positive, and $[rN]$ is the rounded value or rN .

Using the mean values, measured or estimated, for all the random variables while assuming they are all uncorrelated, facilitate the analytical calculation. This approach is known as the mean field approximation, which originated from physics [29–31] and is now also well known in many other fields of research [32–35].

If we further assume that all the probability distributions are exponential, the resulting equations simplify to the following equations:

$$q = q^* = \frac{1}{1 + \frac{\mu}{\Gamma_0}}, \quad (16)$$

$$P = P^* = \begin{cases} 1, & \mu \leq \Gamma_0, \\ \frac{\Gamma_0}{\mu}, & \text{otherwise,} \end{cases} \quad (17)$$

$$P_N^* = \left(\frac{\Gamma_0}{\mu} \right)^N. \quad (18)$$

To connect the above calculations to the probability of active ribosome depletion in the cell, we recall that only a fraction α of the ribosomes are participating in producing copies of themselves, albeit indirectly, by synthesizing ribosomal proteins [4,6–8].

Thus, the probability of depletion of the active ribosome population is given by $P_N^* = \left(\frac{\Gamma_0}{\mu} \right)^{N\alpha}$. We used this formula to calculate the dependence of the depletion probability of the active ribosome population on the ribosome translation speed v_{aa} and on the fraction of ribosomes in assembly ϕ via the equation we derived from our generalized growth law, Eq. (10), to obtain $\alpha_i = (\mu(\phi_i + 1) + \Gamma_0) \frac{L_i}{v_{aa}}$, which leads to

$$P_N^* = \left(\frac{\Gamma_0}{\mu} \right)^{N\alpha_i} = \left(\frac{\Gamma_0}{\mu} \right)^{N(\mu(\phi_i + 1) + \Gamma_0) \frac{L_i}{v_{aa}}}. \quad (19)$$

This equation was used to plot the solid green line in Fig. 3. See Appendix E for the dependency of the active ribosomes depletion probability on both allocation parameter and translation speed.

We compared this simplified model with an event-driven stochastic Monte Carlo simulation we devised. In short, the simulation consists of a finite population of ribosomes. Each ribosome can randomly translate the i th ribosomal protein mRNA with probability α_i . Each ribosome has a random lifetime derived from a shifted gamma probability distribution—although any other realistic distribution, i.e., zero measure at zero time and finite first and second moments, yields similar results, as we verified. We used an average active lifetime of $\Gamma_0^{-1} = 300$ min. The synthesis of a ribosomal

protein that consists of L_i amino acids was also drawn from a shifted gamma distribution with an average synthesis rate of $\frac{L_i}{v_{aa}}$ min. Each time a complete set of ribosomal proteins is synthesized, a new ribosome assembly process is initiated in the simulation. The assembly process ends after a random duration with an average of $\tau_{SA} = 2$ min, following a shifted gamma distribution. Upon completion of the assembly process, the newly formed ribosome joins the pool of existing ribosomes that synthesize various proteins, including more ribosomal proteins. Hence, if there are enough ribosomes, the ribosome population increases exponentially over time.

To empirically estimate the probability of depletion of the active ribosome population, we begin the simulation with 5400 ribosomes in total. For each point in the figure, i.e., for a given translation speed v_{aa} , we found the required allocation parameter α that yielded the same doubling time of 160 min for all the points in the graph. We let each simulation run until the ribosome population became extinct or grew exponentially and crossed the arbitrary limit of 10 times the initial number of ribosomes allocated to ribosomal protein synthesis. We repeat the simulation 1024 times to obtain an estimate of the depletion probability, which is plotted in Fig. 3.

Despite the simplicity of the theoretical calculation, which does not account for the intricacies of ribosome assembly, it agrees well with the simulation, as seen in Fig. 3.

IV. DISCUSSION

In industrial engineering, adjusting production to meet demand when both fluctuate randomly requires buffers. There are three types of buffers [16]: (1) a time buffer, which allocates extra time to a bottleneck process; (2) an inventory buffer, which retains raw material or partially manufactured goods, or finished goods in excess for usage whenever demand increases beyond the momentary ability to satisfy it via production; and (3) a capacity buffer, which allows machines along the production line to increase their speed assuming they are not operating at their maximum speed.

A cell can be viewed as a complex factory whose product is another factory—an autocatalytic network [4]. As discussed, all self-reproducing cells have a unique autocatalytic network structure known as the von Neumann architecture.

In a given environment, a cell can maximize its growth rate by finding an internal allocation of its machines, particularly of the machine that makes all the machines—the universal constructors—the transcription-translation machinery. The allocation parameter α describes the fraction of universal constructors dedicated to manufacturing more universal constructors. The remaining fraction of universal constructors $1 - \alpha$ is allocated to construct all other machines that are required to maintain the entire operation, e.g., to supply the universal constructors with raw material, energy, instructions, and a protected environment to function within, i.e., a membrane that creates a difference between an internal and an external environment. Note that some of the UCs can be allocated to be inactive, e.g., by modulating the initiation of transcription or of translation (or both). Interestingly, as shown in Ref. [36], the optimal allocation for maximizing growth can be readily obtained via a product feedback mechanism.

In principle, all three types of buffers can be utilized to control the manufacturing rate and adjust it to the external, possibly random, supply. In a cellular context, the inventory buffer probably received more attention. Inventory buffers in metabolism, known as metabolic pools [37], allow the streamlined production of essential metabolites, e.g., amino acids, nucleotides, and oligosaccharides, even when the production rate of individual metabolites fluctuates. As an additional example, RNA polymerase is known to be in excess in *E. coli*, and this excess can be viewed as an inventory buffer, which, as suggested in Ref. [13], is required to mitigate noise in gene expression and facilitate growth. Free ribosomes can also be considered as an inventory buffer.

A capacity buffer occurs when control over the speed of molecular machines is exerted so that they do not operate at their maximum rate under all circumstances, thereby allowing modulation (either an increase or a decrease) of their rate when required. As seen in Fig. 3, using a capacity buffer reduces the probability of depletion without affecting the growth rate; therefore, at slow growth rates, hybrid control of both inventory and capacity is better than using just one of them. Interestingly, the elongation speed of RNA polymerase is controlled by the highly conserved protein *spt4* [38]. In Ref. [39], a measurement of the elongation speed of RNA polymerase demonstrated that it decreases as the growth rate decreases. Transcription slowdown at low growth rates can also fit our theory, as it may reduce the probability of depletion of the RNA polymerase population at slow growth rates and is also consistent with the observed translation reduction—an interesting future direction for further research.

A time buffer can be relevant when the allocation parameter is controlled temporally. For example, a circadian clock can regulate gene transcription, resulting in scheduled protein production [40]. Suppose there is a delay in the production of essential proteins. In that case, the circadian clock can be delayed, e.g., by a feedback regulator, to allow the essential proteins to be synthesized and the shortage gap to close—a time buffer [16]. As presented in Sec. III, the growth law that takes into account both ribosome active lifetime and the finite duration of the ribosome assembly process is given by

$$\mu = \left(\frac{\alpha_i}{\tau_i} - \Gamma_0 \right) \frac{1}{1 + \phi_i}. \quad (20)$$

This equation can be interpreted from the point of view of buffer allocation. Modulating the allocation parameter over time using a predefined schedule and modifying that schedule when critical processes lag behind schedule is analogous to time buffer control [16]. If the allocation is not over time but rather asynchronous, controlling the growth rate can be achieved by controlling the level of inventory of ribosomes in assembly via ϕ_i 's, which is known to be locked to a constant value via a translational feedback loop first discovered by Nomura and co-workers [27,28]. Thus, we conclude that controlling translation speed to regulate growth rate is an example of using a capacity buffer in a cellular contest.

Next, following Ref. [14], we focus on the relation between the inactive fraction of ribosomes and the growth rate. Without control over the inactive fraction, the inactive ribosomes diffuse “in between jobs” from one mRNA to another. Without

specific control, e.g., over the initiation of translation, it is expected that as the allocation of ribosomes to ribosomal protein synthesis decreases, the allocation to the synthesis of other proteins will increase. To break this negative correlation, the inactive fraction must be under control, i.e., to serve as a buffer. In slow growth, as the ribosome allocation parameter α alongside the translation speed v_{aa} decreases, the only way to prevent an unwarranted increase in synthesis of other proteins is to increase the inactive fraction. Although the exact mechanism behind this requires further study, the inactive fraction is experimentally observed to increase as the growth rate slows [14]. Note that if controlling the fraction of inactive ribosomes is possible, e.g., by increasing the hibernation fraction [41], this would enable another experimental test of our model. As our model predicts, a controlled increase in the inactive ribosome fraction will either further slow translation or increase the number of cells unable to sustain growth.

As shown, in harsh environments, there is a limit to how small the allocation parameter α_i can be set since the finite number of ribosomes and their finite active lifetime put a shot noise limit on how low we can safely allocate them without risking their depletion. Indeed, if we want to allocate a small percentage of ribosomes toward ribosomal protein synthesis, such that the average number of ribosomes allocated per essential ribosomal protein is on the order of 1, random fluctuation in the actual number of ribosomes allocated can lead to a drop in the ribosome synthesis rate, below their depletion rate thus risking a cascade to total depletion of the entire active ribosome population.

As discussed in the results section, an interesting alternative for controlling ribosome allocation is to temporally allocate a significant portion of the ribosome population to synthesize ribosomal proteins in a predefined schedule. We postulate that in minute cells, like SAR11 [42,43] or *P. Marinus* [23], the small number of ribosomes, ~ 100 in SAR11, forces the cell to employ this control mechanism—temporal ribosome buffer management, to sustain its growth, since any other method will significantly increase the risk of active ribosome population depletion.

In an autocatalytic network, all cycles share the same growth rate in steady state. In a bacterial cell, this observation [4] suggests that there exist two generic “control knobs” for the growth rate: (1) the allocation parameter α that controls which fraction of the transcription-translation machinery is dedicated to self-replicate itself and (2) the translation speed of ribosomes together with the transcription speed of RNA polymerase [4,44] controlling how fast these central molecular machines drive autocatalysis.

There is no *a priori* reason to believe that all autocatalytic cycles employ the same control strategies. However, at a low growth rate, we claim that all cycles will face the shot noise limit, and to mitigate it, they will employ either the speed control knob or the temporal allocation control knob. For example, to maintain high enough levels of RNA polymerase well above the depletion threshold, using similar arguments, we expect transcription speed to decrease in harsh environments when the growth rate is low. Indeed, in *E. coli*, the transcription speed of RNA polymerase decreases with the growth rate as measured in Ref. [39]; see also Ref. [12].

The shot noise depletion threshold is also observed in other autocatalytic systems. A well-known historical example, albeit dual, comes from the Manhattan Project. When calculating the critical mass of U235, legend tells that Feynman noticed that as a subcritical mass approaches criticality from below, the risk that a random chain reaction will lead to a nuclear explosion increases significantly. Similarly, if the mass is above criticality but close to it, an initiated nuclear reaction can be extinct and die out without achieving conversion of most of the fissile material.

In epidemiology, an epidemic outbreak with a reproductive ratio $R_0 > 1$ can be extinct due to stochasticity, before infecting a substantial percentage of the population [45]. Conversely, an epidemic with $R_0 < 1$ can grow exponentially and infect a large percentage of the population before becoming extinct [45].

The question of the minimal machinery required to sustain life, or the physiological determinants of spore viability, is also related. We hypothesize that the stochastic nature of spore germination stems from the limited number of ribosomes and RNA polymerase in the spore. Misallocation of ribosomes, for example, in the early stages of germination, can cause premature depletion rather than growth.

Interestingly, when the challenge is rebooting life, i.e., re-initiating growth after a period of stasis rather than sustaining slow growth, the order in which genes are transcribed and translated can significantly affect cell fate. In this regime, the small number of essential molecular machines, such as ribosomes, RNA polymerases, metabolic proteins, and transporters, increases sensitivity to random fluctuations.

For example, suppose that the cell initiates the synthesis of ribosomal proteins before expressing enough transporters and metabolic proteins. In that case, the cell can get trapped in a starved state, lacking the essential nutrients and ATP required to continue. However, a cell that first expresses metabolic transporters and metabolic proteins and then ribosomal proteins can escape this fate after a lag phase. In Ref. [46], it was shown that the expression of metabolic genes, which are part of a metabolic pathway, is matched to their order in the pathway. Based on our model, we predict that the reversed order will not be essential for cell survival under fast growth conditions but may be critical when the growth rate is low and the number of ribosomes is small. We thus predict that reversing the order of expression can cause significant lag and lead to increased growth arrest and even cell death, compared to the wild type in harsh environments but not in nutrient-rich environments.

In Ref. [47], a fixed number of ribosomes was measured across a wide range of growth rates. Based on our model, we predict that the translation speed of this single-cell organism will change linearly with the growth rate, in accordance with Eq. (10). Based on our view regarding the evolutionary reason for choosing such a work point, we predict that this *Archea* has a small number of ribosomes, at least an order of magnitude less than *E. coli*. We hypothesize that this work point has been chosen to maintain a safe distance from the ribosome depletion threshold while allowing growth rate control via the translation rate.

ACKNOWLEDGMENTS

This research was supported by a grant from the United States-Israel Binational Science Foundation (BSF, Grant No. 2022763), Jerusalem, Israel, and the United States National Science Foundation (NSF), by the Israel Science Foundation (Grant No. 776/19), and also by a grant from the United States-Israel Binational Science Foundation, Jerusalem, Israel (Grant No. 2024038). We acknowledge Oren Raz and Fangwei Si for their useful comments on the manuscript. R.P. would like to thank Avital Siman Tov Erel, who catalyzed the final committing steps of this work, for her analytic skills and firm holding.

DATA AVAILABILITY

The data that support this study's findings are available on Zenodo [21].

APPENDIX A: THE RELATION BETWEEN RNA-TO-PROTEIN RATIO AND THE RIBOSOME ALLOCATION PARAMETER

The RNA-to-protein ratio, $\frac{M_R}{M_P}$, is the ratio between the total RNA mass in the cell to the total protein mass in the cell. The ribosomal RNA is 90% of the total RNA mass in the cell [26] and about two-thirds of the mass of the ribosome [25]. Assuming the translation rate of all the proteins, ribosomal and nonribosomal, is the same, the allocation parameter is the ratio between the mass of the ribosomal proteins, P_R , to the mass of all the proteins in the cell, M_P . The mass of the ribosomal protein, P_R , can be calculated from the RNA mass M_R by

$$P_R = 0.9 \times M_R \frac{1 - \frac{2}{3}}{\frac{2}{3}} = 0.9 \times \frac{M_R}{2}, \quad (\text{A1})$$

the allocation parameter α is

$$\alpha = \frac{P_R}{M_P} = \frac{0.9 \times \frac{M_R}{2}}{M_P} = \frac{0.9}{2} \times \frac{M_R}{M_P}. \quad (\text{A2})$$

APPENDIX B: NUMBER OF RIBOSOMES IN THE CELL AS A FUNCTION OF THE GROWTH RATE

Using the data from Ref. [12], we find the best quadratic fit to the number of ribosomes in the cell as a function of growth rate, in min^{-1} :

$$N_r = a \times \mu^2 + b \times \mu + c. \quad (\text{B1})$$

The coefficients we got are $a = 96\,282\,277$, $b = -504\,604$, and $c = 5981$. R^2 of the fit was 0.9997. Using these coefficients, we found that for a doubling time of ~ 176 min, the number of ribosomes is 5400.

APPENDIX C: USING SETS OF RIBOSOMAL PROTEINS TO FIND MODEL PARAMETERS FROM DATA

The ribosome growth law that we present,

$$\mu = \left(\frac{\alpha_i}{\tau_i} - \Gamma_0 \right) \frac{1}{1 + \phi_i}, \quad (\text{C1})$$

is written for one critical ribosomal protein. One can write a similar equation to a group of ribosomal proteins, i.e., all the

proteins at one operon or the proteins that combine the large subunit of the ribosome. Let G be the subgroup of proteins of the ribosomal proteins. From Eq. (C1), one gets

$$\mu(1 + \phi_i)L_i = (\alpha_i v_{aa} - \Gamma_0 L_i). \quad (C2)$$

Here, L_i is the length of protein i in amino acid and $\tau_i = \frac{L_i}{v_{aa}}$. Summing both side of Eq. (C2) over the group G , we find

$$\mu \left(\sum_{i \in G} L_i + \sum_{i \in G} \phi_i L_i \right) = \left(v_{aa} \sum_{i \in G} \alpha_i - \Gamma_0 \sum_{i \in G} L_i \right). \quad (C3)$$

Defining $L_G = \sum_{i \in G} L_i$ the total number of amino acids of the proteins in the group, $\alpha_G = \sum_{i \in G} \alpha_i$ the total allocation parameter, and $\hat{L}_G = \sum_{i \in G} \phi_i L_i$ the actual part of amino acid belonging to the proteins in the group that are not part of completed ribosome, one gets

$$\mu = (v_{aa} \alpha_G - \Gamma_0 L_G) \frac{1}{(L_G + \hat{L}_G)}. \quad (C4)$$

In the inset of Fig. 1 in the main paper for the *C. glutamicum*, we use Eq. (C4) with G equal to all the ribosomal proteins to predict the linearity of $v_{aa} \alpha_G$ as a function of μ .

APPENDIX D: EXTENDED ANALYSIS OF THE EFFECT OF BACTERIOSTATIC ANTIBIOTICS TARGETING THE RIBOSOME ON ACTIVE RIBOSOME LIFETIME IN DIFFERENT ENVIRONMENTS

To obtain the fits presented in Fig. 2, we used a least squares fit of the growth rate as a function of the RNA-to-protein ratio Φ , and an ansatz that the ribosome active lifetime is modulated by a Micahelis-Menten term:

$$\mu = \frac{\Phi}{2\tau_U} - \Gamma_0 \left(1 + \frac{Ac}{k+c} \right), \quad (D1)$$

where $\Gamma^{-1}(c) = \Gamma_0^{-1} (1 + \frac{k+c}{Ac})$ is the mean active lifetime of the ribosome as a function of c —the concentration of antibiotics. τ_U , A , and k are fitting parameters. Because we can estimate τ_U to be between 6 and 12 min—the translation duration of all the ribosomal proteins by a single ribosome, we can use it as a check that the fit did not diverge to unrealistic values. The minimum inhibitory concentration of chloramphenicol is from 16 up to 256 μM [18], and the MIC for erythromycin is from 50 to 400 μM [19]. All the values of k obtained by the fits were within these ranges.

The mechanism of action of chloramphenicol and erythromycin is to inhibit ribosome translation. Chloramphenicol inhibits the transfer of amino acids to the growing peptide chain within the ribosome. Erythromycin disrupts tRNA translocation from the A site to the P site within the ribosome. Because the A site stays occupied, it prevents new charged tRNA from entering the ribosome, rendering it inactive.

Because ribosomes that are affected by these antibiotics will halt translation and become inactive, we model the effect of these antibiotics by reducing the active lifetime of ribosomes by an MM factor, i.e., the active lifetime decreases as the concentration of antibiotics increases, until saturation, where cellular growth halts. Note that because the cellular response to the presence of antibiotics was measured in the experiments [17], we did not model the control law that governs how cellular allocation of ribosomes is changing as a function of antibiotics concentration. Instead, we used the measured response Φ by equating $\alpha \approx \frac{\Phi}{2}$ (see Appendix A for further details).

We found that the MM coefficient k is approximately identical in different environments and only depends on the antibiotic type (see the table below). In particular, we found for erythromycin $k_{\text{Er.}} = 460 \pm 40$ (1σ) μM and for chloramphenicol $k_{\text{Ch.}} = 48 \pm 5$ (1σ) μM . Both are consistent with recent measurements of MIC [18,19].

Environment	Drug	τ_U (min)	k (μM)	A	R^2
TSB	Chloramphenicol	7.6	50	74	0.79
MOPS glucose synthetic rich	Chloramphenicol	8.8	51.1	120.7	0.75
MOPS glucose + 12 a. a.	Chloramphenicol	7	50	135.6	0.37
MOPS glucose + 6 a. a.	Chloramphenicol	8.2	50.2	81.3	0.52
MOPS glucose	Chloramphenicol	7.5	50	131.1	0.73
MOPS glucose + casamino acids	Chloramphenicol	8.5	50	53.2	0.46
MOPS glycerol	Chloramphenicol	8.3	50	103.3	0.86
MOPS sorbitol	Chloramphenicol	11	48.4	68.3	0.46
MOPS manose	Chloramphenicol	11.5	35	81.7	0.67
MOPS glucose + 6 a. a.	Erythromycin	9.2	493.5	29.2	0.89
MOPS glycerol	Erythromycin	8.9	434.6	24.6	0.95

In the table above, we present the parameters of our least mean square fits and their respective R^2 , employing Eq. (D1). The names of the environment, as well as the data, are taken from Ref. [17]. In 7 of 11 environments, the R^2 exceeds 0.66, indicating that our model reasonably fits the response to antibiotics. The lower R^2 values in environments such as MOPS glucose supplemented with amino acids may suggest that,

in these complex settings, an isogenic population can split into subpopulations with different active metabolic pathways, each responding differently [i.e., with different A ; see Eq. (D1)] to varying concentrations of chloramphenicol. Further research and experiments are needed to determine whether our model can be adapted to explain these observations or if it requires modifications. In one environment, MOPS glycerol,

we did not include the first dataset, which seemed to be an outlier.

In Fig. 2, we present the results of our model fit to the data taken from Ref. [17]. We used the same names used in Ref. [17] to define the nutritional environments. Figure 2 inset (A) from top to bottom: chloramphenicol: TBS, MOPS glucose, MOPS glycerol, MOPS sorbitol, MOPS mannose (bottom). Figure 2 inset (B) from top to bottom: chloramphenicol: MOPS glucose synthetic rich, MOPS glucose + casamino acids, erythromycin: MOPS glucose + 6 amino acids, MOPS glycerol (bottom). Figure 2 inset (C) from top to bottom: chloramphenicol: MOPS glucose + 12 amino acids, MOPS glucose + 6 amino acids (bottom) [17]. Data in inset (C) was separated for clarity, to prevent overlaps between the graphs.

APPENDIX E: ACTIVE RIBOSOME DEPLETION PROBABILITY AS FUNCTION OF MODEL PARAMETERS

In Fig. 4 we show the dependency of the depletion probability as a function of both translation speed v_{aa} and allocation parameter α for different growth rates. We concentrate on the vicinity of growth rate close but higher than the inactivation rate of the ribosomes, where the inactivation probability is higher.

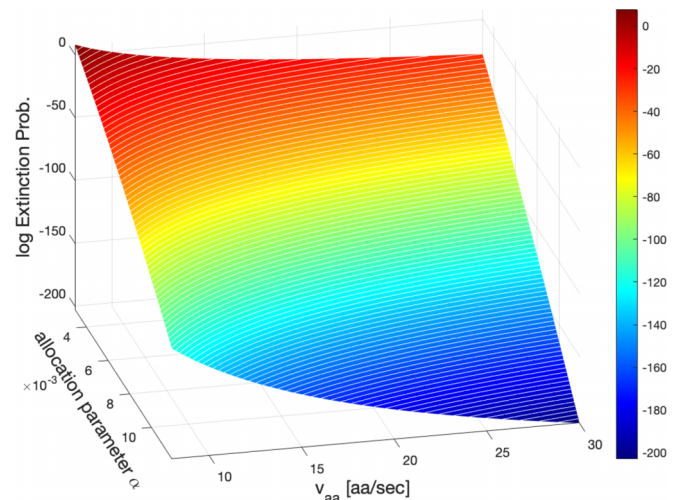


FIG. 4. Active ribosome depletion probability as a function of both the allocation parameter α and the translation speed v_{aa} . All the rest of the parameters we used to plot this graph are as in Fig. 3, i.e., $\Gamma_0 = \frac{1}{300}$, $\phi_i = 0.1$, and $L_i = 250$. This graph should be viewed in cross section of constant growth rate, as we did in the paper.

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