Fundamental limitations on the rate of bacterial cell division

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1 Introduction

The range of bacterial growth rates can be enormous. In natural environments, doublings occur approximately once per year whereas in comfortable laboratory conditions, growth can be rapid with several divisions per hour. This remarkable diversity illustrates the intimate relationship between environmental conditions and the rates at which cells convert nutrients into new cellular material. This relationship between the environment and cellular growth rate has remained a major topic of inquiry in bacterial physiology for over a century (**?**). In 1958, Schaecter, Mall{oe, and Kjeldgaar reported the discovery of a strong, linear relationship between the total cellular protein content and growth rate, revealing a fundamental relationship between the total cellular protein content and the composition of the intracellular milieu (**?**). Over the past decade, a remarkable body of work has examined this relationship with single-protein resolution using modern methods of mass spectrometry (**???**) and ribosomal profiling (**?**) which permit a quantitative investigation of the relationship between gene expression and growth rate. This body of experimental data places us in the auspicious position to explore how the abundance of fundamental protein complexes are related to the growth rate of the population and interrogate what biological processes may set the growth rate in a particular growth condition.

In this work, we seek to leverage a collection of proteomic data sets of *Escherichia coli* across 31 growth conditions to quantitatively explore what biological processes may set the speed limit of bacterial growth. Broadly speaking, we entertain three classes of hypotheses illustrated in categories. First, we consider potential limits on the transport of nutrients into the cell. We address this hypothesis by performing an order-of-magnitude estimate for how many carbon atoms would be needed to build a cell and consider how many transporters may be needed to facilitate this requirement given a 6000 second division time. As a second hypothesis, we consider there exists a fundamental limit on how quickly the cell can generate ATP. We approach this hypothesis from two angles, considering how many ATP synthase complexes must be needed to churn out enough ATP to power protein translation followed by an estimation of how many electron transport complexes must be present to maintain the proton motive force. Our third and final class of hypotheses centers on the synthesis of a variety of biomolecules. Our focus is primarily on the stages of the central dogma as we estimate the number of protein complexes needed for DNA replication, transcription, and protein translation.

With estimates in hand for each of these processes, we turn to our collection of data sets to assess the accuracy of our estimates. In broad terms, we find that the majority of our estimates are exceeded by the experimental observations, allowing us to systematically scratch off the hypotheses diagrammed in categories as setting the speed limit. Ultimately, we find that protein translation (particularly the generation of new ribosomes) acts as the rate limiting step of bacterial division. We again leverage the quantitative nature of this data set and present a quantitative model of the relationship between the fraction of the proteome devoted to ribosomes and the speed limit of translation, revealing a fundamental tradeoff between the translation capacity of the ribosome pool and the maximal cellular growth rate.

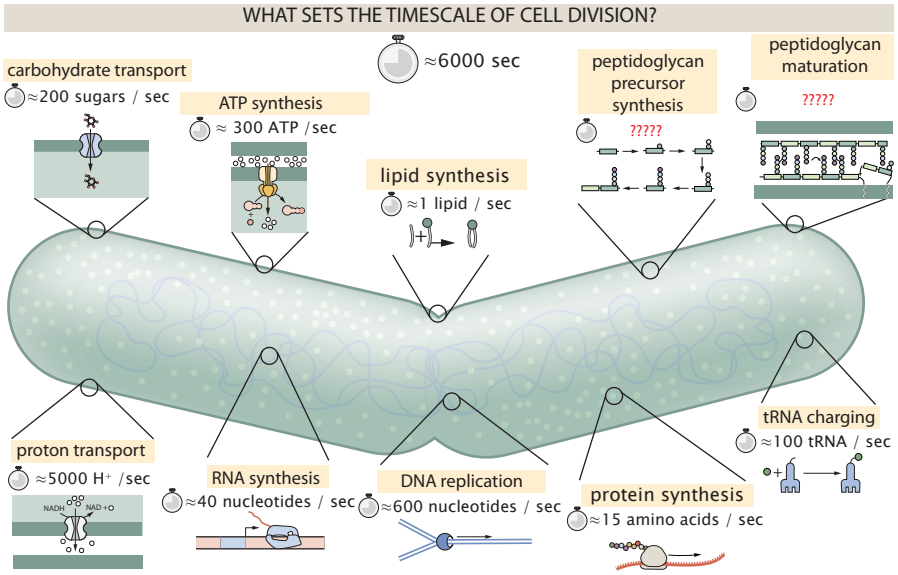

Figure 1: **Transport and synthesis processes necessary for cell division.** We consider an array of processes necessary for a cell to double its molecular components. Such processes include the transport of carbon across the cell membrane, the production of ATP, and fundamental processes of the central dogma namely RNA, DNA, and protein synthesis. A schematic of each synthetic or transport category is shown with an estimate of the rate per macromolecular complex. In this work, we consider a standard bacterial division time of $\approx$ 6000 sec.

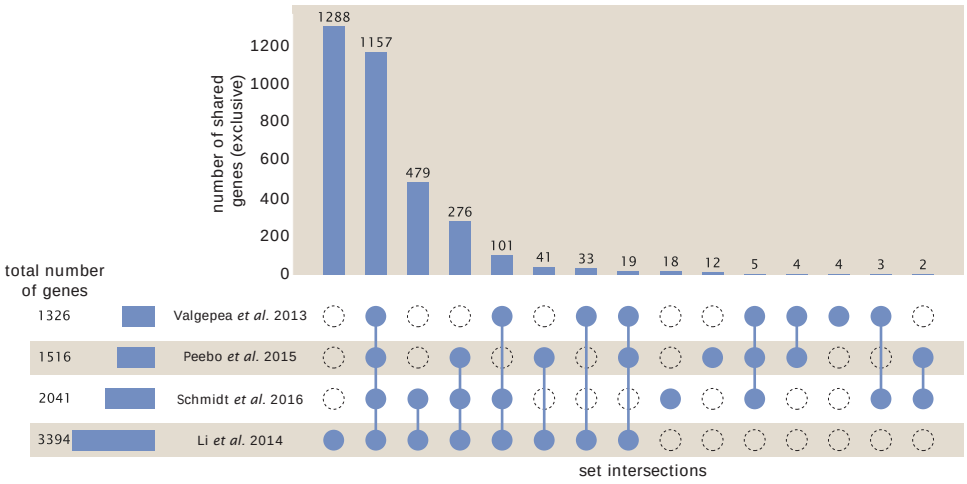

Figure 2: **Summary of the compiled datasets.**

2 A comprehensive examination of the *E. coli* proteome

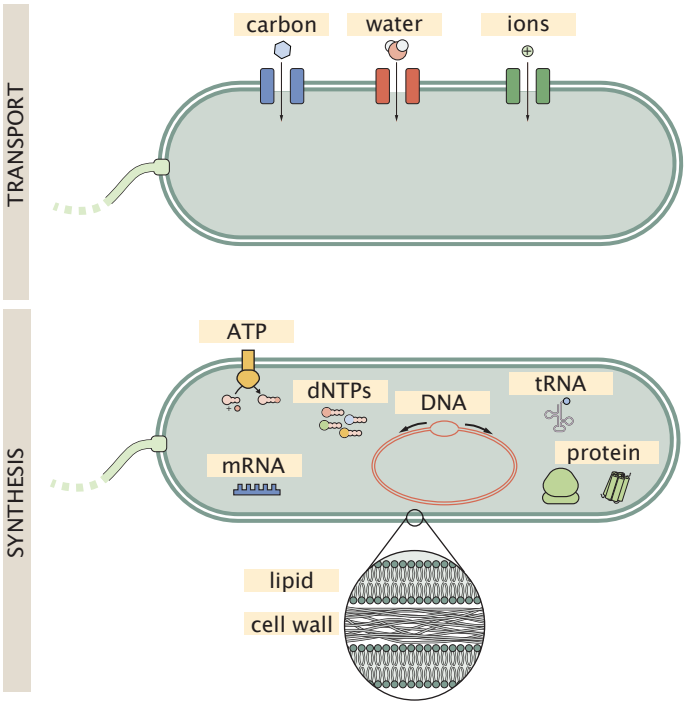

Figure 3: **Potential bottlenecks for bacterial growth.** The growth rate of *Escherichia coli* may be limited by the transport of biochemical precursors across the cell membrane (top panel) or by the synthesis of various molecules and macromolecules. Illustrative examples of the potential bottlenecks are provided in both panels.

3 Transport of Biochemical Building Blocks

We begin with an interrogation of some of the myriad transport systems bacteria use to bring extracellular materials (such as carbon, water, and ions) into the cell.

3.1 Carbon Transport

All macromolecules synthesized by cells include carbon as the primary elemental constituent. It is therefore reasonable to consider the acquisition of carbon from the environment, typically in the form of sugar, as a candidate process which sets the bacterial speed limit. We can a combination of biological intuition and the vast literature on the molecular composition of *E. coli* to make an order-of-magnitude estimate for the total number of sugar transporters needed double.

For convenience, we will consider a condition in which glucose is the primary carbon source in a growth medium. In standard laboratory conditions, a minimal medium supplemented with only glucose will have a growth rate of $\approx 1 \text{generation} \cdot \text{hr}^{-1}$ which is an approximate doubling time of ≈ 3000 seconds. During this time, the cell must be able to import enough carbon molecules to double all macromolecules.

We can begin by using the well justified estimate that the typical *E. coli* cell is $\approx 70\%$ water by mass, yielding a $\approx 30\%$ dry mass (BNID: 109049, **?**). Exponential growth of *E. coli* in a glucose based medium results in an average cell volume of 1 fL, bringing our total dry mass to 30 pg. Assuming half of this dry mass is protein (0.15 pg), we can estimate how many carbons are present in the protein pool.

Let's assume that a standard protein is approximately 300 amino acids long, which comes out a mass of ≈ 30 kDa. From this, we estimate that the total number of amino acids (incorporated into protein) is

$$N_{\text{AA}} = \left(1.5 \times 10^{-13}\,\text{g}\right) \times \left(\frac{1\,\text{protein}}{3 \times 10^4\,\text{Da}}\right) \times \left(\frac{6 \times 10^{23}\,\text{Da}}{1\,\text{g}}\right) \times \left(\frac{3 \times 10^2\,\text{AA}}{\text{protein}}\right) \approx 2.5 \times 10^8\,\text{Amino Acids}. \tag{1}$$

The typical amino acid consists of a two-carbon backbone with a ≈ 3 carbon side-chain. Thus, assuming the typical amino acid contains ≈ 5 carbons, the total mass of carbon in the protein pool can be calculated as

$$N_{\text{C}}^{(\text{protein})} = N_{\text{AA}} \times \frac{\text{C}}{\text{AA}} = 2.5 \times 10^8\,\text{AA} \times \frac{5\,\text{C}}{\text{AA}} \approx 5 \times 10^9\,\frac{\text{C}}{\text{cell}}. \tag{2}$$

Since we approximated that about half of the dry mass is protein, it's reasonable to assume that the remaining half of the dry mass has a similar composition, permitting us to say that

$$N_{\text{C}}^{(\text{cell})} \approx 2 \times N_{\text{C}}^{(\text{protein})} = 10^{10}\,\frac{\text{C}}{\text{cell}}. \tag{3}$$

With a handle on the total number of carbons needed per cell, we can now try to estimate how many sugar transporters would be needed to transport 100 billion carbon atoms per standard cell doubling time of ≈ 3000 seconds. With 6 carbon atoms per glucose molecule, we can estimate the minimum glucose flux across the membrane to be

$$J_{\text{glucose}} = \frac{10^{10}\,\text{C}}{\text{cell}} \times \frac{1\,\text{glucose}}{6\,\text{C}} \times \frac{1\,\text{cell}}{3 \times 10^3\,\text{s}} \approx 5 \times 10^6 \frac{\text{glucose}}{\text{s}}. \tag{4}$$

The chief glucose transport system of *E. coli* (the PTS system) can transport $\approx 200\,\text{glucose} \cdot \text{s}^{-1}$ (BNID: 103693 **?**) meaning that the *minimum* number of glucose transporters per cell needed to double the cellular carbon content is

$$N_{\text{tpr.}} \approx \frac{J_{\text{glucose}}}{J_{\text{transporter}}} = \frac{5 \times 10^6 \text{glucose / s}}{2 \times 10^2\,\text{glucose / s}} \approx 2 \times 10^4\,\text{tpr. / cell}. \tag{5}$$

3.2 Water Transport