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Minireview

MINIREVIEW

Rethinking cell growth models

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One sentence summary: The minimal description of a growing cell as self-replicating ribosomes is discussed, highlighting the possible interplay between achieving maximal growth and coordinating different growth-related processes.
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ABSTRACT

The minimal description of a growing cell consists of self-replicating ribosomes translating the cellular proteome. While neglecting all other cellular components, this model provides key insights into the control and limitations of growth rate. It shows, for example, that growth rate is maximized when ribosomes work at full capacity, explains the linear relation between growth rate and the ribosome fraction of the proteome and defines the maximal possible growth rate. This ribosome-centered model also highlights the challenge of coordinating cell growth with related processes such as cell division or nutrient production. Coordination is promoted when ribosomes don't translate at maximal capacity, as it allows escaping strict exponential growth. Recent data support the notion that multiple cellular processes limit growth. In particular, increasing transcriptional demand may be as deleterious as increasing translational demand, depending on growth conditions. Consistent with the idea of trade-off, cells may forgo maximal growth to enable more efficient interprocess coordination and faster adaptation to changing conditions.

Keywords: transcription; translation; growth rate; protein burden; ribosome; microorganisms

INTRODUCTION

'Everything should be made as simple as possible, but no simpler' (Albert Einstein)—cells are highly complex entities. Even the simplest bacteria express hundreds of genes and protein types, which perform distinct functions and are engaged in numerous interactions. Is it necessary to fully characterize each and every protein in order to understand how cells work? Or is it possible to formulate general models that rely on basic principles and capture the essence of cell function with fewer parameters? Which questions require detailed knowledge on molecular processes and which can be answered with a more general approach? In this review, we touch upon these questions focusing on the growth of single-cell microorganisms such as bacteria and yeast. Growth is a basic physiological property of cells, which depends on the coordination of multiple proteins and processes, as well as environmental conditions. Can simple models guide us in understanding its basic determinants?

MINIMAL MODEL OF CELL GROWTH: THE SELF-REPLICATING RIBOSOME

At a very basic level, growing cells can be described as self-replicating entities that grow and divide, with the entire cellular content being duplicated at each and every division. Cellular functions are performed by proteins and can therefore be accelerated, with no apparent limit, by increasing protein levels. An exception is protein synthesis itself. Protein translation depends on ribosomes, which, critically, synthesize themselves. This need for self-duplication limits the ability to increase protein production indefinitely, as increased synthesis would require more ribosomes, the production of which will compete with the synthesis of non-ribosomal proteins. The synthesis of ribosomes might therefore be the key to understand cell growth. Indeed, classical studies, as well as more recent reports, formulated models of cell growth that are centered on the ribosome as a self-replicating entity (Neidhardt and Magasanik 1960; Maaløe

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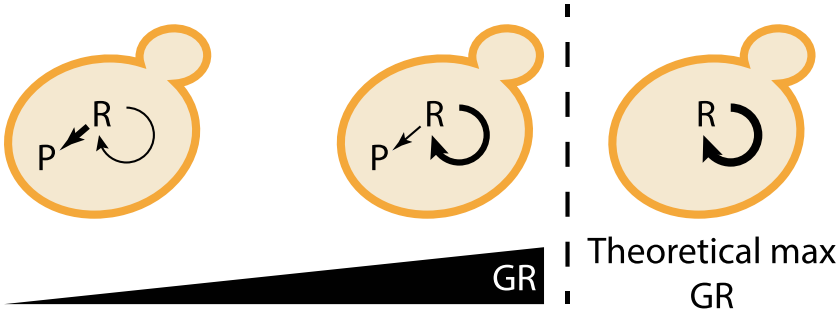

Figure 1. Modeling cell growth rate (GR) as a self-replicating ribosome.

1979; Koch 1988; Marr 1991; Hernandez and Bremer 1993; Zaslaver *et al.* 2009; Scott *et al.* 2010, 2014; Shachrai *et al.* 2010; Scott and Hwa 2011).

The minimal model of cell growth considers the ribosomes and the proteins they produce. Some of the ribosomes are occupied by making new ribosomes, while the rest translate other proteins. In this model, cell size is proportional to the total protein content, and cell growth is set by the rate of protein accumulation. Since for a given number of ribosomes, growth rate is maximized when all ribosomes are actively translating, it is generally assumed that ribosomes are saturated, e.g. working at full capacity (Fig. 1). The model is therefore defined by the equation:

$$dP/dt = \gamma\ R \tag{1}$$

where R is the number of ribosomes, while P is the total mass of amino acids in both ribosomal and non-ribosomal proteins. γ denotes the translation rate. Ribosome production is similarly written as:

$$dR/dt = \gamma'\ r\ R \tag{2}$$

where r denotes the fraction of ribosomes that are producing proteins required for ribosome functions. Note that the effective translation rate used here, $\gamma\prime = (\gamma\ /\ N^R)$, reflects the need to translate N^R amino acids in order to obtain a functional ribosome.

Together, these equations define exponential growth: $P(t) = P_0 e^{\mu t}$, where the amount of proteins made in a given time is proportional to the amount of proteins present at the initial time. Furthermore, protein concentration remains constant over time, as each individual protein increases with the same exponential dynamics. In particular, r (Eq. 2) defines the ribosomal fraction of the proteome,$r = P^R/P$, with $P^R = N^R\ R$. Similar to all other concentrations, r remains constant throughout the dynamics.

The model therefore predicts that the specific growth rate, μ, is determined by the ribosomal fraction:$\mu = \gamma'\ r$. Furthermore, the minimal division time is given $\log(2)/\gamma r$: the time it takes a single ribosome to translate one additional ribosome. This theoretical limit is reached when all ribosomes are busy duplicating themselves, with practically no other proteins being made. Clearly, this limit is not feasible, yet rapidly growing bacteria such as *Escherichia coli* may live not far from this limit. The division time of these cells is 20 min, which is comparable to the 6–10 min theoretical limit (Scott *et al.* 2010) and suggests that ~30% of their proteome is composed of ribosome-related proteins. Similarly, minimal division time in budding yeast is ~90 min, ~3–4-folds higher than the ~24-min theoretical limit (Kafri *et al.* 2016).

THE SELF-REPLICATING RIBOSOME MODEL PROVIDES A POWERFUL PLATFORM FOR INTERPRETING BACTERIAL GROWTH LAWS

In addition to explaining the doubling time of rapidly growing cells, the ribosome-centered model provides a useful platform for interpreting the cellular response to perturbations (Maaløe 1979; Dekel and Alon 2005; Zaslaver *et al.* 2009; Scott *et al.* 2010; Shachrai *et al.* 2010; Keren *et al.* 2013; You *et al.* 2013). At the very basic level, the model predicts a direct correlation between the specific growth rate μ and the ribosomal fraction r: $\mu\ = \gamma' r$. As cells adapt to new conditions, they adjust their proteome composition, e.g. induce proteins for compulsory metabolic functions and change the ribosomal fraction r. To maximize growth rate, ribosomes should remain translation limiting to keep the linear relation between the growth rate and the ribosome fraction.

Consistent with this prediction, studies in bacteria, as well as in eukaryotes, revealed a tight correlation between cell growth rate and the ribosome/protein ratio measured under different growth conditions (Schaechter, Maaløe and Kjeldgaard 1958; Neidhardt and Magasanik 1960; Maaløe 1979; Bremer and Ehrenberg 1995). Quantitative analysis showed that this dependency, measured under multiple conditions, complies with the linear relationship: $\mu = \kappa r + r_0$ (Scott *et al.* 2010). Consistent with the model-based interpretation of κ as translation rate, translation-inhibiting drugs reduced κ (and increased the ribosome fraction to partially compensate for the reduced translation efficiency). Using this data, it was further possible to account for the reduced growth rate of *Escherichia coli* cells forced to express unneeded proteins (Andrews and Hegeman 1976; Bentley *et al.* 1990; Vind *et al.* 1993; Dong, Nilsson and Kurland 1995, 1996; Dekel and Alon 2005; Stoebel, Dean and Dykhuizen 2008), attributing this growth attenuation to the decrease in the ribosomal fraction of the proteome, r, due to the addition of unneeded proteins (Scott *et al.* 2010).

We note that while the ribosome-centered model provided a powerful platform for explaining changes in growth rate, the measured linear relation $\mu = \kappa r + r_0$ deviates from the straightforward prediction of the model. First, the model does not predict a finite intercept $r_0 > 0$. To account for that, it was suggested that the r_0 fraction of the proteome consists of ribosomes that are not actively translating (Scott *et al.* 2010). The role of this function, and why it remains constant between conditions and growth rates, is less intuitive. Second, the interpretation of κ as the ribosomal translation rate also implies that the translation

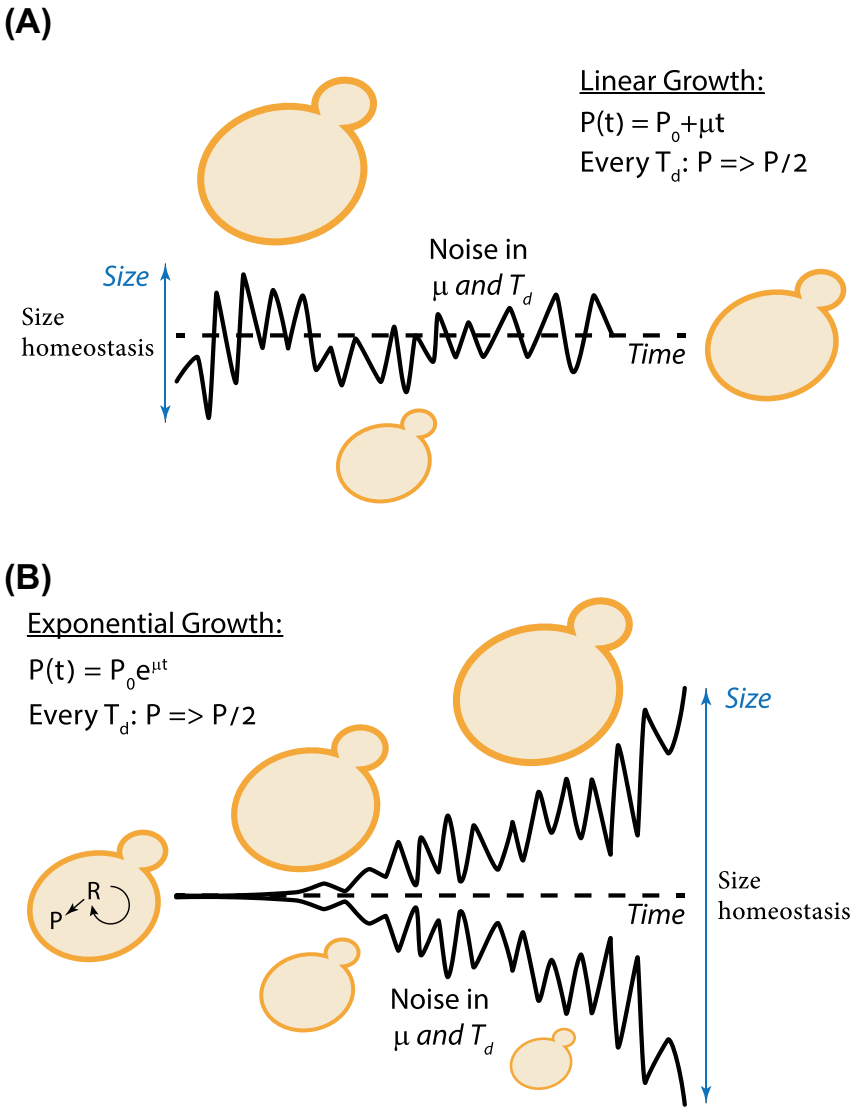

Figure 2. Size homeostasis. (**A**) When growth is linear, cell size approaches a well-defined steady state. (**B**) Size fluctuations accumulate when cells growth exponentially.

rate is condition independent, while experiments show slowing down of translation elongation at low growth rates (Dalbow and Young 1975; Pedersen 1984). As a possible solution, it was suggested that tRNA flux becomes limiting in slow-growing cells, thereby affecting the relation between growth rate and the ribosomal fraction (Klumpp *et al.* 2013).

COORDINATING PARALLEL GROWTH-RELATED PROCESSES

The ribosome-centered model captures key properties of cell growth, and highlights the challenge of coordination, namely, the need to precisely adjust different growth-related processes to maintain stable proliferation. Maintaining robust coordination becomes difficult in exponentially growing cells, a central outcome of the model.

The challenge of coordination is best illustrated by considering the need to maintain size homeostasis. Stable cell size requires that cells double their biomass at each and every cell division. If cells were to grow linearly, e.g. $P(t) = P(0) + \mu_1 t$, the cellular protein content would flow towards a stable steady state $P^{st} = \mu_1 T^d$ for any (arbitrary) division time T^d and growth rate μ_1 (Fig. 2A). Size homeostasis can therefore be obtained for any combination of doubling time and growth rate, and fluctuations of these parameters are compensated for by the dynamics itself. The situation is quite different when cells grow exponentially. Indeed, when the protein levels increase exponentially $P(t) = P(0)e^{\mu t}$, the division time must be adjusted to precisely match the growth rate $T^d = \ln(2)/\mu$. For any other parameter values, protein mass will either continuously increase or continuously decrease in time, losing size homeostasis. Furthermore, even when tuning exists, fluctuations in growth rate or division time will accumulate, again due to the lack of natural scale for cell size, leading to uncontrollable size variation (Fig. 2B). Therefore, strict exponential growth lacks an inherent method for coordinating cell division time and biomass production, requiring additional mechanisms to adjust these processes and control cell size in the event of fluctuations.

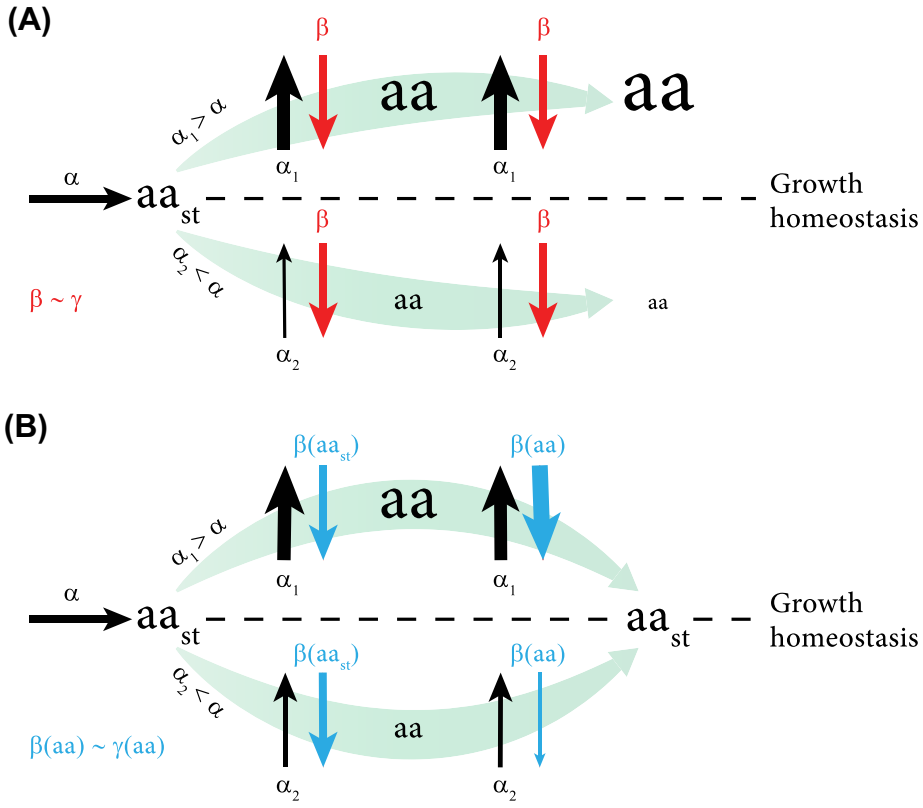

Figure 3. Amino acids (aa) homeostasis. (**A**) Fluctuations in aa concentration accumulate when cells grow exponentially. (**B**) aa approach is a well-defined steady state when translation rate depends on aa acid level.

This challenge of coordination in exponentially growing cells is not limited to cell division time, but in fact extends to other properties. Consider for example the production or transport of nutrients required for protein production, such as amino-acids concentration. Production of these nutrients is proportional to the concentration of various proteins, while their consumption depends on the rate by which proteins are made, which in exponentially growing cells is again proportional to the total protein content. Therefore, denoting amino-acids concentration as aa, we obtain $daa/dt = P(\alpha - \beta)$, where α, β are size-independent constants proportional to the proteome fractions dedicated for nutrient production and protein translation, respectively. Clearly, the two parameters α, β must be fine-tuned to ensure stable concentrations of the amino-acids pool (aa_{st}). Furthermore, even when such tuning exists, additional mechanisms are required to buffer fluctuations in nutrient production or consumption (Fig. 3A).

Within the ribosome-centered model, the need for coordinating different growth-related processes is accounted for by optimizing the proteome partitioning. This would ensure that the proteome fraction dedicated to nutrient production, for example, is tuned to the respective ribosomal fraction, so that, e.g., in the example above $\alpha = \beta$ is ensured (Hernandez and Bremer 1993; Kalisky, Dekel and Alon 2007; Zaslaver *et al.* 2009; Scott *et al.* 2010, 2014; Shachrai *et al.* 2010; Klumpp *et al.* 2013; You *et al.* 2013). Notably, also here, tuning by itself is not sufficient to control fluctuations in nutrient transport or growth rate, again due to the lack of steady state or scale. Specialized feedback mechanisms are required to ensure stable coordination and control for fluctuations for each deviation of homeostasis.

Feedback mechanisms between different growth-related processes have been proposed. A size checkpoint, for example, adjusts cell division with cell growth by preventing a certain cell cycle transition until cells pass a minimal size or translation rate threshold (Nurse and Thuriaux 1977; Johnston *et al.* 1979; Dolznig *et al.* 2004; Jorgensen and Tyers 2004; Tzur *et al.* 2009; Son *et al.* 2012; Turner, Ewald and Skotheim 2012). Other size control mechanisms, which effectively regulate division time depending on cell size, were also proposed (Cooper 2004; Aldea 2007; Campos *et al.* 2014; Schmidt-Glenewinkel and Barkai 2014; Soifer and Barkai 2014; Schmoller and Skotheim 2015; Schmoller *et al.* 2015; Taheri-Araghi 2015; Soifer, Robert and Amir 2016). Similarly, feedback mechanisms may render translation rates dependent on nutrient levels. Examples for such feedbacks include the ppGpp-dependent decrease in ribosome production in response to amino-acid depletion (Marr 1991; Bremer and Ehrenberg 1995; Shachrai *et al.* 2010; Scott *et al.* 2014).

This challenge of coordination is exclusive to exponentially growing cells, as any deviation from strict exponential growth will ensure a stable steady state. The ribosome-centered model predicts exponential growth; in order to maximize growth rate, translation depends only on ribosomes that are working at full capacity. Deviation from exponential growth is possible if ribosomes were not strictly limiting. For example, if translation rates also depend on amino-acid levels, these would flow towards a well-defined steady state, providing a natural way to control for fluctuations in amino-acids levels (Fig. 3B). Similarly, if growth rate was dependent on the total protein content, cell size would also be well defined. In both cases, the improved

ability to control for fluctuations would come at the expense of lowering growth rate, as ribosomes will no longer work at maximal capacity.

In particular, perhaps the simplest way to escape from strict exponential growth is by allowing translation rates to depend not only on ribosomes but also on mRNA availability. Since mRNA is produced by one to two DNA copies, its production may not scale precisely with cell size. This would naturally introduce dependence between protein production rate and size, promoting the coordination of division cycle and cell size with cell growth.

DOES mRNA PRODUCTION AFFECT PROTEIN PRODUCTION AND CELL GROWTH?

Following the above discussion, we asked whether there is an experimental justification for assuming that not only protein translation, but also other processes and in particular mRNA transcription, is limiting for protein production and growth rate. Notably, in budding yeast, the estimated number of ribosomes (~200,000 (Milo *et al.* 2010, BNID 100267)) is larger than the estimated number of mRNAs (~30,000 (Milo *et al.* 2010, BNID 104516)).

To address this question, we forced cells to almost only transcribe or also translate increasingly high amounts of inert proteins (mCherry), and measured their growth rate using a sensitive competition assay (Kafri *et al.* 2016). The results supported the concept that growth rate is limited by multiple processes, including the initiation and elongation phases of protein translation and mRNA transcription. Notably, the relative contribution of the different processes depended on the environmental conditions: when cells were provided with standard medium, transcriptional and translational burden had an equivalent effect. Burdening protein translation was significantly more deleterious than burdening only transcription when growing cells in low nitrogen, or when providing them with a non-fermentable carbon source (glycerol).

Most strikingly, perhaps, when phosphate was low, the growth rate was highly sensitive to transcriptional demands, but largely robust to additional translational burden. Under this condition, the specific growth rate was significantly reduced by increasing mRNA production, despite negligible changes of the proteome composition. Notably, since the proteome fraction dedicated to gene transcription is small, a possible increase of this fraction to compensate for the induced burden would have a negligible effect on the ribosome fraction and could not explain the reduced growth rate.

These results suggest that the specific growth rate, and therefore the total protein translation rate, depends not only on the proteome composition but also on the process of mRNA production, at least in certain environmental conditions. The mechanistic basis of this effect is still unknown. However, we noted that the proteome composition in the transcriptionally burdened cells remained highly stable, while cell size increased. One possible explanation is that mRNA concentration decreases, thereby directly limiting translation speed. Alternatively, reduced growth rate could result from depletion of certain nutrients (e.g. phosphate), although no indication for such limitation could be detected in gene expression profiling. Other possibilities include competition for limited transcription factors that could similarly introduce regulatory effects. Further studies are required to reveal the mechanistic basis of the observed limitation.

CONCLUDING REMARKS

Complex systems, such as cell growth, can be studied at different levels of simplifications (Neidhardt and Magasanik 1960; Maaløe 1979; Okamoto and Savageau 1984; Koch 1988; Marr 1991; Hernandez and Bremer 1993; Price, Reed and Palsson 2004; Tadmor and Tlusty 2008; Molenaar *et al.* 2009; Zaslaver *et al.* 2009; Feist and Palsson 2010; Lewis *et al.* 2010; Scott *et al.* 2010, 2014; Shachrai *et al.* 2010; Scott and Hwa 2011). Here, we described perhaps the simplest approach to model cell growth, which only considers protein production by self-replicating ribosomes. Importantly, in addition to characterizing parameters controlling cell growth, this model emphasizes the potential difficulty to coordinate different growth rate-related processes. Such coordination is particularly challenging in exponentially growing cells, which lack natural scales for e.g. the overall protein or nutrient content.

The concept of trade-off suggests that cells are optimized for multiple constraints, some of which may introduce conflicting demands. The ribosome-centered model highlights this trade-off in the context of cell growth: growth rate is maximal when ribosomes work at full capacity and translation rates depend only on ribosome levels. However, coordination of different growth-related processes is made easier if this assumption is relaxed, allowing additional factors to influence the rate of translation.

A critical next question is how to evolve the ribosome-centered model in a way that will preserve its transparency, while providing additional insights and accounting for further experimental observations. Accounting for gene transcription may be particularly important in this respect, following the recent observations that, depending on growth conditions, transcription plays a major role in cell growth limitation.

A related challenge is to define the objectives that guide the evolution of the growing cell. Maximizing growth rate is clearly one such constraint. Coordination of different growth-related processes may be another. Other constraints may be rapid response to changing conditions, which may also be promoted by maintaining some ribosomes in a non-active state. It is left to be seen whether the complexity of cell growth models be increased to account for such constraints while preserving their transparency, and what experimental approaches could best guide us in this.

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