Fundamental limits on the rate of bacterial growth

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Abstract Recent years have seen an experimental deluge interrogating the relationship between bacterial growth rate, cell size, and protein content, quantifying the abundance of proteins across growth conditions with unprecedented resolution. However, we still lack a rigorous understanding of what sets the scale of these quantities and when protein abundances should (or should not) depend on growth rate. Here, we seek to quantitatively understand this relationship across a collection of *Escherichia coli* proteomic data covering ≈ 4000 proteins and 36 growth rates. We estimate the basic requirements for steady-state growth by considering key processes in nutrient transport, energy generation, cell envelope biogenesis, and the central dogma. From these estimates, ribosome biogenesis emerges as a primary determinant of growth rate. We expand on this assessment by exploring a model of proteomic regulation as a function of the nutrient supply, revealing a mechanism that ties cell size and growth rate to ribosomal content.

Introduction

The observed range of bacterial growth rates is enormously diverse. In natural environments, some microbial organisms may double only once per year (***Mikucki et al., 2009***) while in comfortable laboratory conditions, growth can be rapid with several divisions per hour (***Schaechter et al., 1958***). This six order-of-magnitude difference in time scales of growth encompasses different microbial species and lifestyles, yet even for a single species such as *Escherichia coli*, the growth rate can be modulated over a comparably large scale by tuning the type and amount of nutrients in the growth medium (***Liu et al., 2005***). This remarkable plasticity in growth rate illustrates the intimate relationship between environmental conditions and the rates at which cells convert nutrients into new cellular material – a relationship that has remained a major topic of inquiry in bacterial physiology for over a century (***Jun et al., 2018***).

A key discovery in bacterial physiology of the past 70 years was the identification of bacterial "growth laws" (***Schaechter et al., 1958***); empirical relationships that relate the bacterial growth rate to the protein and RNA composition of the intracellular milieu in a number of different species. Over the past decade, a flurry of work (***Molenaar et al., 2009***; ***Scott et al., 2010***; ***Klumpp and Hwa, 2014***; ***Basan et al., 2015***; ***Dai et al., 2016***; ***Erickson et al., 2017***) have examined these growth laws at a quantitative level, developing a series of phenomenological models from which the growth laws naturally emerge. In parallel, a "molecular revolution" in biology has yielded an increasingly refined molecular census of the cell, particularly for bacteria such as the microbial workhorse *E. coli* (***Schmidt et al., 2016***; ***Davidi et al., 2016***). In light of the now expansive trove of quantitative biological data, we can revisit several of the evergreen questions about bacterial growth and physiology that were originally raised by microbiologists in the middle of the 20th century. Specifically, what biological processes are the primary determinants for how quickly bacterial cells can grow and

reproduce? Why do cells modulate the absolute numbers and relative ratios of their molecular constituents as a function of changes in growth rate or nutrient availability?

In this work, we begin by considering these two questions from two distinct angles. First, as a result of an array of high-quality proteome-wide measurements of *E. coli* under diverse growth conditions, we have generated a census that allows us to explore how the number of key molecular players change as a function of growth rate. Here, we have assembled a singular data set of protein copy numbers using measurements collected over the past decade via mass spectrometry (***Schmidt et al., 2016***; ***Peebo et al., 2015***; ***Valgepea et al., 2013***) or ribosomal profiling (***Li et al., 2014***) of the composition of the *E. coli* proteome across a gamut of growth rates. Due to notable changes in cell size and cellular composition as a function of growth rate (***Bremer and Dennis, 2008***; ***Taheri-Araghi et al., 2015***), as well as differences in normalization and standardization schemes used in each experimental work, substantial care was taken to ensure consistency on a per cellular basis (see the Appendix for a detailed analysis and additional discussion). To our knowledge, this compiled and curated dataset represents the most comprehensive view of the *E. coli* proteome, covering ≈ 4000 proteins and 36 unique growth rates, with the observed abundance of any given protein being directly comparable between data sets and across growth rates. This allows us to interrogate the *E. coli* specific physiology underlying the observed abundances while minimizing the effects of experimental noise, as ≈ 75% of the proteins are observed in at least two separate datasets.

Second, by compiling molecular turnover rate measurements for many of the fundamental processes associated with bacterial growth, we make quantitative estimates of a handful of key cellular processes (schematized in ***Figure 1***) to determine whether our current understanding of the dynamics of these processes are sufficient to explain the magnitude of the observed protein copy numbers across conditions (see **??** describing the philosophy behind this approach). The census, combined with these estimates, provide a window into the question of whether the rates of central processes such as energy generation or DNA synthesis vary systematically as a function of cell growth rate by altering protein copy number.

Throughout our estimates, we consider an archetypal growth rate of ≈ 0.5 hr^{-1} corresponding to a doubling time of ≈ 5000 seconds, as the data sets examined here heavily sample this growth regime. While we formulate point estimates for the protein abundances at this division time, we also consider how these values will vary at other growth rates due to changes in cell size, surface area, and chromosome copy number (***Taheri-Araghi et al., 2015***; ***Harris and Theriot, 2018***). For the majority of the processes considered, we find that the protein copy numbers appear tuned for the task of cell doubling across a continuum of growth rates. Thus, our understanding of the kinetics of various biological processes is sufficient to quantitatively explain the observed abundances of these proteins.

From these estimates, it emerges that translation, particularly the synthesis of ribosomal proteins is a plausible candidate that limits the rate of cellular growth in *E. coli*. We reach this conclusion by considering that ribosome synthesis is 1) a rate limiting step for the *fastest* bacterial division, and 2) the main determinant of bacterial growth rate across nutrient conditions associated with moderate to fast growth rates. In addition, a strict dependence between the maximal growth rate and ribosomal mass fraction coincides with the regime where the growth laws appear most valid (***Amir, 2017***; ***Scott et al., 2010***). This enables us to suggest that the long-observed correlation between growth rate and cell size (***Schaechter et al., 1958***; ***Si et al., 2017***) can be simply attributed to the increased absolute number of ribosomes per cell under conditions supporting extremely rapid growth. To better understand how the observed alterations in absolute protein abundances, and in particular, changes in ribosome copy number, influence growth rate across different nutrient conditions we consider a minimal model of cellular growth. Our conclusions from these analyses provide important insight into how *E. coli* regulates growth across conditions of differing nutrient availability and identifies fundamental constraints in bacterial growth more broadly.

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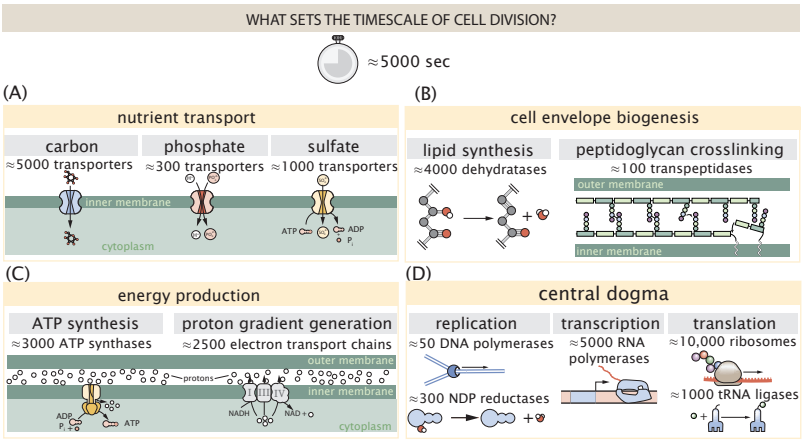

Figure 1. Transport and synthesis processes necessary for cell division. We consider an array of processes necessary for a cell to double its molecular components, broadly grouped into four classes. These categories are (A) nutrient transport across the cell membrane, (B) cell envelope biogenesis, (C) energy production (namely, ATP synthesis), and (D) processes associated with the central dogma. Numbers shown are the approximate number of complexes of each type observed at a growth rate of 0.5 hr^{-1}, or a cell doubling time of ≈ 5000 s.

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