Fundamental limits on the rate of bacterial cell division

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Abstract

Introduction

The range of bacterial growth rates is enormously diverse. In natural environments, some microbial organisms might double only once per year while in comfortable laboratory conditions, growth can be rapid with several divisions per hour. This six order of magnitude difference 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***). As was noted by Jacques Monod, "the study of the growth of bacterial cultures does not constitute a specialized subject or branch of research, it is the basic method of Microbiology." Those words ring as true today as they did when they were written 70 years ago. Indeed, the study of bacterial growth has undergone a molecular resurgence since many of the key questions addressed by the pioneering efforts in the middle of the last century can be revisited by examining them through the lens of the increasingly refined molecular census that is available for bacteria such as the microbial workhorse *Escherichia coli*. Several of the outstanding questions that can now be studied about bacterial growth include: what sets the fastest time scale that bacteria can divide, and how is growth rate tied to the quality of the carbon source. In this paper, we address these two questions from two distinct angles. First, as a result of an array of high-quality proteome-wide measurements of the *E. coli* proteome under a myriad of different growth conditions, we have a census that allows us to explore how the number of key molecular players change as a function of growth rate. This census provides a window onto whether the processes they mediate such as molecular transport into the cells and molecular synthesis within cells can run faster. Second, because of our understanding of the molecular pathways responsible for many of the steps in bacterial growth, we can also make order of magnitude estimates to infer the copy numbers that would be needed to achieve a given growth rate. In this paper, we pass back and forth between the analysis of a variety of different proteomic datasets and order-of-magnitude estimations to determine possible molecular bottlenecks that limit bacterial

growth and to see how the growth rate varies in different carbon sources.

Specifically, we leverage a combination of *E. coli* proteomic data sets collected over the past decade using either mass spectrometry (**Schmidt et al., 2016**; **Peebo et al., 2015**; **Valgepea et al., 2013**) or ribosomal profiling (**Li et al., 2014**) across 31 unique growth conditions. Broadly speaking, we entertain several classes of hypotheses as are illustrated in **Figure 1**. 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, phosphorous, and sulfur atoms are needed to facilitate this requirement given a 5000 second division time. As a second hypothesis, we consider the possibility that 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. A third class of estimates considers the need to maintain the size and shape of the cell through the construction of new lipids for the cell membranes as well as the glycan polymers which make up the rigid peptidoglycan. Our 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.

In broad terms, we find that the majority of these estimates are in close agreement with the experimental observations, with protein copy numbers apparently well-tuned for the task of cell doubling. This allows us to systematically scratch off the hypotheses diagrammed in **Figure 1** as setting possible speed limits. Ultimately, we find that protein translation (particularly the generation of new ribosomes) acts as 1) a rate limiting step for the *fastest* bacterial division, and 2) the major determinant of bacterial growth across all nutrient conditions we have considered under steady state, exponential growth. This perspective is in line with the linear correlation observed between growth rate and ribosomal content (typically quantified through the ratio of RNA to protein) for fast growing cells (**Scott et al., 2010**), but suggests a more prominent role for ribosomes in setting the doubling time across all conditions of nutrient limitation. Here 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 growth rate.

Uptake of Nutrients

In order to build new cellular mass, the molecular and elemental building blocks must be scavenged from the environment in different forms. Carbon, for example, is acquired via the transport of carbohydrates and sugar alcohols with some carbon sources receiving preferential treatment in their consumption (**Monod, 1947**). Phosphorus, sulfur, and nitrogen, on the other hand, are harvested primarily in the forms of inorganic salts, namely phosphate, sulfate, and ammonia (**Jun et al., 2018**; **Assentoft et al., 2016**; **Stasi et al., 2019**; **Antonenko et al., 1997**; **Rosenberg et al., 1977**; **Willsky et al., 1973**). All of these compounds have different permeabilities across the cell membrane and most require some energetic investment either via ATP hydrolysis or through the proton electrochemical gradient to bring the material across the hydrophobic cell membrane. Given the diversity of biological transport mechanisms and the vast number of inputs needed to build a cell, we begin by considering transport of some of the mot important cellular ingredients: carbon, nitrogen, oxygen, hydrogen, phosphorus, and sulfur.

The elemental composition of *E. coli* has received much quantitative attention over the past half century (**Neidhardt et al., 1991**; **Taymaz-Nikerel et al., 2010**; **Heldal et al., 1985**; **Bauer and Ziv, 1976**), providing us with a starting point for estimating the copy numbers of various transporters. While there is some variability in the exact elemental percentages (with different uncertainties), we can estimate that the dry mass of a typical *E. coli* cell is ≈ 45% carbon (BNID: 100649, **Milo et al.** (**2010**)), ≈ 15% nitrogen (BNID: 106666, **Milo et al.** (**2010**)), ≈ 3% phosphorus (BNID:

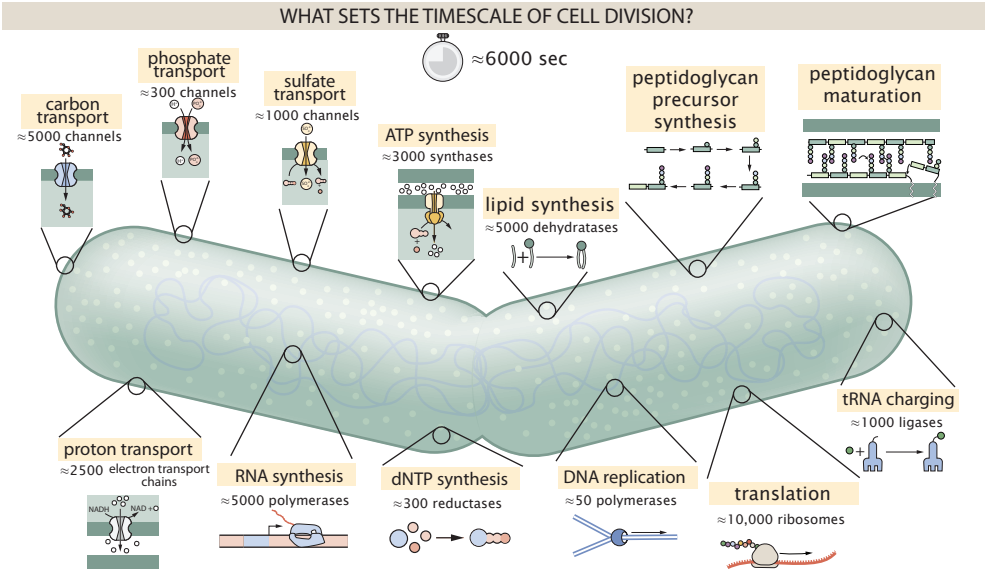

Figure 1. Transport and synthesis processes necessary for cell division. We consider an array of processes necessary for a cell to double its molecular components. Such processes include the transport of carbon across the cell membrane, the production of ATP, and fundamental processes of the central dogma namely RNA, DNA, and protein synthesis. A schematic of each synthetic or transport category is shown with an approximate measure of the complex abundance at a growth rate of 0.5 per hour. In this work, we consider a standard bacterial division time of ≈ 5000 sec.

100653, ***Milo et al.*** (***2010***)), and 1% sulfur (BNID: 100655, ***Milo et al.*** (***2010***)). In the coming paragraphs, we will engage in a dialogue between back-of-the-envelope estimates for the numbers of transporters needed to facilitate these chemical stoichiometries and the experimental proteomic measurements of the biological reality. Such an approach provides the opportunity to test if our biological knowledge is sufficient to understand the scale at which these complexes are produced. Specifically, we will make these estimates considering a modest doubling time of 5000 s, a growth rate of ≈ 0.5 hr^{-1}, the range in which the majority of the experimental measurements reside.

Carbon Transport

We begin with the most abundant element by mass, carbon. Using ≈ 0.3 pg as the typical *E. coli* dry mass (BNID: 103904, ***Milo et al.*** (***2010***)), we estimate that $\approx 10^{10}$ carbon atoms must be brought into the cell in order to double all of the carbon-containing molecules (***Figure 2***(A, top))). Typical laboratory growth conditions, such as those explored in the aforementioned proteomic data sets, provide carbon as a single class of sugar such as glucose, galactose, or xylose to name a few. *E. coli* has evolved myriad mechanisms by which these sugars can be transported across the cell membrane. One such mechanism of transport is via the PTS system which is a highly modular system capable of transporting a diverse range of sugars (***Escalante et al., 2012***). The glucose-specific component of this system transports ≈ 200 glucose molecules per second per channel (BNID: 114686, ***Milo et al.*** (***2010***)). Making the assumption that this is a typical sugar transport rate, coupled with the need to transport 10^{10} carbon atoms, we arrive at the conclusion that on the order of 1,000 transporters must be expressed in order to bring in enough carbon atoms to divide in 6,000 s, diagrammed in the top panel of ***Figure 2***(A). This estimate, along with the observed average number of carbohydrate transporters present in the proteomic data sets (***Schmidt et al., 2016***; ***Peebo et al., 2015***; ***Valgepea et al., 2013***; ***Li et al., 2014***), is shown in ***Figure 2***(A). While we estimate 1,000 transporters are needed, the data reveals that at a division time of ≈ 5000 s there is nearly a ten-fold excess of transporters. Furthermore, the data illustrates that the average number of carbohydrate transporters present is largely-growth rate independent.

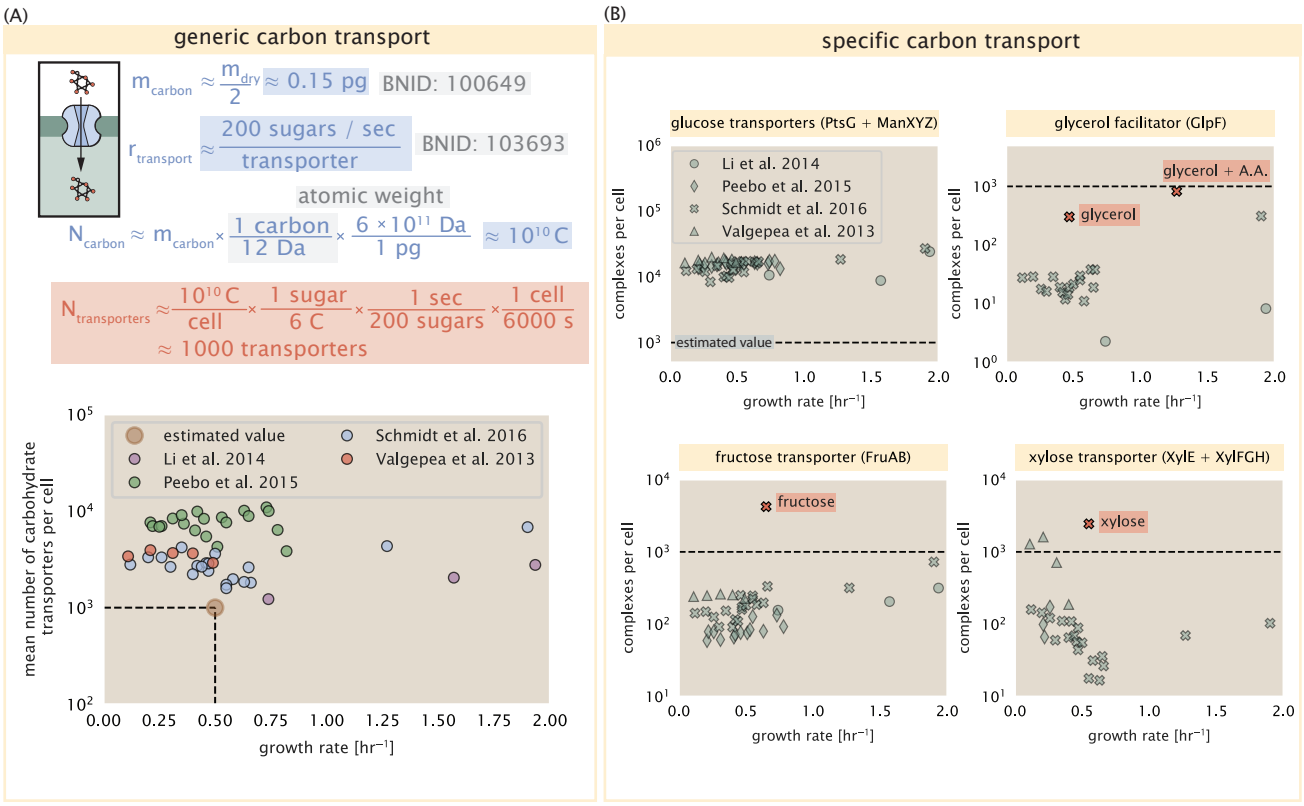

Figure 2. The abundance of carbon transport systems across growth rates. (A) A simple estimate for the minimum number of generic carbohydrate transport systems (top) assumes $\approx 10^{10}$ C are needed to complete division, each transported sugar contains ≈ 6 C, and each transporter conducts sugar molecules at a rate of ≈ 200 per second. Bottom plot shows the estimated number of transporters needed at a growth rate of ≈ 0.5 per hr (light-brown point and dashed lines). Colored points correspond to the mean number of carbohydrate transporters for different growth conditions across different published datasets. (B) The abundance of various specific carbon transport systems plotted as a function of the population growth rate. Red points and red-highlighted text indicate conditions in which the only source of carbon in the growth medium induces expression of the transport system.

The estimate presented in ***Figure 2***(A) neglects any specifics of the regulation of carbon transport system and presents a data-averaged view of how many carbohydrate transporters are present on average. Using the diverse array of growth conditions explored in the proteomic data sets, we can explore how individual carbon transport systems depend on the population growth rate. In ***Figure 2***(B), we show the total number of carbohydrate transporters specific to different carbon sources. A striking observation, shown in the top-left plot of ***Figure 2***(B), is the constancy in the expression of the glucose-specific transport systems (the PtsG enzyme of the PTS system and the glucose-transporting ManXYZ complex). Additionally, we note that the total number of glucose-specific transporters is tightly distributed $\approx 10^4$ per cell, an order of magnitude beyond the estimate shown in ***Figure 2***(A). This illustrates that *E. coli* maintains a substantial number of complexes present for transporting glucose which is known to be the preferential carbon source (***Monod, 1947***; ***Liu et al., 2005***; ***Aidelberg et al., 2014***).

It is now understood that a large number of metabolic operons are regulated with dual-input logic gates that are only expressed when glucose concentrations are low (mediated by cyclic-AMP receptor protein CRP) and the concentration of other carbon sources are elevated (***Gama-Castro et al., 2016***; ***Zhang et al., 2014b***). A famed example of such dual-input regulatory logic is in the regulation of the *lac* operon which is only natively activated in the absence of glucose and the presence of allolactose, an intermediate in lactose metabolism (***Jacob and Monod, 1961***), though we now know of many other such examples (***Ireland et al., 2020***; ***Gama-Castro et al., 2016***; ***Belliveau et al., 2018***). This illustrates that once glucose is depleted from the environment, cells have a means to dramatically increase the abundance of the specific transporter needed to digest the next sugar that is present. Several examples of induced expression of specific carbon-source transporters are shown in ***Figure 2***(B). Points colored in red (labeled by red text-boxes) correspond to growth conditions in which the specific carbon source (glycerol, xylose, or fructose) is present. These plots show that, in the absence of the particular carbon source, expression of the transporters is maintained on the order of $\sim 10^2$ per cell. However, when induced, the transporters become highly-expressed and are present on the order of $\sim 10^4$ per cell, which exceeds the generic estimate given in ***Figure 2***(A). Together, this generic estimation and the specific examples of induced expression suggest that transport of carbon across the cell membrane, while critical for growth, is not the rate-limiting step of cell division.

In the context of speeding up growth, one additional limitation is the fact that the cell's inner membrane is occupied by a multitude of other membrane proteins. Considering a rule-of-thumb for the surface area of *E. coli* of about 6 μm^2 (BNID: 101792, ***Milo et al.*** (***2010***)), we expect an areal density for 1,000 transporters to be approximately 200 transporters/μm^2. For a glucose transporter occupying about 50 nm^2/dimer, this amounts to about only 1 percent of the total inner membrane (***Szenk et al., 2017***). In addition, bacterial cell membranes typically have densities of 10^5 proteins//μm^2 (***Phillips, 2018***), implying that the cell could accommodate more transporters if it were rate limiting.

Phosphorus and Sulfur Transport

We now turn our attention towards other essential elements, namely phosphorus and sulfur. Phosphorus is critical to the cellular energy economy in the form of high-energy phosphodiester bonds making up DNA, RNA, and the NTP energy pool as well as playing a critical role in the post-translational modification of proteins and defining the polar-heads of lipids. In total, phosphorus makes up ≈3% of the cellular dry mass which in typical experimental conditions is in the form of inorganic phosphate. The cell membrane has remarkably low permeability to this highly-charged and critical molecule, therefore requiring the expression of active transport systems. In *E. coli*, the proton electrochemical gradient across the inner membrane is leveraged to transport inorganic phosphate into the cell (***Rosenberg et al., 1977***). Proton-solute symporters are widespread in *E. coli* (***Ramos and Kaback, 1977***; ***Booth et al., 1979***) and can have rapid transport rates of 50 molecules per second for sugars and other solutes (BNID: 103159; 111777, ***Milo et al.*** (***2010***)). In *E. coli* the PitA phosphate

transport system has been shown to very tightly coupled with the proton electrochemical gradient with a 1:1 proton:phosphate stoichiometric ratio (***Harris et al., 2001***; ***Feist et al., 2007***). Illustrated in ***Figure 3***(A), we can estimate that ≈ 300 phosphate transporters are necessary to maintain an ≈ 3% dry mass with a 6,000 s division time. This estimate is again satisfied when we examine the observed copy numbers of PitA in proteomic data sets (plot in ***Figure 3***(A)). While our estimate is very much in line with the observed numbers, we emphasize that this is likely a slight over estimate of the number of transporters needed as there are other phosphorous scavenging systems, such as the ATP-dependent phosphate transporter Pst system which we have neglected.

Satisfied that there are a sufficient number of phosphate transporters present in the cell, we now turn to sulfur transport as another potentially rate limiting process. Similar to phosphate, sulfate is highly-charged and not particularly membrane permeable, requiring active transport. While there exists a H+/sulfate symporter in *E. coli*, it is in relatively low abundance and is not well characterized (***Zhang et al., 2014a***). Sulfate is predominantly acquired via the ATP-dependent ABC transporter CysUWA system which also plays an important role in selenium transport (***Sekowska et al., 2000***; ***Sirko et al., 1995***). While specific kinetic details of this transport system are not readily available, generic ATP transport systems in prokaryotes transport on the order of 1 to 10 molecules per second (BNID: 109035, ***Milo et al.*** (***2010***)). Combining this generic transport rate, measurement of sulfur comprising 1% of dry mass, and a 6,000 second division time yields an estimate of ≈ 1000 CysUWA complexes per cell (***Figure 3***(B)). Once again, this estimate is in notable agreement with proteomic data sets, suggesting that there are sufficient transporters present to acquire the necessary sulfur. In a similar spirit of our estimate of phosphorus transport, we emphasize that this is likely an overestimate of the number of necessary transporters as we have neglected other sulfur scavenging systems that are in lower abundance.

Nitrogen Transport

Finally, we turn to nitrogen transport as the last remaining transport system highlighted in ***Figure 1***. Unlike phosphate, sulfate, and various sugar molecules, nitrogen in the form of ammonia can readily diffuse across the cell membrane and has a permeability on par with water ($\approx 10^5$ nm/s, BNID:110824 ***Milo et al.*** (***2010***)). In particularly nitrogen-poor conditions, *E. coli* expresses a transporter (AmtB) which appears to aid in nitrogen assimilation, though the mechanism and kinetic details of transport is still a matter of debate (***van Heeswijk et al., 2013***; ***Khademi et al., 2004***). Beyond ammonia, another plentiful source of nitrogen come in the form of glutamate, which has it's own complex metabolism and scavenging pathways. However, nitrogen is plentiful in the growth conditions examined in this work, permitting us to neglect nitrogen transport as a potential rate limiting process in cell division.

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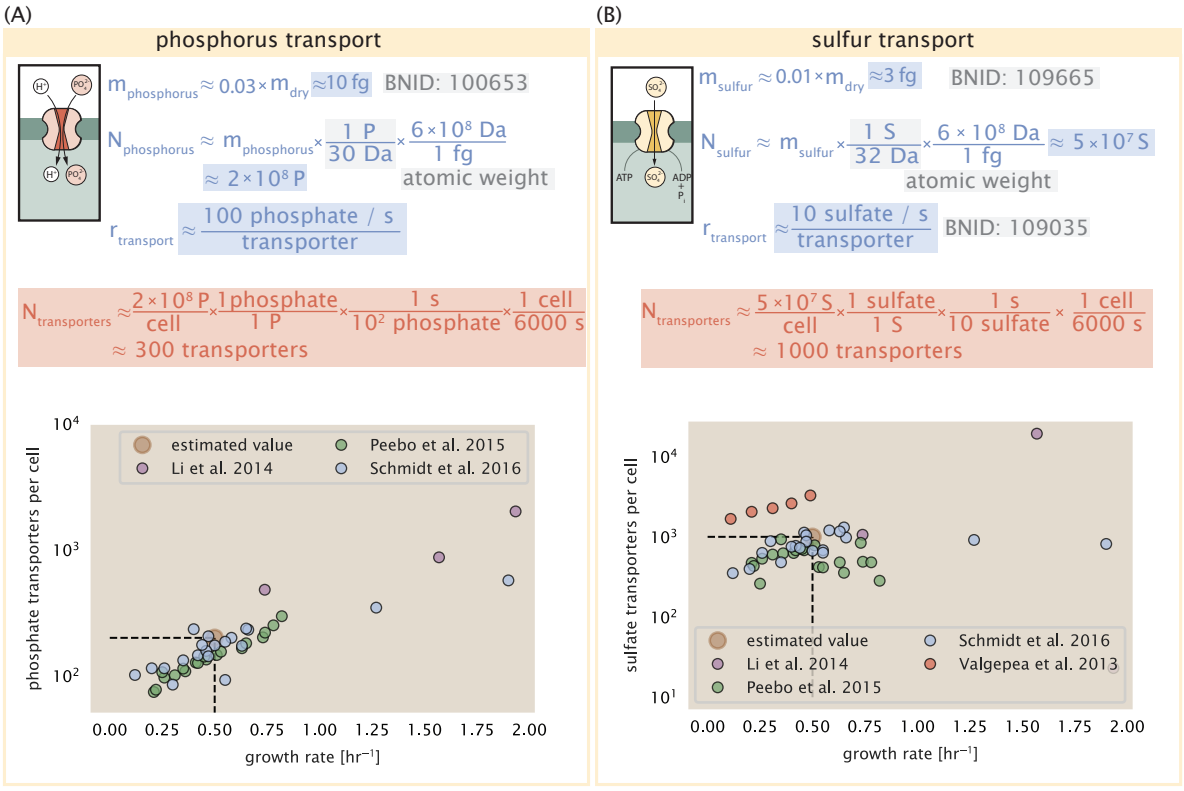

Figure 3. Estimates and measurements of phosphate and sulfate transport systems as a function of growth rate. (A) Estimate for the number of PitA phosphate transport systems needed to maintain a 3% phosphorus *E. coli* dry mass. Points in plot correspond to the the total number of PitA transporters per cell. (B) Estimate of the number of CysUWA complexes necessary to maintain a 1% sulfur *E. coli* dry mass. Points in plot correspond to average number of CysUWA transporter complexes that can be formed given the transporter stoichiometry $[CysA]_2[CysU][CysW][Sbp/CysP]$.

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