Transcription-translation machinery — an autocatalytic network coupling all cellular cycles and generating a plethora of growth laws.

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Recently discovered simple quantitative relations known as bacterial growth laws hint on the existence of simple underlying principles at the heart of bacterial growth. In this paper, we provide a unifying picture on how these known relations, as well as new relations which we derive, stem from a universal autocatalytic network common to all bacteria, facilitating balanced exponential growth of individual cells. We show that the core of the cellular autocatalytic network is the transcription-translation machinery, which by itself is an autocatalytic network composed of several coupled autocatalytic cycles like ribosome, RNA-polymerase and tRNA charging cycles. We derive two kinds of growth laws per autocatalytic cycle, relating growth rate to the relative fraction of the catalyst and its catalysis rate and relating growth rate to all the time-scales in the cycle. The autocatalytic network structure defines multiple growth regimes, determined by the limiting components. The number of growth regimes can be very large, while the number of growth laws obtained can be much smaller. We derive a growth law that accounts for RNA-polymerase auto-catalytic cycle, which we use to explain the dependence of growth rate on an inducible expression of rpoB and rpoC genes that code for RpoB and C protein subunits of RNA-polymerase, and to explain how the concentration of rifampicin — a drug that targets RNA-polymerase — affects growth rate without changing the RNA to protein ratio. We further derive growth laws for RNA synthesis and charging and predict how perturbations to ribosome assembly or membrane synthesis affect the growth rate.

Bacterial growth laws | transcription translation machinery | coupled auto-catalytic cycles | bacterial physiology | bacterial metabolism

The transcription-translation machinery is a universal set of molecular machines at the core of all known self-reproducing single-cell organisms. It can be considered as an embodiment of von-Neuman’s concept of a universal constructor — a machine capable of making other machines, self included, by reading an instruction set and consuming raw materials (2, 23).

The transcription-translation machinery is composed of two key molecular machines, RNA-polymerase and the ribosome. According to the central dogma, all cellular proteins are synthesized by this core machinery in a two-step process. First, RNA-polymerase transcribes a gene to form the mRNA instructions. The mRNA instructions are translated by ribosomes which synthesize the coded protein.

To qualify as a universal constructor, the transcription-translation machinery should also be capable of replicating itself. While the details of this process are complex, they are also universal. In all single-cell organisms capable of self-replication the transcription-translation machinery self-replicates in the same manner via two prominent coupled autocatalytic cycles — the RNA-polymerase auto-catalysis cycle, and the ribosome auto-catalysis cycle. Both cycles are coupled because de-novo synthesis of new ribosomes cannot occur without RNA-polymerase transcribing rRNA. Similarly, de novo synthesis of RNA-polymerase cannot occur without ribosomes translating mRNAs of rpo-genes coding for the RNA-polymerase protein subunits from which RNA-polymerase is composed. Both cycles also involve a self-assembly step.

Both these cycles rely on a third auto-catalytic cycle responsible for charging tRNA with amino-acids (aa) and in assisting the ribosomes to initiate, translocate and successfully end the translation process. The autocatalytic nature of the third cycle is less familiar and becomes more evident when we consider each of its elements e.g. tRNA as catalyzing itself with the help of RNA-polymerase and ribosomes as we explain below.

All three aforementioned autocatalytic cycles are interwound and require each other to perform auto-catalysis. Removing any key-catalyst from any one of these cycles breaks autocatalysis in all three cycles.

Recently, the “ribo-centric” view which focuses on the ribosome auto-catalytic cycle — ‘ribosomes makes ribosomes’, have lead to the discovery of a bacterial growth law that quantitatively relate bacterial growth rate to the ribosomal protein fraction and the ribosome translation rate (8, 14). A recent study have focused on the relation between growth rate, translation rate and the transcription of rRNA (16).

Despite its successes the “ribo-centric” approach also have shortcomings, as it disregards an important pillar in the central dogma — transcription, as well as other auto-catalytic cycles in the cell. Some of the cycles can significantly affect growth rate, and in some cases without changing the RNA to protein ratio. In order to explain this apparent deviation from the simple growth law presented in (8) a more general approach is required.

Here we take a more general approach by considering both transcription and translation on equal footing and derive growth laws that are based on the autocatalysis of the transcription-translation machinery. We further show that transcription and translation also couples all other auto-catalytic cycles in the bacterial cell, leading to balanced exponential growth of all components with the same growth rate without requiring complex feedback mechanisms.

We demonstrate that each auto-catalytic cycle leads to two types of growth laws. The first which we call relative...
abundance growth law, connect the growth rate to the relative abundances of the catalysts that drives the cycle and their respective rate. The other type of growth law which we call closed cycle growth laws relates the growth rate to all time scales and allocation parameters within a given auto-catalytic cycle. Using this formalism we derive and discuss new growth laws, as well as re-deriving existing growth laws, demonstrating that merit of a holistic picture of cellular growth.

Our modeling approach offers a simple way to recognize ‘growth regimes’ characterized by a list of catalysts and substrates that are limiting for growth. Despite the elegant simplicity and experimental success of bacterial growth laws (4, 8, 14), the combinatorial explosion in the number of growth regimes as the level of details in the model increases to better model real cells reveals the limits of simple growth-laws in elucidating the evolutionary shaped controls that allows the cell to navigate to a specific growth regime depending on the external environment and its internal state. Finding general principles — evolutionary ‘design logic’ remains an interesting open challenge (4).

Results

The transcription-translation machinery self-replicates using three coupled auto-catalytic cycles. In all single-cell organisms capable of self-replication, the transcription-translation machinery self-replicate in the same manner, using three coupled auto-catalytic cycles, one for RNA-polymerases, one for ribosomes, and one for tRNA-charging and other translation facilitating functions. These coupled auto-catalytic cycle are depicted in figure 2.

The ribosome auto-catalytic cycle To de-novo synthesize new ribosomes, existing ribosomes are required to create more than 50 types of ribosomal proteins. The mRNAs for the ribosomal proteins are transcribed by RNA-polymerases. A sub-group of the ribosomal proteins directly bind to rRNA which is also transcribed by RNA-polymerase, other groups subsequently bind to the sub-assembled ribosome, in a pre-defined partial order which is summarized by the well-known small and large ribosome subunits assembly maps (15, 17). The ribosomal protein auto-catalytic cycle is derived by assuming rRNA is abundant, and focusing on the fraction of ribosomes that are allocated to synthesize ribosomal proteins. The ribosomal proteins spend some time free-floating and eventually enter the ribosome assembly line, spend some time in the assembly process and exit embedded in the ribosome’s small or large subunits. The newly synthesized small and large subunits of the ribosomes spend some time free-floating before binding to mRNA and becoming engaged in translation.

We derive both abundance and closed-cycle growth laws by writing a set of coupled ODEs for the rate of change of abundance of ribosomal proteins, the rate of conversion to actively assembling state, the rate of assembly of new ribosomes, and rate of interconversion between resting and active states. The resulting closed cycle growth law is

\[
\left(\mu + \frac{1}{\tau_{f}\alpha_{f}}\right) \left(1 + \mu \tau_{p}\alpha_{p}\beta_{p}^{\alpha_{p}} + 1\right) \left(1 + \frac{1}{\tau_{p}\alpha_{p}\beta_{p}^{\alpha_{p}} + 1}\right) = \frac{R}{\mu R_{p}}
\]

where \(\mu\) is the growth rate, \(\alpha_{f}\) is the ribosome assembly time, \(\tau_{p}\) is the ribosomal protein time in its assembly
precursor pool, \( R \) is the total number of ribosomes, \( R_0 \) is the total number of active ribosomes, \( \tau_{\text{ife}(R)} \) is the ribosome lifetime, \( \alpha_{\text{R}} \) is the fraction of ribosomes that are allocated to synthesize ribosomal protein \( R_P \) and \( \tau_{\text{R}} = L_{\text{R}} P_{\text{R}} \tau_{\text{aa}} \) is the translation duration of ribosome \( R_P \), whose lengths is \( L_{\text{R}} P_{\text{R}} \) amino-acids, and \( \tau_{\text{aa}} \) is the elongation rate.

By further assuming that both the ribosome assembly time and the duration spent by ribosomal proteins in their precursor pools as free ribosomal proteins are negligible compared to the doubling time, i.e. \( \mu \ll 1/\tau_{\text{SA}(R)} \) and \( \mu \ll 1/\tau_{\text{pool}(R_P)} \), we obtain

\[
\mu \tau_{\text{R}} + \frac{\tau_{\text{R}}}{\tau_{\text{ife}(R)}} = \alpha_{\text{R}} R_0 \phi_b,
\]

where we define the fraction of active ribosomes as \( \phi_b = \frac{R_b}{R_0} \).

The term \( \alpha_{\text{R}} R_0 \phi_b \) in the equation above is the fraction of active ribosomes that are allocated to translate ribosomal proteins. Thus, the second term on the left hand side, stands for the allocation of active ribosomes towards translation of ribosomal proteins at zero growth \( \mu = 0 \).

Equation 2 is thus equivalent to the well known bacterial growth law, \( \dot{\xi} = \phi + \phi_0 = \theta \varphi \) presented and experimentally tested in \((8, 14)\) under the conditions \( \mu \tau_{\text{SA}(R)} \ll 1 \) and \( \mu \tau_{\text{pool}(R_P)} \ll 1 \). In this approximation, the ribosomal protein mass fraction is equivalent to the allocation parameter (SI). Interestingly, the validity of these assumptions and the agreement between ribosome allocation and ribosome mass fraction can be tested experimentally since ribosome allocation can be directly measured in ribosome profiling experiments and the ribosomal protein mass fraction can be measured using RNA-seq combined with mass-spectrometry \((14)\). In 20 minutes doubling time, measuring the ribosomal mass fraction yields \( 30\% \) \((8)\) while ribosome profiling measures \( 28.5\% \) of ribosomes are engaged in translating ribosomal proteins \((18)\) — a close results considering the noises in both measurements.

Nevertheless, deviations from this correspondence are predicted by our model when one or more of the aforementioned assumptions breaks down, e.g. when the ribosome assembly time increases, as in \((22)\).

Since ribosomes are synthesized by self-assembly of rRNA and ribosomal proteins, we can also write a ribosome growth law that is based on the production of rRNAs by RNA-polymerases. The resulting abundance and closed cycle growth laws are obtained by writing a set of coupled ODEs, for the rate of change of abundance of RNA-polymerase subunits, the rate of conversion to actively transcribing state, the rate of transcription of new ribosomal RNA by RNA-polymerases and rate of production of new ribosomes by these ribosomal RNAs after assembly (SI). The closed cycle growth law we obtain is

\[
\mu R + \frac{R_0}{\tau_{\text{ife}(R)}} = \frac{R_0 \alpha_{\text{R}}}{\tau_{\text{RNA}} \tau_{\text{R}} \alpha_{\text{R}}} R_0 \phi_b
\]

where \( \tau_{\text{RNA}} \) is the transcription time of the \( j \)th ribosomal RNA, \( \tau_{\text{R}} \) is the translation time of \( j \)th RNA-polymerase subunit, \( \tau_{\text{SA}(R)} \) is the assembly time of RNA-polymerase, and \( \tau_{\text{ife}(R)} \) is its lifetime. \( \alpha_{\text{R}} \) is the fraction of active ribosomes translating \( R_{\text{po}} \) protein subunit of RNA-polymerase.

\[
\alpha_{\text{trans}(\text{RNA})} \text{ is the fraction of active RNA-polymerases transcribing RNA,} \quad \alpha_{\text{R}} \text{ and } \text{Rpo}_j \text{ is the number of RNA-polymerases that are actively transcribing.}
\]

If we assume further that \( \mu \ll 1/\tau_{\text{SA}(R)} \) and \( \mu \ll 1/\tau_{\text{pool}(R_{\text{po}})} \) and that the lifetime of ribosomes and RNA-polymerases are long compared to the doubling time, we obtain

\[
\mu^2 = \frac{\alpha_{\text{RNA}} \alpha_{\text{R}} R_0 R_0 \text{Rpol}_0}{\tau_{\text{RNA}} \tau_{\text{R}} \text{Rpol}_0 R_{\text{pol}}}
\]

which is equivalent to the ribosome RNA auto-catalysis growth law presented in \((16)\).

### The RNA-polymerase auto-catalytic cycle

Fig. 3. RNA-polymerase growth law. Inset (A) Illustration of the RNA-polymerase auto-catalytic cycle. Among the active RNA-polymerases, a fraction \( \alpha_{\text{RNA}} \text{Rpo}_j \) is allocated to transcribe rpoA-D and rpoZ genes (rpoD not shown). The transcribed mRNAs produce rpoA-D and rpoZ protein subunits during their life time. The rate of protein synthesis by mRNA is equal to \( \mu_{\text{R}} + \frac{R_0}{\tau_{\text{ife}(R)}} \) rRNA upon depletion of the RpoB pool, the synthesis rate of RpoB decreases. We find that when the concentration of rifampicin \( c \approx 17 \mu \text{g/mL} \) growth rate is reduced by half compared to the nominal \( c = 0 \) case. The measured RNA to protein ratio stays constant, indicating that ribosomes are not limiting growth rate in this experiment. This is because RNA transcription becomes limiting and this equally attenuates both ribogenesis and protein synthesis due to a global shortage in all forms of mRNA, as explained in the main text.

In bacteria, RNA-polymerase is made from four core protein subunits — rpoA (\( \alpha \)), rpoB (\( \beta \)), rpoC (\( \beta' \)) and rpoZ (\( \omega \)) and an interchangeable \( \sigma \)-factor. Consider the RNA-polymerase...
makes RNA-polymerase’ autocatalytic cycle, which operates as follows. A fraction $\alpha_{rpo}$ of the active RNA-polymerases are allocated to transcribe mRNAs from the rpo genes. A fraction $\alpha_{Rpo}$ of the active ribosomes are allocated to translate these mRNAs and synthesize the RNA-polymerase protein subunits, as long as the mRNA exists — since it has a finite lifetime of $\sim 3$ minutes (13). The protein subunits subsequently self-assemble to form new RNA-polymerases.

The closed-cycle RNA-polymerase growth law is obtained by writing a set of coupled ODE equations for the rate of change of the mRNAs transcribed from the rpo genes while accounting for their finite lifetime, the rate of change of Rpo protein subunits, the rate of production of new RNA-polymerase from the Rpo subunits after assembly, and the fraction of active RNA-polymerase that are (re-)allocted to transcribe rpo genes thus sustaining the exponential growth of RNA-polymerase in the cell. This leads to the following closed cycle growth law,

$$
\mu \tau_{poo} (\mu \tau_{SA}(Rpol) + 1)(\mu \tau_{Sf}(m(Rpoo)) + 1) = \alpha_{rpo} \phi_b R_m \tau_{Sf(m(Rpoo))} \tau_{Rpo}
$$

where $\tau_{Sf(m(Rpoo))}$ is the mRNA’s lifetime, $\alpha_{rpo}$ is the fraction of active RNA-polymerases transcribing the mRNAs of the RNA-polymerase subunit Rpo, $R_m$ is the average number of ribosomes translating this mRNA, and $\phi_b = \frac{R_{pol}}{\tau_{pol}}$ is the fraction of busy RNA-polymerases.

To demonstrate the merit of our RNA-polymerase growth law, we used data from two recent experiments (7, 25). In the first experiment, the researchers developed a reversible growth switch in E. coli by removing rpoBC genes from its genome and placing them on a plasmid with an inducible promoter and a fluorescent reporter (7). By controlling the external concentration of the inducer — IPTG, the expression of rpoBC genes were controlled. When the IPTG levels were high, nominal growth rates were observed. However, when the IPTG concentration dropped, a rapid decrease in growth rate was observed.

To explain the steep drop in growth rate we use our RNA-polymerase growth law, Eq. 5, with RpoB as the limiting factor in the assembly process. As long as the levels of RpoB are sufficient at steady growth conditions are non-zero, growth rate is not affected. As soon as the free RpoB pool vanish due to the induced reduction in its expression, the assembly time of new RNA-polymerase starts to increase. To model this we assume that the increase in the assembly time equals to the delay in the delivery of RpoB.

The measured fluorescence of the reporter protein is not proportional to the size of the free Rpo pool, since the RpoB proteins are consumed by the assembly reaction at a faster rate compared to the decay rate of the fluorescence. We thus expect the fluorescence level to be monotonically increasing with the expression level. The equation for the assembly time we use is $f_{SA}(Rpol) = \tau_{SA}(Rpol) \frac{\phi_b}{\tau_{pol}}$, with $\tau_{SA}(Rpol)$ being the assembly in the nominal (high IPTG or wild-type) case.

For the three environments tested in the experiment, we fitted the RNA-polymerase allocation parameter to yield the nominal growth rate without limiting the expression of the rpoB and rpoC genes. All other parameters were taken from (19, 24) see SI for more details. We then fitted $K$ and $H$ values to the growth rate as a function of the fluorescence in the M9+glucose media. We found that the values of $K$ and $H$ remained valid for the other two environments, without further fitting, which supports the hypothesis that our model is consistent.

In the second experiment (25), rifampicin — a drug that targets DNA-bound RNA-polymerases just beginning to transcribe RNA, was administered in sub-lethal dosages to E. coli. The effect on growth rate as a function of the concentration as well as the RNA to protein ration were both measured. The growth rate was found to reduce as the concentration of rifampicin was increased. The RNA to protein ratio however, was found to remain constant, in marked difference to the ribosome growth law.

We explain this by using our RNA-polymerase growth law (Eq. 5). Our model naturally explains these observations by assuming that rifampicin makes the RNA-polymerase autocatalytic cycle the limiting cycle, by reducing the number of active RNA-polymerases. As the concentration of rifampicin goes up, the fraction of active RNA-polymerases decreases, and hence growth rate of RNA-polymerases decrease. Moreover, the decrease in the number of active RNA-polymerases causes a global decrease in RNA transcription. The reduction in available rRNA and mRNA equals affects ribogenesis and protein synthesis. This explains why RNA to protein ratio remains constant. We also derived this result by rewriting the ribosome growth law under the assumption that mRNA is limiting (SI).

Most RNA transcription in a growing cell is of rRNA. It is thus natural to ask if rifampicin actually reduce growth by preventing rRNA transcription and thus ribogenesis. Using our model we can calculate the growth rate and RNA to protein ratio for various growth regimes. We consider three relevant regimes; (i) rRNA is limiting but mRNA is not; (ii) both RNA and mRNA are limiting; (iii) rRNA is not limiting but mRNA is.

First, rRNA can limit ribosome production, because RNA-polymerases are limited, but mRNA is not limiting translation. The reduction in rRNA synthesis will be accompanied by a reduction in ribosomal protein translation. Partially, this is due to a remarkable mechanism discovered by Nomura (5), who found that ribosomal proteins that are primary rRNA binders that fail to find their target rRNA sequence subsequently bind to a similar region on their own mRNA, thus stopping their own translation as well as all downstream ribosomal proteins that reside on the same operon.

This translational feedback as well as other mechanisms keeps the levels of ribosomal protein precursor pools in sync with rRNA transcription, which, in turn is governed by modulating RNA-polymerase transcription via the stringent response (33). Thus, a limitation on rRNA transcription without an accompanied limitation in mRNA is expected to cause a reduction in ribosomal protein translation and hence increase the number of ribosome that will be allocated to make other proteins. We conclude that the regime of rRNA limitation without mRNA limitation is expected to reduce the RNA to protein ratio.

The second regime to consider is that both rRNA and mRNA are limiting both ribogenesis and translation simultaneously. This is consistent with a constant RNA to protein ratio, as the drop is common to all sectors, and the ribosomes that are freed from making ribosomal proteins cannot instead...
make other proteins, since mRNA is in shortage. However, a global shortage in mRNA also means that the RNA-polymerase autocatalytic cycle is limiting, since the shortage in mRNA is eventually the result of a shortage in RNA-polymerase. We thus conclude that if RNA-polymerase is limiting, growth rate will be determined by it, irrespective whether or not rRNA is also limiting.

The third regime to consider is that rRNA is not limiting and mRNA is limiting. This regime is also consistent with a constant RNA to protein ratio, since the global shortage in mRNA implies a reduction in protein synthesis that includes ribosomal proteins.

An alternative explanation for the reduction in growth rate as a function of rifampicin concentration one might want to consider is that shortage in mRNA makes a particular metabolic autocatalytic cycle limiting. However, if that was the case, we would expect the stringent response to respond and reduce the production of rRNA which in turn would reduce the translation of ribosomal proteins as discussed. Thus, if a metabolic autocatalytic cycle was limiting, we would expect the RNA to protein ratio to decrease.

**tRNA-Synthetase and tRNA autocatalytic cycles**

In order to translate, ribosome relies on a series of essential protein that facilitate their binding to mRNA, tRNA charging and transfer into the ribosome A-site, translocating the ribosome along the mRNA, and release of ribosomes from mRNA upon completing the translation process.

In any auto-catalytic cycle, any of the catalysts that are part of the cycle can be seen as the pivot catalysts around which the autocatalytic cycle is constructed. To exemplify this point we present here two growth laws — aminoacyl-tRNA-synthetase growth law and tRNA growth law (Fig. 4).

Consider first the aminoacyl-tRNA-synthetase (aaS) for a particular amino acid i. The aaSₐ catalyzes the loading of an amino-acid of type i onto its corresponding tRNA. The loaded tRNA binds to EF-Tu — the most abundant protein in E. coli, and proceed to enter a ribosome and deposit the amino-acid to the elongating peptide-chain which subsequently fold to form a new protein. A fraction of the proteins formed will be aaSᵢ proteins, thus closing the cycle.

Similarly, consider tRNA synthesis by RNA-polymerase. After maturation, the tRNAs transcribed by RNA-polymerases will be charged with amino-acids and transferred to ribosome’s A-site. A fraction of the tRNAs will contribute their amino-acids to Rpo proteins. The Rpo proteins self-assemble to form new RNA-polymerase, some of which are allocated to make tRNAs thus closing the cycle.

More generally, using this method allows us to connect almost any two processes in the cell e.g., transcription, translation, tRNA charging, metabolic rate, DNA synthesis rate, membrane synthesis and assembly times of protein complexes.

In the current context of tRNA charging, we find a connection between the number of tRNAs and the number of RNA-polymerases required to sustain growth at a given rate µ given the allocation of RNA-polymerase towards transcription of tRNA and rpo-genes.

We find that the growth rate µ is equal to the fraction of active RNA-polymerases transcribing mRNAs divided by the transcription time multiplied by the tRNA to active RNA-polymerase ratio, 

$$\mu = \frac{rRNA_{\text{transcribed}}}{tRNA_{\text{charged}}} \times \frac{tRNA_{\text{active}}}{Rpo_{\text{active}}}$$

Traditionally, the ratio of tRNA to ribosomes was studied since tRNA are serving ribosomes with amino-acids. Also, RNA-polymerase to ribosomes ratio was studied since RNA-polymerases writes mRNA instructions for ribosomes to translate. However, evidently, tRNA is also serving RNA-polymerases since RNA-polymerase is made of proteins, and tRNA had to deliver the amino-acids to the ribosomes that synthesized these proteins.

The aminoacyl-tRNA-synthetase growth law we derived by

$$\mu = \frac{\alpha_{aaS_j}}{L_j f_{usage}(i) \tau_{charging}(i)}$$

where as before µ is the growth rate, $\alpha_{aaS_j}$ is the fraction of active ribosomes translating aaSᵢ proteins, L is the number of amino-acids in aaSᵢ. fusage(i) is the fraction of amino-acids of type i used in the entire proteome and $\tau_{charging}(i)$ is aaSᵢ’s charging rate.

The tRNA growth law we derived is given by

$$\mu^2 = \frac{\alpha_{tRNA_i} \alpha_{Rpo_j} L_j f_{usage}(i) \tau_{transfer}(i)}{\phi_b}$$

where $\alpha_{tRNA_i}$ is the fraction of active RNA-polymerases transcribing tRNAᵢ, $\alpha_{Rpo_j}$ is the fraction of active ribosomes translating the jth Rpo protein, $\tau_{transfer}(i)$ is the charging cycle time of tRNAᵢ, and $\phi_b$ is the fraction of active RNA-polymerases.

We also found that the ratio between the tRNA and its aa-tRNA-synt. is inversely proportional to the rate of tRNA amino-acid transfer cycle to the rate of aa-tRNA-synt charging. Thus, the faster the charging of a particular aa-tRNA-synt., the less tRNAs per aa-tRNA-synt. are required.

In order to verify our tRNA growth laws, accurate measurements of the fraction of active RNA-polymerase transcribing each gene is required, with accurate measurements of tRNA abundances and the growth rate, all under the same conditions and preferably on the same experiment.
In Fig. 4 we present these two autocatalytic cycles and the resulting growth laws. It is interesting yet beyond the scope of this work to investigate how the different cycles are balanced among themselves using a few common control points.

**Fig. 5.** Effect of two antibacterial agents — triclosan and Lamotrigine on E. coli growth rate. Upper figure shows the effect of triclosan — common antibacterial agent, on the growth rate of E. coli. Data taken from (25). Triclosan mechanism of action is to disrupt the protein FabI which catalyzes an essential step in fatty acid bio-synthesis. Fatty acid bio-synthesis is necessary for building the bacterial membrane. To coarsely model this we decreased the utilization of the model’s membrane synthesis proteins (depicted in black in the illustration above the graph). This is a simplification since in practice there are several such proteins that collectively synthesize the membrane. The membrane synthesis rate $\tau^{-1}$ is unaffected for concentrations $c < c_{HM} = 0.15 \mu g/mL$. Administering triclosan reduces the size of the pool until a critical concentration the pool is depleted. Thereafter, any further delay in the supply of fatty-acid precursors cause a delay in the insertion of material to the elongating membrane by the membrane synthesis proteins. Thus, after the critical concentration $c_{HM}$ is surpassed, growth rate starts to decrease. To accommodate this switch-like behavior we used a Hill function with two fitting parameters, $K = 0.15$ and Hill’s coefficient $h = 2.6$. Inset (A) schematically depicts three membrane synthesis protein complexes $C = 3$ inserting new membrane patches of width $W_{in}$ into the existing membrane. The growth law equates the rate of growth $\mu$, the area of the inserted patch $W$, the surface concentration of the membrane synthesis proteins $\phi$ and the insertion rate $\tau^{-1}$. Lower figure shows the effect of Lamotrigine, an anti-convulsive drug that was recently found to adversely affect ribosome assembly process in E. coli (12). The growth law we used to fit the data is based on Eq. 1 in the main text. We neglect both the ribosomal protein pool time and the free ribosome idling time in order to obtain a simple quadratic equation. Interestingly, for assembly time significantly shorter than the doubling time, assembly time has a tiny correction to the bacterial growth-law. Only when the assembly time becomes comparable or larger than the bare growth rate $\frac{\mu}{\tau_{R}}$ that we start to see non-negligible corrections to the standard bacterial growth law. In the lamotrigine experiment this occurs at concentrations above $0.03 \mu g/mL$.

**Further applications of autocatalytic growth laws**

**Effect of lamotrigine on ribosomal assembly time of E. coli** As presented in Eq. 1, the ribosome growth law can be used to obtain the relation between growth rate and all the time-scales in the cycle — ribosome resting time $\tau_0$, translation time of all ribosomal proteins $- \tau_R$, assembly time $- \tau_{AS}$ and the allocation parameter $\alpha_{RP}$ and the percentage of actively translating ribosomes that translate ribosomal proteins. Upon the approximation that $\mu \tau_0 \ll 1$, we obtain a second order equation for the growth rate which is readily solved,

$$\mu = \sqrt{\frac{1}{2} \left[ \frac{\alpha_{RP} \tau_{SA}}{2 \tau_{AS}} - 1 \right]}, \quad [8]$$

where as before $\tau_{SA}$ is the ribosomal assembly time, $\tau_{RP}$ is the translation duration of a ribosomal protein that is a primary rRNA binder, and $\alpha_{RP}$ is the fraction of active ribosomes allocated to translating this ribosomal protein.

Eq. 8 demonstrates that the relation between growth rate $\mu$ and ribosome assembly duration $\tau_{SA}$ is only important to second order in $\frac{\alpha_{RP}}{\tau_{R}}$. Indeed by expanding the square root in Eq. 8 we see that the first order contribution leads to $\mu \approx \alpha_{RP} \tau_{RP}$, which is identical to the bacterial growth law when setting $\Phi_0 = 0$. Recently, an anti-convulsion drug — Lamotrigine, was found to adversely affect the ribosome assembly process in E. coli (12). We can use this model to fit the observed dependence of the growth rate on the concentration of lamotrigine by equating $\tau_{SA} = \tau_{SA} (1 + \frac{c}{c_{HM}})$, and then fitting $c_{HM}$ and $h$. We find that $c_{HM} = 150 \mu g/mL$ which is in accord with the empirical concentration at which the growth rate reduces by half. We also found that $h = 1$, indicating non-cooperative effect of lamotrigine on the ribosome self-assembly process. In figure 5 we present the resulting fit.

A more detailed theory for the assembly process is required in order to obtain a mechanistic understanding of the effect of lamotrigine on the assembly process, which we leave for a future study.

**Effect of triclosan on cell-wall synthesis of E. coli** Cylindrically shaped bacteria such as E. coli are known to elongate at an exponential rate (3). If the width of the bacteria does not change significantly over the doubling time, this also implies exponential growth of the volume and surface area. However the process of synthesizing new membrane is linear — membrane is inserted through an insertion site, a process hypothesized to be coordinated by proteins belonging to the ‘elangosome’, with MreB and penicillin-binding proteins that serve a key role, which is still subject to research (27, 28).

A mechanism that can coarsely account for the observation of exponential elongation is asynchronous threshold initiation of new insertion sites by constitutive proteins. At balanced growth conditions these proteins are expected to grow exponentially — due to the inherent coupling with the transcription-translation machinery that synthesize these constitutive cell-wall synthesis proteins. If either the initiation is asynchronous or there is an inhomogeneity in the rate of insertion between different insertion sites, a smooth exponential growth of the membrane ensues.

We devised a simple mathematical description of this coupling, and obtained the following relative abundance growth law relating the abundances of insertion sites and instantaneous surface area. We obtain the following growth law

$$\mu = \frac{\phi S \frac{W_{in} h}{\tau_{R}}}{\tau_{m}} \quad [9]$$

where $\mu$ is the growth rate, $\phi_S$ is the surface density of the cell-wall insertion sites, $W_{in}$ is the width of the insertion, and $\tau^{-1}$ is the speed of insertion in units of length over time. Eq. 9 was used in (28) to obtain the width of insertion site as a function of the growth rate.

Membrane bound volume and membrane surface area are also physical resources that are allocated in the cell, since all the cellular constituents occupy some volume, and surface area is required by many cellular processes mainly in metabolism in order to exchange metabolites and heat with the surrounding environment (9, 26). Closing this cycle is more involved than the other cycles and goes beyond the scope of this work.
What happens when membrane synthesis is disrupted? Consider the material triclosan — an antibacterial agent that targets fatty-acid biosynthesis. Triclosan mechanism of action is to disrupt the protein FabH which catalyzes an essential step in fatty acid bio-synthesis. Fatty acid bio-synthesis is necessary for building the bacterial membrane. To account for the effect of triclosan on the growth rate we use our simple modeling approach as follows. We modulate the rate of insertion of new membrane surface area by a Hill function that is monotonically decreasing with the triclosan concentration to account for the decreased speed caused by the dwindling of the fatty acid supply. Thus, the new membrane synthesis rate is \( \tau_m = \tau_m^{\text{max}} \frac{1}{1 + c_{HM}} \). Using data from (12) we found by fitting that \( c_{HM} = 0.0385 \text{ nM/mL} \) and that \( h = 1 \) — indicating a non-cooperative effect. See inset A in figure 5.

**Discussion.** The existence of simple mathematical relations between growth rate to the rate of cellular processes such as transcription, translation, metabolism and membrane synthesis seems to suggest that understanding the causal relations between growth rate and these processes is also at hand. Unfortunately, this is not the case.

The two kinds of growth laws obtained per auto-catalytic cycle — the closed cycle growth law and the relative abundance growth law, require local knowledge about the time scales and relative abundances of catalysts in the cycle, they also require global knowledge regarding the allocation parameters — fractions of RNA-polymerase and ribosomes dedicated to transcribe and translate the relevant proteins. Understanding the causal relation between these variables and in relation to the growth rate requires a global understanding regarding the manner in which the allocation parameters are determined. This amounts to knowing how the cell allocates its resources among all its auto-catalytic cycles. In particular, it requires understanding the design-logic — evolutionarily speaking, behind transcription, translation and metabolic control mechanisms that function across time scales from milliseconds to hours.

While much is already known about these control mechanisms, a holistic picture is yet to form. A few general principles do seem to emerge, such as allosteric regulation to control gene expression in response to change in external metabolite composition (32) and product feedback inhibition e.g. in metabolism, that enables the cell to optimally tune gene-expression and protein content (31).

Lack of causal explanation already exists in the ‘ribosome makes ribosome’ bacterial growth law. Understanding how the ribosome fraction and elongation rate change when there is a change in the nutritional composition of the environment, requires a causal model that accounts for how the cell controls the global allocation of its resources among all coupled auto-catalytic cycles. In the absence of such a mechanistic model, one cannot distinguish different mechanisms that lead to the same growth rate.

For example, in harsh environments, the cellular response can be to modify the allocation towards ribosomal proteins, while keeping the rate of translation fixed. Alternatively, the cellular response can be to modify the allocation towards ribosomal proteins in a manner that will cause the elongation rate to slow down compared to a permissive environment.

To distinguish between these two possibilities thus requires a measurement of the elongation rate and ribosome fraction together, while a causal model would have been able to predict the interdependence of the elongation rate and ribosome fraction as a function of the growth rate.

To demonstrate both the power and the limitation of working with coarse-grained growth laws we present another simple model for the dependence of growth rate on temperature. In contrast to previous models that finds a nice fit but offer no mechanism (29) or offer a mechanism, but are over-detailed, our model attempts to strike a balance between over-simplification to over-ramification.

Our starting point is the bacterial growth law \( \mu = \gamma_{\text{transl.}}(\Phi_R - \Phi_0) \) experimentally measured at 37°C. We recall a few experimental facts. First observation is the existence of an Arrhenius regime which is a range of temperature between \( T_{Ac} = 20°C \) to \( T_{Ah} = 40°C \) in E. coli where the RNA to protein ratio is not changing while the elongation rate changes with an Arrhenius temperature dependence, i.e. \( \gamma_{\text{elong.}} = \gamma_{\text{elong.}} e^{\frac{\Delta G}{kT}} \). The fact that growth rate scales with temperature with an Arrhenius type dependence which is typically relevant to a single chemical reaction might seem surprising, however, if the ribosome cycle locks all other auto-catalytic cycles, the scaling is a natural consequence of the increase in the elongation rate with temperature. Furthermore, the constancy of the ribosome fraction along the Arrhenius regime comes as a natural consequence of the fact that this cycle is the leading cycle across the entire Arrhenius regime. What happens beyond the Arrhenius regime? Both above and below the regime, the cell cannot sustain growth, since an increasing amount of proteins denature, if \( T > T_{Ah} \) or misfold \( T < T_{Ac} \) and more ribosomes has to be allocated to mitigate the leakage in functioning proteins.

It is possible, although not essential to assume that metabolic proteins are the first to cause such reallocation of ribosomes, due to the stringent response. We model this reallocation by a linear change of the ribosome fraction from \( \Phi_R \) at the hot \( T = T_{Ah} \) and cold \( T = T_{Ac} \) edges of the Arrhenius regime down towards \( \Phi_0 = 3.5\% \) — the minimal allocation at zero growth rate. We use measurements that found that E. coli ceases to grow at \( T_{cd} = 8°C \) and at \( T_{hd} = 49°C \) (19).

A simple model that can recreate the growth rate dependence on temperature can be obtained by adapting the ribosomal growth law (8) to account for the above observations. First, we scale the elongation rate for all temperatures by the Arrhenius factor, \( \gamma_{\text{elong.}} = \gamma_{\text{elong.}} e^{\frac{\Delta G}{kT}} \). For the Arrhenius regime, we write \( \mu = (\Phi_R - \Phi_0)\gamma_{\text{elong.}} e^{\frac{\Delta G}{kT}} \), where \( \Phi_R \) is the ribosome fraction at the maximal growth conditions at 37°C, \( \Phi_0 \approx 0.3 \) (8). Finally for temperatures above \( T_{Ah} \) until \( T_{hd} \) and from \( T_{Ah} \) down to \( T_{cd} \) we scale the ribosome fraction \( \Phi_R \) linearly from its maximal value down to \( \Phi_0 \approx 0.035 \), which is the allocation below which growth is brought to a halt.

In Fig. 6 we plot the results of such a model which is capable of obtaining an excellent fit with the measured data without any fitting parameters. This coarse-grained model exhibits both the merit and the pitfall of such a simple approach. On the one hand, this approach qualitatively explains why growth rate scale like Arrhenius in the Arrhenius regime. It is because cellular growth rate is locked to the ribosome auto-catalytic cycle which in turn scales like Arrhenius because elongation rate scales like Arrhenius. When the temperature passes above or below the Arrhenius regime, another cycle, e.g. the metabolic cycle becomes limiting, and the cell has to compensate by
given by $\gamma_{\text{elong}}$ and $\Delta \hat{G} = \Delta G(T) - \Delta G(T = 37^\circ C)$. We calculated $\Delta G(T)$ using the formula developed in (20) following (30), and using an average protein size of $N = 364$ amino-acids which we calculated using ribosome profiling data (18).

To understand the causal relation between growth rate and any sub-cellular process requires knowledge regarding how cells monitor, adjust and allocate their resources among all the auto-catalytic cycles that drive cellular growth. A well known and apparently universal cellular strategy for such control is to use multiple feedback loops on the level of work-in-process in various loops via product feedback inhibition. This mechanism enable the cell to control the transcription of enzymes that belong to the amino-acid synthesis pathway by preventing over-expression. Similarly, the cell use feedback inhibition on the level of translation to down-regulate the production of ribosomal proteins if the level of free ribosomal proteins rise due to failure to connect to rRNA, which is regulated by another feedback mechanism known as the stringent response, that prevent over-expression of rRNA by RNA-polymerase when amino-acids are in shortage, via the RelA protein that synthesize ppGpp, when it is bound to a starved ribosomes. The alarmon ppGpp subsequently inhibits RNA-polymerase transcription.

On the other side, a mechanism that facilitates a cellular decision to increase the growth rate by committing to produce more ribosomes and RNA-polymerases is also beneficial for the cell.

Finally, we would like to emphasize the while the growth laws obtained by our method are simple enough, even when they are empirically known to be hold, they still do not provide us with the full information regarding the cellular physiological state. This is because multiple growth regimes can lead to the same growth law.

In the case of our RNA-polymerase growth law in an environment with a non-zero concentration of rifampicin we saw that two different growth regimes — mRNA alone is limiting but not rRNA and both mRNA and rRNA are limiting, led to the same growth law and RNA to protein ratio.

We thus conclude that the satisfaction of a growth law does not provide full characterization of the physiological state of the cell. Such characterization requires more detailed information.

**Methods**

A standard approach to model kinetics is to focus on concentrations, and write ordinary differential equations (ODEs) for their rate of change. A well known example is the Michaelis-Menten form $rac{dN}{dt} = \frac{km[N]}{K + [N]}$ which is non-linear.

Instead of using coupled non-linear ODEs for concentrations we focus on the rate of increase of the absolute number of molecules. This has two main advantages over other approaches, which are also approximations. First, by counting molecules, the hallmark of auto-catalysis — exponential growth becomes evident. Second, catalysts in the broader sense of the word, i.e. materials that are required to facilitate a reaction but are not consumed by it, and substrates which are materials required by a reaction and are consumed by it, acquire equal footing in the equations, allowing us to better understand which cycle is limiting and which is not, under different circumstances.

We begin by explaining how we translate the graphical...
Fig. 7. Two-step model buildup. Inset (A). Coupling of two autocatalytic cycles (compare with (11)). Two minimum functions imply 4 different growth regimes. Inset (B) Adding idle and active states and an intermediate self-assembly step. Equations provide a quantitative description of the graphical model.

language presented in Figures 1 and 2 into a set of coupled ordinary differential equations which are piecewise linear, and how we derive from them various growth laws that are valid in different growth regimes — which we also define.

For the sake of clarity we use a simplified toy model to elucidate the two-step process that we used in order to derive more complex models that bare biological relevance such as the ones presented in the main text. The first step was to identify all autocatalytic cycles. The second step is to insert more internal states per autocatalytic cycle to account for idling vs. active catalysts, self-assembly step and precursor pools, as we explain further below.

We recall the proverb attributed to G. Box ‘All models are wrong, but some are useful’. We argue that the usefulness of our modeling approach lies in its modularity, and in its ability to unravel simple growth laws as well as the complex circumstances that renders them valid.

Consider then a drastically simplified model shown in inset (A) of Fig 7. In this inset, a fraction of machines of type $U$ auto-catalyse themselves. The remaining Us synthesize a second type machines of type $P$. The $P$ machines convert an external substrate $f$ to an internal substrate $F$ that is used by $U$ to make both more copies of itself and new $P$s. The $U$ machines have a lifetime $\tau_u$, while the $P$ machines have an infinite lifetime. To catalyze a new $U$ machine, $F_U$ units of $F$ are required. To catalyze a new $P$ machine, $F_P$ units of $F$ are required.

The rate at which $U$ catalyze either $P$ or $U$ is by incorporating one unit of $F$-substrate per $\tau_u$ units of time, assuming $F$ is abundant. The rate at which $P$ converts external substrate $f$ to internal substrate $F$ is $\tau_F$, assuming $F$ is abundant. The minimum function in the equations presented in the figure, allows us to uncover four different growth regimes in this model. Regime (i) with $f \geq P$ and $F \geq U$ where both $P$ and $U$ catalyze at their fastest rate, since at any given moment, both $U$s and $P$s are not starved for substrate. In regime (ii) $f \geq P$ and $F < U$, i.e. $P$s are not starved for their substrate, but $U$s are starved for their substrate. As a consequence, the rate at which $U$s operate is reduced by a factor $\eta_U = \frac{P}{F}$. Regime (iii) $f < P$ and $F > U$ where despite $P$s being starved, $U$s are not. Finally, regime (iv) $f < P$ and $F < U$ where both $P$s and $U$s are starved for their respective substrates.

To find the growth rate as a function of the time-scales in the model, it is instructive to formulate the equations using matrix algebra. Define the column state vector $S = (U, P, F)^\dagger$. Then the dynamics per region can be described as $\frac{dS}{dt} = M_i S_i$, $i \in \{1, 2, 3, 4\}$. For example, the matrix for region (i) $M_1$ is defined as $(M_1)_{11} = \frac{\alpha}{\tau_u} + \frac{1}{\tau_F}$, $(M_1)_{12} = \frac{\tau_u}{\tau_u}$, $(M_1)_{21} = \frac{1}{\tau_u}$ and $(M_1)_{23} = \frac{1}{\tau_F}$ while all other elements of $M_i$ are zero. It is important to set the external substrate $f$ to model the appropriate experimental circumstances. For example, growth in a chemostat would be modeled by an exponentially growing $f$ over time, with a fixed growth rate $D$ which is equal to the dilution rate. In this regime, at steady growth conditions $f$ will be less than $P$, i.e. $f < P$. In contrast, in turbidostat conditions, $f(t)$ will grow exponentially at a growth rate that is higher than the maximal obtained growth rate and $f \geq P$.

Since per regime, the matrix $M_i$ is constant, the steady growth solution will be $S(t) = S_i e^{\mu t}$ where $\mu$ is the largest eigenvalue of $M_i$ and $S_i$ is the corresponding eigenvector i.e. $M_i S_i = \mu S_i$.

When $U$ is not starved for its substrate, the growth rate $\mu$ can be analytically calculated to be $\mu = \frac{F}{U} - \frac{P}{F}$. To yield positive growth, $U$’s must be catalyzed at a rate that is faster than the decay rate $\frac{1}{\tau_F}$. This implies a minimal value for the allocation parameter $\alpha$ above which exponential growth begins. In the region where $U$ is starved, the second autocatalytic cycle — the ‘$P$ catalyze $F$, $F$ produces $P$’ cycle, determines the growth rate. In this regime, the largest eigenvalue once again can be calculated analytically and is given as the positive root of the quadratic characteristic equation, $\mu = \frac{1}{2\tau_u} \left(1 + \frac{4(1-\alpha)\tau_u}{\tau_F} - 1\right)$.

Finally, in order to find if the growth rate we found is consistent with the conditions that we used in order to derive it, we need to check if the ratio between the components of the eigenvector $S_i$ satisfies the conditions that defined it. For example, $M_1$ was defined for the regime $F > U$ and $f > P$. If the eigenvector components satisfy $(S_{i1}) > (S_{i2})$, and $(S_{i1}) > (S_{i2})$ we will say that this region is consistent, otherwise, the region will be inconsistent and the growth law derived from the matrix that correspond to this region will not be valid — another one will.

Assuming $f$ is abundant e.g because it is constantly replenished, like in a turbidostat, the simple growth law $\mu = \frac{F}{U}$ will be valid as long as $\alpha \leq \alpha_{opt}$ — the optimal allocation that leads to maximal growth. $\alpha_{opt}$ can be found by equating the two growth laws $\mu = \frac{F}{U}$ and $\mu = \frac{1}{2\tau_u} \left(1 + \frac{4(1-\alpha)\tau_u}{\tau_F} - 1\right)$, and solving for $\alpha$. When $\alpha > \alpha_{opt}$ region one eventually becomes inconsistent.

It is important to note that in experiments on wild-type bacteria, when a parameter equivalent to the parameter $\tau_u$ is measured, there is no a-priori way of knowing in which growth regime the measurement was performed. Hence, what is actually being measured is not the ‘bare’ $\tau_u$ but rather $\tau_u'$ which can be different due to a utilization factor that measures the percentage of time $U$ is waiting for substrate. Thus, if $\tau_u'$ is given, it is still true that $\alpha = \frac{\tau_u'}{\tau_u}$ simply because in the regime where $F < U$ this equation will switch to the eigenvalue equation that is valid in this regime.

The growth law obtained from solving the characteristic polynomial is the closed cycle growth law — a growth law
that depends on all the time scales in the cycle. The relative abundance growth law is much simpler to derive and is obtained from using the eigenvector. For example, assuming region i is consistent we have that $M_i S_j = \mu S_i$. Dividing by the $k^{th}$ component of the eigenvector $S_i$ namely by $(S_i)_k$ we obtain that for the $l^{th}$ component $\mu = \sum_j (M_{ij})_{kl} ((S_j)_l / (S_k)_l)$. This is useful if the relative abundances $(S_j)_l / (S_k)_l$ can be measured experimentally, as in the case of ribosomal protein mass fraction. Evidently, many more relative abundance growth laws can be derived, growth laws that involve other types of abundances, e.g. protein to mRNA, or RNA-polymerase to mRNA and so on.

Before moving to explain inset B in Fig. 7, we note that using ODEs with switchings is a well-known practice in control theory, but also in standard classical physics — when considering e.g. the bouncing of a basketball on the floor. In particular, the use of a minimum function is considered standard in input-output economy, and is called Leontif’s production function (21).

So far we explained how we devise a model that couples just two autocatalytic cycles using the minimum function to define the various regimes that support in principal steady exponential growth. Next, we wish to explain how we further add more biological details to the model, in order to facilitate its use for deriving biologically relevant growth laws. For that purpose we turn our attention to inset (B) in Fig. 7 which depicts the next step in building our model. For the sake of simplicity, in order to explain the second step, we simplify the model by taking $\alpha = 1$ and assuming $F \gg U$. In this simplified model, there is only one autocatalytic cycle. However, we add to this cycles new generic states in order to make it more realistic. First we differentiate between two modes of $U$ — idling and being active. The number of idling $U$’s is denoted by $U_0$. The idling state is characterized by a time scale $\tau_0$ which is the average idling duration. When $U$’s are not idling they are active. The number of active $U$’s is denoted by $U_b$. The time scale for being active in principal is a complex function of the allocation towards all the synthesis tasks $U$ is allocated to, and their respective work-load. In this simplified picture, since we took $\alpha = 1$ there is only one task that $U$ is allocated to perform which is replicate itself, and hence the time scale for being active is the time scale to make new $U$’s — $\tau_U = F_0 \omega U$. Finally, we also add a self-assembly step, by breaking the process of making new $U$’s further, to the process of making subunits $a_1$, $a_2$ and $a_3$ and the process assembling them to form a new $U$. This self-assembly process proceeds at a rate $\tau_A$. In our model, we use what we dub a ‘tetrus’ model which assumes that every time a stoichiometric series of a’s (i.e. $a_1 = a_2 = a_3 = 1$) is formed for the first time, the assembly of a new $U$ is initiated. The assembly will be completed on average after $\tau_A$ time units after assembly initiation.

In the results section we combine the autocatalytic cycles of the transcription-translation machinery, and other cycles while accounting for the couplings between the different cycles, the existence of different cytoplasmic chemical reaction, mRNA-polymerase and ribosomes, the existence of idling and busy periods, ribosome and RNA-polymerase self-assembly steps and the finite lifetime of mRNA, ribosomes and proteins. The resulting model is also piecewise linear, albeit much larger, and we derive from it the various growth laws we present in the paper by algebraically calculating the characteristic polynomial roots (closed-cycle growth law), or by using the eigenvector equation (relative abundance growth law) per a specific regime.

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