A unified coarse-grained theory of bacterial physiology explains the relationship between cell size, growth rate and proteome composition under various growth limitations

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Abstract

Microbial cells are able to reach balanced exponential growth in a variety of environmental conditions. At the single-cell level, this implies coordination between the processes governing cellular growth, cell division and gene expression. A fundamental goal of microbiology is to build a quantitative and predictive understanding of how such coordination is achieved. It is known since long that the size of E. coli cells grown in media of different nutrient quality increases with growth rate. Recent data, however, shows that this relationship is of another nature when growth is modulated by other types of limitations, such as translation inhibition or forced expression of useless proteins. Here, we present a single-cell coarse-grained model of bacterial physiology that unifies previous efforts (i.e. the proteome allocation theory and the structural model of division control) into a simple and low-parametric model. We show that this model quantitatively explains the observed relationship between cell size and growth rate for various types of growth limitations with a single free parameter. In addition, predicted proteome fractions agree with observations, and when noise is included in the model, the recently discovered 'adder' principle of cell size homeostasis is correctly predicted. Thus, our minimalistic coarse-grained model successfully recapitulates the most fundamental aspects of bacterial physiology, and could serve as a guide for the development of more detailed and mechanistic whole-cell models.

Introduction

Understanding how bacterial cells adapt their gene expression, cellular growth and cell size in order to grow in a variety of environmental conditions is a fundamental question of longstanding interest. This question entails several aspects, such as proteome allocation (every time a new protein is produced, what should it be?), division control (which aspects of cell state govern the decision to divide?) and cell size homeostasis (how to ensure a steady distribution of cell size despite deviations from the average division size in single cells?).

Proteome allocation. The question of proteome allocation received high interest in recent years and our understanding strongly progressed, notably under the impulsion of the Hwa group (Scott et al. (2010), Scott et al. (2014), Hui et al. (2015)). The current theory of proteome allocation explains why the fraction of ribosomal proteins increases with growth rate when the nutrient quality is improved, and why it increases when the growth rate is decreased by translation inhibition: this results from an optimal allocation of protein synthesis towards metabolic and ribosomal proteins so as to match and maximize the rate of amino-acids synthesis and the rate of protein synthesis (Scott et al. (2014)). Recently, more mechanistic coarse-grained models of bacterial physiology able to reproduce the same observations on proteome allocation have been proposed (Weisse et al. (2015), Pandey and Jain (2016)). However, those models cannot predict how cell size changes under different types of growth limitations, since the underlying theories ignore cell division and its control.

Control of cell division. The question of division control and how division size depends on environmental conditions has a long history. Schaechter and colleagues (Schaechter, Maaloe, and Kjeldgaard (1958)) observed that size increases with the nutritional quality of the medium, and that this change depends primarily on the growth rate supported by a given medium rather than the specific nutrients it contains. Of note, this relationship can be broken by defects in central carbon metabolism or fatty acid biosynthesis (Vadia and Levin (2015)).
Fantes and colleagues (Fantes et al. (1975)) considered a range of theoretical models for cell division control and assessed their consistency with experimental knowledge and their robustness to perturbations. They notably proposed a class of models, called the 'structural' models, in which the absolute amount of a given factor (rather than its concentration) controls cell division. In principle, division control mechanisms relying on absolute amounts of a given factor could enable a form of size control, because maintaining the concentration of this factor constant leads to absolute amounts scaling with cell size.
Because cell division must be linked to DNA replication and segregation, significant work has been devoted to studying how the initiation of DNA replication is controlled and how it is linked to cell division (Donachie and Blakely (2003), A. Amir (2014), Ho and Amir (2015)). A recent model (the multiple origins accumulation model, Ho and Amir (2015)) proposes that replication initiates upon the accumulation of a critical amount of initiator molecules per origin of replication. Initiator molecules are then assumed to be destroyed, and to each replication initiation event corresponds a division triggered after a constant duration (the $C + D$ period, C and D referring respectively to the time required for replication and the time required for chromosome segregation and septum formation (Cooper and Helmstetter (1968))). In fact, this model relies on the same strategy as structural models of division control but applied to the control of DNA replication. Division control simply follows because division events are assumed to be tightly linked to replication events. Interestingly, if the $C + D$ period does not depend on growth rate, this model predicts an exponential increase of cell size with growth rate, in agreement with observations for nutrient-based growth modulation. However, this model cannot make direct predictions on the relationship between size and growth rate for different types of growth modulations without making assumptions on how the $C + D$ period is affected.

Cell size homeostasis. While the changes in average cell size for different environmental or genetic perturbations certainly inform about division control, how deviations (from population average) in the division size of individual cells are corrected must also provide rich and complementary insights into division control mechanisms (Kennard et al. (2016)). Recently, advances in single-cell techniques led to the discovery that most bacterial cells achieve cell size homeostasis via an adder principle: the added size between birth and division is statistically independent of the birth size (Campos et al. (2014), Taheri-Araghi et al. (2015)). Interestingly, the multiple origins accumulation model predicts the adder principle of size homeostasis (Ho and Amir (2015)), because the accumulation of initiator molecules follows the added volume (assuming that their expression follows the expression of proteins maintained at a constant concentration, as in the model by Sompayrac and Maaloe (1973)).

Surprising cell size - growth rate relationships for orthogonal growth modulations. Very recently, surprising data on the relationship between growth rate and cell size has been obtained (Basan et al. (2015)). In addition to the classic nutrient-based modulation, the authors explored the impact of two other growth limitations on cell size: forced expression of useless proteins (*LacZ*), and translation inhibition with chloramphenicol. Interestingly, while cell size increased with growth rate for nutrient-based modulation, as expected, it strongly increased with decreasing growth rate for forced expression of useless proteins, and remained relatively constant with decreasing growth rate for translation inhibition with chloramphenicol. Using the structural model to interpret their data, they found that for forced expression of useless proteins, the relationship between the total protein amount per cell and the growth rate is consistent with the proteome fraction of the hypothetical structural protein decreasing linearly with growth rate. It is indeed the case for most proteins under

this growth modulation (Hui et al. (2015)). However, no explanation for the cell size - growth rate relationship for the two other growth modulations was proposed. Conversely, the multiple origins accumulation model is hard to reconcile with the useless expression data, as it will require a very strong increase of the $C + D$ duration as growth rate decreases. To explain the chloramphenicol data, the $C + D$ period must also change with growth rate in a certain way (more precisely, it should be inversely proportional to the growth rate), and it is unclear why this would be the case.

In summary, new data highlights that the relationship between cell size and growth rate is still poorly understood, and a theoretical framework connecting proteome allocation and division control, as well as the adder principle for size homeostasis, is still lacking. Here, we present a coarse-grained theory of bacterial physiology that unifies the proteome allocation theory with the structural model of division control. We show that it quantitatively predicts the size - growth rate relationships for the three growth modulations applied in Basan et al. (2015) with a single free parameter, that it is compatible with the observed changes in ribosomal proteome fractions, and that it correctly predicts the adder principle underlying cell size homeostasis.

Results

A simple coarse-grained model of bacterial growth, proteome allocation and cell division

We propose a simple coarse-grained model of bacterial growth, proteome allocation and cell division (Figure 1 and Methods). It considers three classes of proteins (E, R and X) and one class of protein precursors. E represent metabolic proteins, which transform external nutrients into protein precursors. R are ribosomal proteins, which transform precursors into proteins. X are structural proteins, which need to reach a certain amount before the cell can divide.

We note by f_E, f_R and f_X the fraction of the total protein synthesis devoted to each protein class. In other terms, and adopting a stochastic view, each time a new protein is created, with probability f_E it is a metabolic enzyme E, with probability f_R it is a ribosomal protein, etc. We assume that the protein synthesis allocation to structural proteins f_X is a constant, while the allocation of the remaining capacity is such that growth rate is maximal (Figure 1B). Under those assumptions, the steady-state growth rate is given by $\alpha = (1 - f_X) \frac{k}{\left(1+\sqrt{k/\sigma}\right)^2}$ (see Methods).

Note that our model is directly inspired from a recent work (Pandey and Jain (2016)), with the addition of cell division control via the additional class of structural proteins (X). Moreover, we make the assumption that cells maintain a constant mass density, meaning that cell volume and cell mass are proportional; and we choose our coarse-grained proteins and precursors such that they all have the same mass.

This minimalistic model has only four parameters: the nutritional efficiency k, which represents how fast one metabolic enzyme can produce one precursor in a given medium; the ribosomal efficiency σ, which represents how fast one ribosomal protein can produce one protein (at a saturating precursor concentration); the structural investment f_X, which represents the fraction of produced proteins that are structural proteins; and finally the division threshold X_{div}, which represents the amount of structural proteins triggering cell division.

An example stochastic simulation of the model is shown in Figure 1C. Balanced growth is reached after some time independently of the initial conditions (provided the minimal components are present), and mass fractions simply fluctuate around steady-state values of the deterministic model.

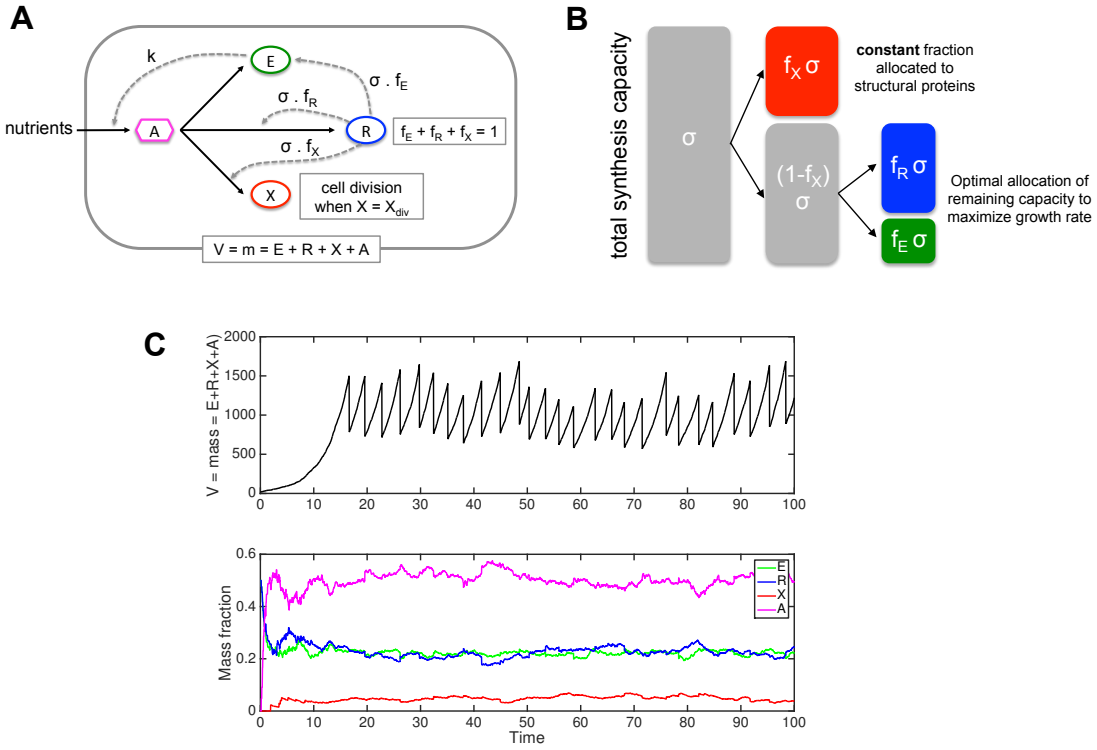

Figure 1: **A simple coarse-grained model of bacterial growth, proteome allocation and cell division. (A)** Schematic of the model. Metabolic proteins E transforms nutrient into protein precursors A. Precursors are transformed by ribosomal proteins R into either metabolic, ribosomal or structural (X) proteins. Division occurs when a threshold amount X_{dviv} of structural proteins is reached. Importantly, the total cell mass is the sum of all its components E, R, X, A, and cell volume is proportional to cell mass. **(B)** Allocation of total protein synthesis capacity between metabolic (E), ribosomal (R) and structural (X) proteins. A main model assumption is that the fraction allocated to structural proteins is constant, while the remaining capacity is optimally allocated between metabolic and ribosomal proteins. **(C)** Example stochastic simulation of the model. Parameters are $k = \sigma = 1$, $f_X = 0.1$, $X_{div} = 70$. At division only one of the daughter cells is kept, mimicking a mother machine.

Model quantitatively explains cell size change with growth rate for nutrient-based modulation

Despite its simplicity and low parameter number, the holistic nature of our model enables to reproduce various types of growth rate modulations. First, from a given, reference medium, increasing or decrease nutrient quality can be modeled by increasing or decreasing k (Figure 2). It can be shown that the resulting change in cell size as a function of the change in growth rate depends on a single parameter: the ratio $\frac{k}{\sigma}$ for the reference medium. Remarkably, fitting this single parameter leads to a quantitative agreement with the observed size - growth rate relationship observed for all media with different nutrient quality (Figure 3, green). Qualitatively, the size increase with nutrient quality arises because the precursor pool size (a) allowing a maximal growth rate increases with the ratio $\frac{k}{\sigma}$, hence reducing the concentrations of all proteins, including structural proteins. As a result, a higher size is required to reach the division threshold.

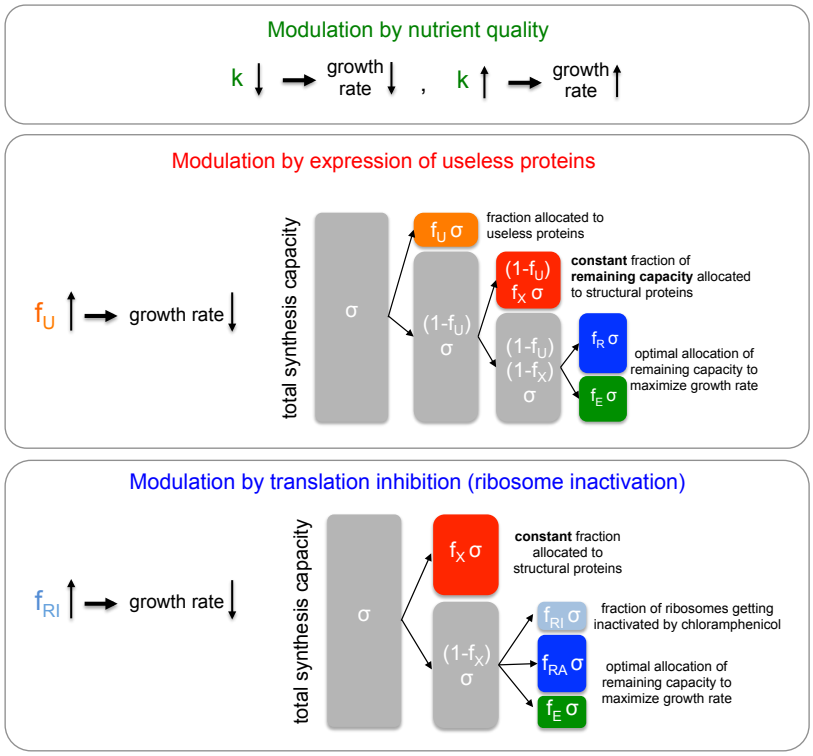

Figure 2: **Reproducing various growth limitations with our model.** Nutrient modulation is modeled by changing the nutritional efficiency k. Modulation by forced expression of useless proteins is modeled by assuming that a fraction f_U of the total synthesis capacity is allocated to useless proteins and that the remaining capacity is allocated between X, R and E as if it were the full synthesis capacity. Finally, we model the action of chloramphenicol by ribosome inactivation: a fraction of each newly synthetized ribosome gets inactivated. The synthesis of structural proteins is unperturbed, and we assume that the ability to maximize growth rate is not affected by ribosome inactivation (i.e., f_R will increase to maintain an optimal ratio $\frac{f_{RA}}{f_E}$).

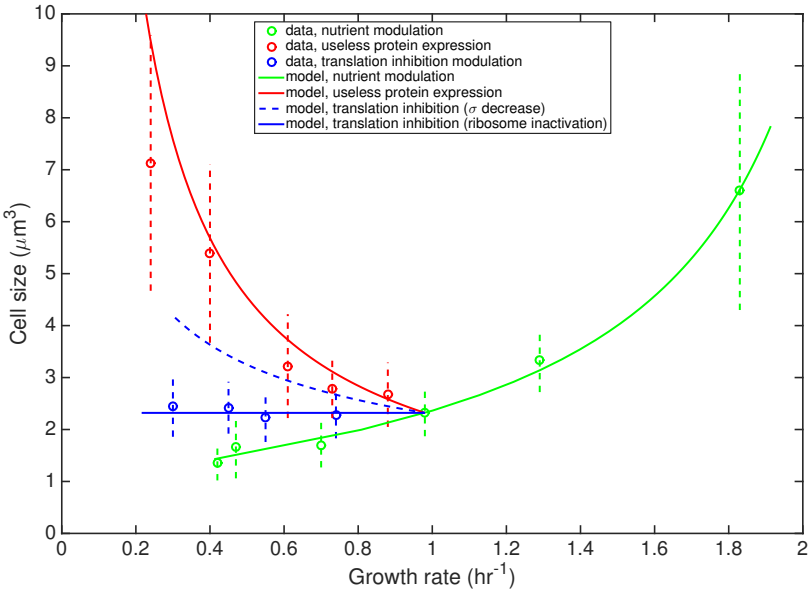

Figure 3: **Predicting the relationship between cell size and growth rate for the three growth modulations.** Data points and error bars are from Basan et al. (2015). These authors applied three growth modulations (nutrient modulation, green; forced expression of useless proteins, red; and translation inhibition with chloramphenicol, blue) from a reference medium (minimal media with glucose, growth rate $\sim 1\,hr^{-1}$) and measured the changes in cell size and growth rate. Our model quantitatively predicts the relationship between cell size change and growth rate change for all modulations after fitting a single parameter ($\frac{k_{ref}}{\sigma} = 3.14$) to the nutrient modulation curve.

Model quantitatively predicts cell size change with growth rate for forced expression of useless proteins

What would then our model predict for growth modulation by forced expression of useless proteins? First, the strength of the growth limitation will be given by the fraction f_U of newly produced proteins that are useless. Growth rate will decrease because 'useful' protein synthesis will be reduced to the fraction $f_X + f_E + f_R = 1 - f_U$ of total protein synthesis. But how the remaining fraction is allocated between X, E and R?

We reasoned that because the limitation consists in hijacking global synthesis capacity towards production of useless proteins, the relative synthesis of E, R and X should not be affected, but just reduced by the same ratio (i.e. $f_X = (1 - f_U) f_X^{ref}$, $f_E = (1 - f_U) f_E^{ref}$, etc., see Figure 2). This is consistent with Hui et al. (2015) data, which displayed a linear decrease of most protein fractions with growth rate for useless protein expression limitation. It is also expected mechanistically: the fractions $f_{X,E,R,U}$ can be interpreted as reflecting the relative levels of the mRNAs coding for the corresponding proteins, which are mechanically reduced by the same factor when useless protein mRNAs are produced. Strikingly, under that assumption, the observed strong size increase with decreasing growth rate is quantitatively predicted by the model (Figure 3, red curves). Qualitatively, cell size increases with f_U because the concentration x of structural proteins is reduced, hence requiring a higher size to reach the division threshold. In fact, here cell size scales with the inverse of the growth rate (see Methods).

Model quantitatively predicts cell size change with growth rate for translation inhibition

What would our model predict for growth modulation by translation inhibition? At first, it seems reasonable to model the effect of translation inhibition by chloramphenicol by decreasing the ribosomal efficiency σ, all other parameters remaining unchanged. Under that assumption, our model predicts a slight increase of cell size as growth rate decrease (Figure 3, dashed blue line), in disagreement with data, which displays almost no change of cell size.

We then reasoned that mechanistically, chloramphenicol inhibits translation by binding to and inactivating ribosomes, while the remaining active ribosomes can produce proteins at the normal rate. Thus, a more appropriate way to model the action of chloramphenicol is to assume that a fraction of each newly produced ribosome gets inactivated. Remarkably, if we further assume that the ratio between active ribosomes and metabolic enzymes remain optimal for growth (see Figure 2), our model then predicts that size should not change when growth rate is decreased by chloramphenicol, in quantitative agreement with data (Figure 3, solid blue curve).

In summary, with a single free parameter, our model can quantitatively explain the relationship between cell size and growth rate for three different kinds of growth modulation: change of nutrient quality, forced expression of useless proteins, and translation inhibition with chloramphenicol.

Model correctly predicts changes in proteome fractions

Our model can also predict the change in proteome composition for the three growth modulations applied in (Basan et al.) and for which proteome data also exist (Scott et al., 2010; Hui et al., 2015). Model predictions agree with observations (Figure 4): the ribosome protein fraction increases with growth rate for nutrient-based modulation, increases with decreasing growth rate for translation inhibition with chloramphenicol, and decreases with decreasing growth rate for forced expression of useless proteins.

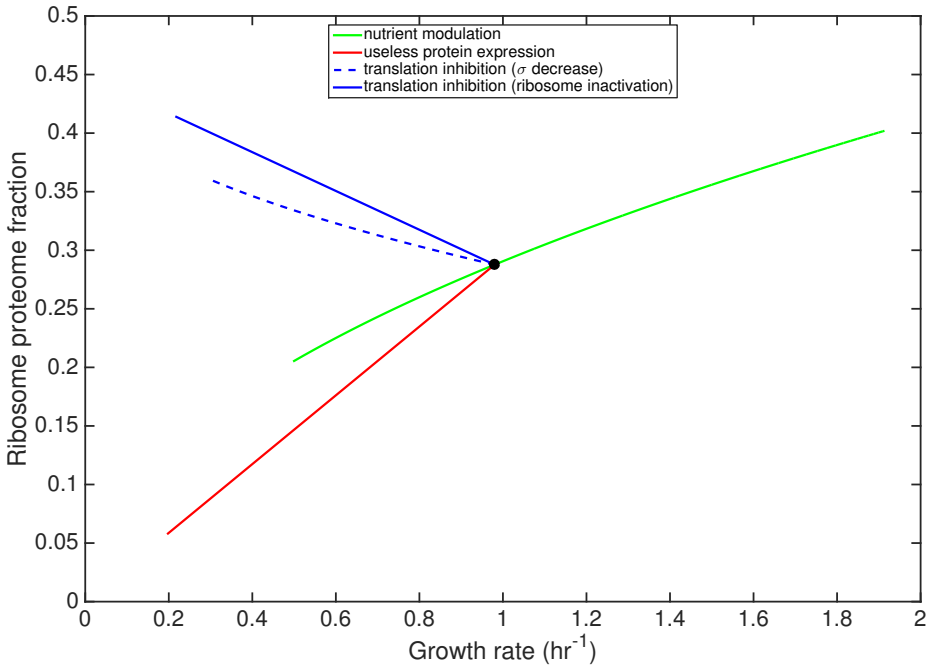

Figure 4: **Predicted changes in ribosomal protein fractions.** The black circle indicate the reference medium from which the growth modulations are applied. The same parameters as in Figure 3 were used. Additionally, f_X, which is not constrained by the size - growth rate relationship shown in Figure 3, was set to 0.55. For translation inhibition by chloramphenicol, both inactive and active ribosome were taken into account. Note that the proteome fraction can differ from the mass fraction because the precursor pool (A) accounts for the mass but is not part of the proteome.

Model predicts the adder principle of cell size homeostasis

To investigate the change of average cell size as a function of growth conditions (Figure 2 and 3), we have used our model at steady-state and in the deterministic limit. However, as already shown in Figure 1C, we can also perform dynamic and stochastic simulations of the model to study fluctuations around the steady-state, allowing us to adress the question of cell size homeostasis. Remarkably, simulations show that our model predicts the adder principle of cell size homeostasis (Figure 5).

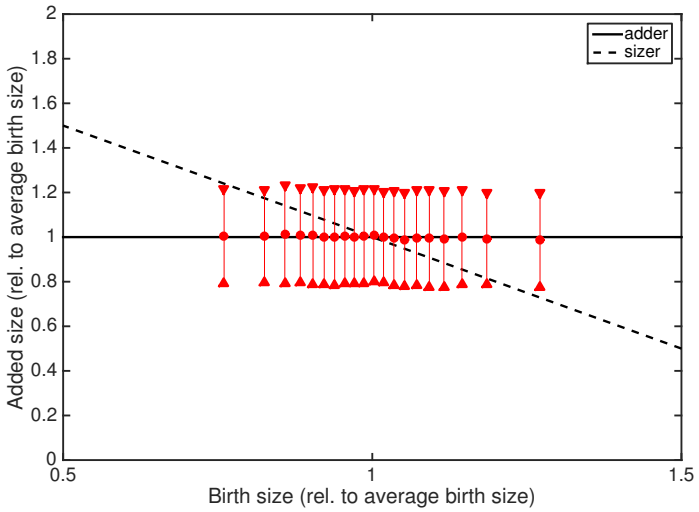

Figure 5: **Model predicts the adder principle of size homeostasis.** Added size as a function of birth size as predicted by the model. The same parameters as in Figure 1C were used. Simulation time was such that approximately 30K cell cycles were obtained. The variability of size at division (CV) was 11%. Cell cycles were binned according to birth size and the average (circles) and standard deviation (triangles) of the added size were computed.

Discussion

We have proposed a very simple coarse-grained model of bacterial physiology that unifies previous efforts to understand proteome allocation (Scott et al., 2010) and division control (Fantes et al., 1975). Remarkably, despite its simplicity, our model quantitatively predicts the relationship between cell size and growth rate for three types of growth rate modulations. More precisely, the size increase with growth rate for nutrient-based modulation is quantatively captured with a single parameter. In addition, both size - growth rate relationships for forced expression of useless protein and translation inhibition with chloramphenicol are quantitatively predicted. Thus, bacterial cell size can be explained using three fundamental principles: 1) a constant fraction of the protein synthesis capacity is allocated to structural proteins, 2) cell division can proceed as soon as a given amount of such structural proteins is produced, and 3) allocation of the remaining capacity between metabolic and ribosomal enzymes is regulated such that the growth rate is maximal.

The role of amino-acid pool size in cell size increase with increasing nutrient quality. The observation that cell size increases exponentially with growth rate for nutrient-based modulation (the 'Schaechter law') is very ancient (Schaechter, Maaloe, and Kjeldgaard (1958)) but the underlying mechanisms are still mysterious. In our model, cell size increase with increasing nutrient quality is a consequence of the optimal allocation between metabolic and ribosomal proteins: as already

noted by Scott and colleagues (Scott et al. (2014)), the resulting amino-acid pool size increases with the nutritional efficiency (k in our model). Together with a constant investment of the total protein synthesis capacity towards structural proteins, this leads to a cell size increase with growth rate in quantitative agreement with experimental data. This explanation seems quite different from the one proposed by A. Amir (2014): cells initiate DNA replication at an average size independent of growth rate and cell division events follow replication initiation events after a duration $(C + D)$ also independent of growth rate. Although our model does not account for DNA replication, it has predictive power for two other types growth limitations, and is more holistic: growth rate is not a parameter, it is instead predicted from parameters describing the nutritional efficiency of the medium (k), the maximum protein synthesis rate per ribosome (σ), the intensity of the forced expression of useless proteins (f_U) and the ribosome inactivation efficiency f_{RI} of chloramphenicol. Interestingly, the predicted relationship between cell size and growth rate is not strictly exponential. It follows an exponential trend when k and σ are comparable, but deviates from that trend when $k << \sigma$ or $k >> \sigma$. This prediction might be tested experimentally, although it might be difficult to reach such regimes.

Connection between the adder principle for size homeostasis, protein synthesis scaling with cell size, and the structural model of division control. In our model, the total rate of protein synthesis naturally scales with size as a result of balanced exponential growth: concentrations of proteins and precursors are steady, hence the protein synthesis rate per unit volume is $\sigma \times a \times r$. When considering a single protein (i.e., with negligible contribution to cell size) expressed in exponentially growing and dividing cells, it can be shown that if the synthesis rate of that protein scales with cell size, then its concentration is constant during the cell cycle. For such proteins, absolute amounts follow size, and added amounts follow added size. If a threshold amount acts as a trigger for cell division, we have found using stochastic simulations that size homeostasis in the presence of protein synthesis noise will be achieved via the adder principle, a result also recently obtained by others (Ghusinga, Vargas-Garcia, and Singh (2016)). Of note, as opposed to what has been often suggested, there is no need for destruction of the structural protein between divisions. Indeed, at steady-state, cells will start the cell cycle with half of the threshold and will have to reach the threshold, which is equivalent to start with zero and have to reach a threshold corresponding to half the initial threshold.

Structural proteins as DNA replication initiators or surface material. It seems that our simple model of division control is a powerful effective representation of the detailed mechanisms controlling cell division. In fact, it is likely that the control of DNA replication plays a major part in cell division control (Donachie and Blakely (2003), Wallden et al. (2016)). Perhaps that the structural proteins in our model correspond in part to proteins controlling DNA replication initiation. Recent single-cell data on DNA replication for cells growing in media of different quality (Wallden et al. (2016)) provided rich information on DNA replication control and its relationship with cell division. Notably, in slow growth conditions, the $C + D$ period was found to depend on growth rate and a deviation from the adder principle of size homeostasis was observed. Still, an issue with growth and division control models relying on DNA replication control is that they assume a highly phenomenological timer between replication initiation and cell division (the $C + D$ period). In fast growing cells, multiple instances of such timer can function in parallel without interferring with one another, and each timer is unaffected by division triggered by other timers. Because there is no model predicting how those timers behave for different growth rates and types of growth modulations, they cannot be readily used in general-purpose coarse-grained models of bacterial physiology. Alternatively, a recent study proposed that accumulation of surface material during the cell cycle could serve as a trigger for cell division (Harris and Theriot (2016)). Here, the structural proteins in our model would therefore localize at the cell surface. To investigate further this idea, our model can be extended to predict both volume and surface area, by describing which coarse-grained proteins are constituents of the cell surface.

Towards more mechanistic and detailed models. Because of its simplicity, holistic nature and predictive power, our unified coarse-grained theory of bacterial physiology could be used as a starting point for the construction of increasingly mechanistic and detailed whole-cell models (Shahrezaei and Marguerat (2015)). For example, key steps would be to model transcription and translation as separate processes, to introduce DNA replication, and to incorporate regulatory mechanisms responsible for the optimal allocation of the synthesis capacity. A guiding principle will then be to explain additional data describing the behavior of those processes while preserving the original predictive power.

Methods

Differential equations for absolute amounts

The model is defined by the following differential equations:

$\frac{dA}{dt} = kE - \sigma \frac{AR}{V}$

$\frac{dE}{dt} = f_E \sigma \frac{AR}{V}$

$\frac{dR}{dt} = f_R \sigma \frac{AR}{V}$

$\frac{dX}{dt} = f_X \sigma \frac{AR}{V}$

with $V = A + E + R + X$ and $f_E + f_R + f_X = 1$.

It follows that: $\frac{dV}{dt} = k\,E$.

Computing steady-state growth rate and mass fractions

We note the mass/volume fractions with lowercase (i.e. $a = A/V$, $e = E/V$, etc.).

Assuming balanced growth at a rate α, we obtain after several calculations:

$\alpha = \frac{\sigma\, f_R\, k\, f_E}{\sigma\, f_R + k\, f_E}$

$a = \frac{k\, f_E}{\sigma\, f_R + k\, f_E}$

$e = f_E\,(1-a)$, $r = f_R\,(1-a)$ and $x = f_X\,(1-a)$

Optimal allocation of synthesis between metabolic and ribosomal proteins

One can derive the expression of f_E^* and f_R^* maximizing the growth rate α (but respecting the constraint $f_E^* + f_R^* = 1 - f_X$). We find:

$f_E^* = (1 - f_X)\,\frac{1}{1+\sqrt{k/\sigma}}$ and $f_R^* = (1 - f_X)\,\frac{\sqrt{k/\sigma}}{1+\sqrt{k/\sigma}}$

The corresponding maximum growth rate is then $\alpha^* = (1 - f_X)\,\frac{k}{\left(1+\sqrt{k/\sigma}\right)^2}$

We also have $a^* = \frac{\sqrt{k/\sigma}}{1+\sqrt{k/\sigma}}$ and hence $x = f_X \frac{1}{1+\sqrt{k/\sigma}}$

Because we have $V_{div} = \frac{X_{div}}{x}$ we find that the size at division is: $V_{div} = \frac{X_{div}}{f_X}\left(1 + \sqrt{k/\sigma}\right)$.

Size and growth rate for useless protein expression

If f_U impacts f_X and $f_E + f_R$ equally, we have $f_X^U = (1 - f_U) f_X$ and $\alpha^{U,*} = (1 - f_U)\alpha^{ref,*}$

We also have $V_{div}^U = \frac{V_{div}^{ref}}{1-f_U} = V_{div}^{ref} \frac{\alpha^{ref,*}}{\alpha^{U,*}}$: size is now inversely proportional to growth rate.

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