

Fundamental limits on the rate of bacterial cell division

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Abstract

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 (Jun *et al.*, 2018). In 1958, Schaecter, Malløe, and Kjeldgaard reported the discovery of a strong, linear dependence of the total cellular protein content on the growth rate, revealing a fundamental relationship between the environment and the composition of the intracellular milieu (Schaechter *et al.*, 1958). Over the past decade, a remarkable body of work has examined this relationship with single-protein resolution using modern methods of mass spectrometry (Valgepea *et al.*, 2013; Peebo *et al.*, 2015; Schmidt *et al.*, 2016) and ribosomal profiling (Li *et al.*, 2014) 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 speed limit of bacterial growth.

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 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 atoms 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

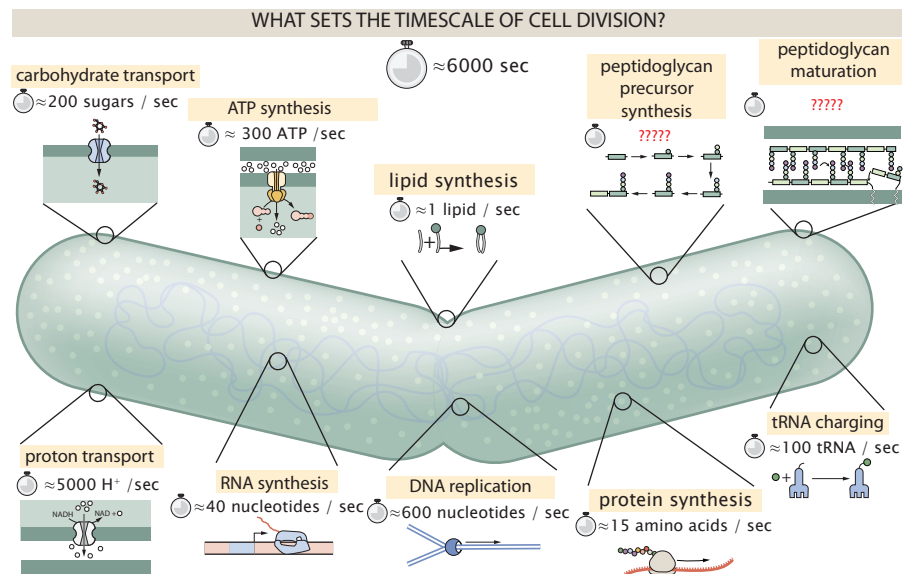


Figure 1. Transport and synthesis processes necessary for cell division. We consider an array of processes necessary for a cell to double its molecular components. Such processes include the transport of carbon across the cell membrane, the production of ATP, and fundamental processes of the central 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 ≈ 6000 sec.

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 **Figure 1** 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.

Nutrient Transport

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 atoms present in the cell, we begin by considering transport of elemental requirements as a possible rate-limiting step of

68 bacterial cell division.

69 The elemental composition of *E. coli* has received much quantitative attention over the past
 70 half century (Neidhardt et al., 1991; Taymaz-Nikerel et al., 2010; Heldal et al., 1985; Bauer and Ziv,
 71 1976), providing us a starting off-point for estimating the copy numbers of various transporters.
 72 While there is some variability in the exact percentages (with different uncertainties), we can esti-
 73 mate that the dry mass of a typical *E. coli* cell is $\approx 45\%$ carbon (BNID: 100649, Milo et al. (2010)), \approx
 74 15% nitrogen (BNID: 106666, Milo et al. (2010)), $\approx 3\%$ phosphorus (BNID: 100653, Milo et al. (2010)),
 75 and 1% sulfur (BNID: 100655, Milo et al. (2010)). In the coming paragraphs, we will examine how
 76 many transporters and/or channels must be present to maintain these elemental compositions
 77 with a moderate doubling time of 6000 s.

78 Carbon Transport

79 We begin with the most abundant element by mass, carbon. Using ≈ 0.3 pg as the typical *E. coli*
 80 dry mass (BNID: 103904, Milo et al. (2010)), we estimate that $\approx 10^{10}$ carbon atoms must be brought
 81 into the cell in order to double all of the carbon-containing molecules (Figure 2(A, top)). Typical
 82 laboratory growth conditions, such as those explored in the aforementioned proteomic data sets,
 83 provide carbon as single class of sugar such as glucose, galactose, or xylose to name a few of many.
 84 *E. coli* has evolved myriad mechanisms by which these sugars can be transported across the cell
 85 membrane. One such mechanism of transport is via the PTS system which is highly modular system
 86 capable of transporting a diverse range of sugars (Escalante et al., 2012). The glucose-specific
 87 component of this system is highly-conductive, transporting ≈ 200 glucose molecules per second
 88 per channel (BNID: 114686, Milo et al. (2010)). Making the assumption that this is a typical sugar
 89 transport rate, coupled with the need to transport 10^{10} carbon atoms, we arrive at the conclusion
 90 that on the order of 1000 transporters must be expressed in order to bring in enough carbon
 91 atoms to divide in 6000 s, diagrammed in the top panel of Figure 2(A). This estimate, along with
 92 the observed average number of carbohydrate transporters present in the proteomic data sets
 93 (Schmidt et al., 2016; Peebo et al., 2015; Valgepea et al., 2013; Li et al., 2014), is shown in Figure 2(A).
 94 While we estimate 1000 transporters are needed, the data reveals that at a division time of ≈ 6000 s
 95 there is nearly a ten-fold excess of transporters. Furthermore, the data illustrates that the average
 96 number of carbohydrate transporters present is largely-growth rate independent.

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

109 It is now understood that a large number of metabolic operons are regulated with dual-input
 110 logic gates which are only expressed when glucose concentrations are low (mediated by cyclic-AMP
 111 receptor protein CRP) and the concentration of other carbon sources are elevated (Gama-Castro
 112 et al., 2016; Zhang et al., 2014b). A famed example of such dual-input regulatory logic is in the regu-
 113 lation of the *lac* operon which is only natively activated in the absence of glucose and the presence
 114 of allolactose, an intermediate in lactose metabolism (Jacob and Monod, 1961), though we now
 115 know of many other such examples (Ireland et al., 2020; Gama-Castro et al., 2016; Belliveau et al.,
 116 2018). This illustrates that once glucose is depleted from the environment, cells have a means to
 117 dramatically increase the abundance of the specific transporter needed to digest the next sugar

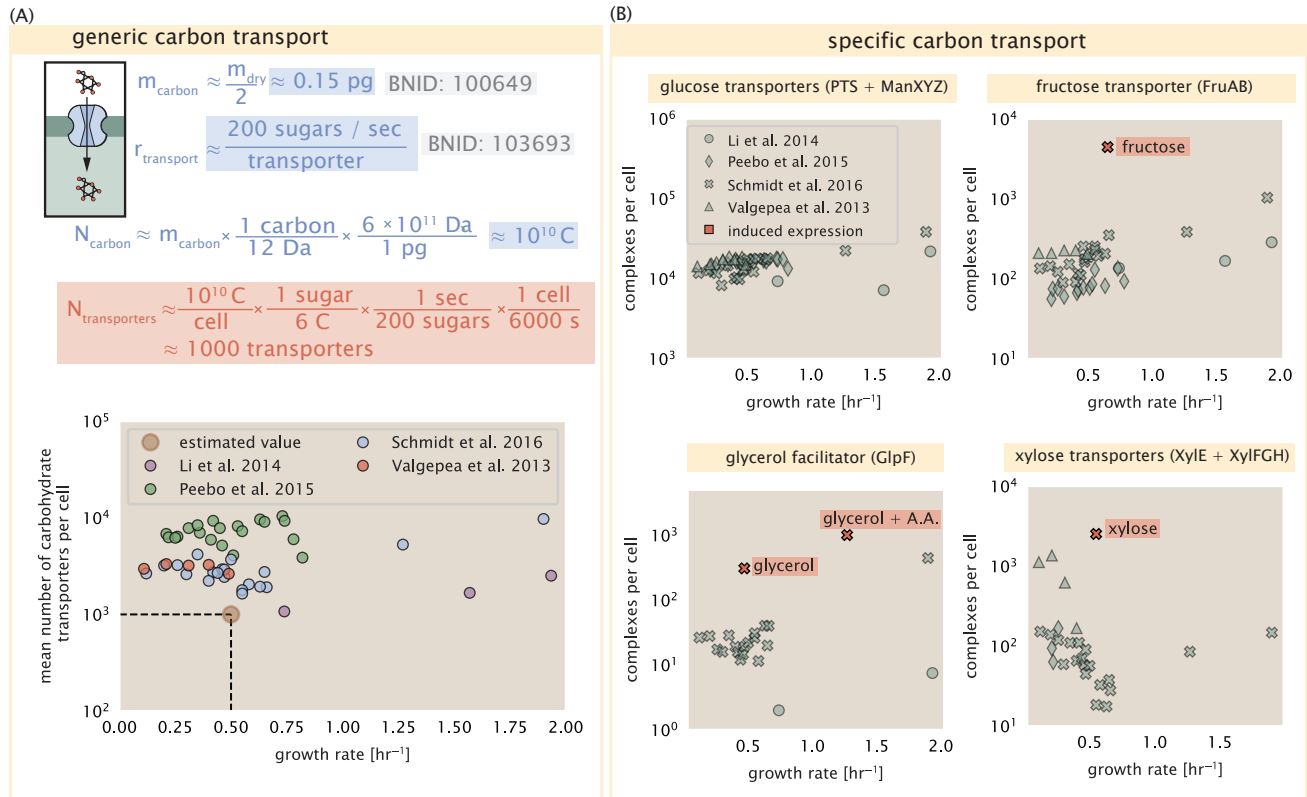


Figure 2. The abundance of carbon transport systems across growth rates. (A) A simple estimate for the minimum number of generic carbohydrate transport systems (top) assumes $\approx 10^{10}$ C are needed to complete division, each transported sugar contains ≈ 6 C, and each transporter conducts sugar 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.

that is present. Several examples of induced expression of a 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 in abundance. 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.

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 XTP 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 $\approx 3\%$ 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 $\approx 3\%$ dry mass with a 6000 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 sulfur transport as another potentially rate limiting process. Similar to phosphate, sulfate is highly-charged 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 are 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 6000 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 over-estimate of the number of necessary transporters as we have neglected other sulfur scavenging systems that are considerably 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 trans-

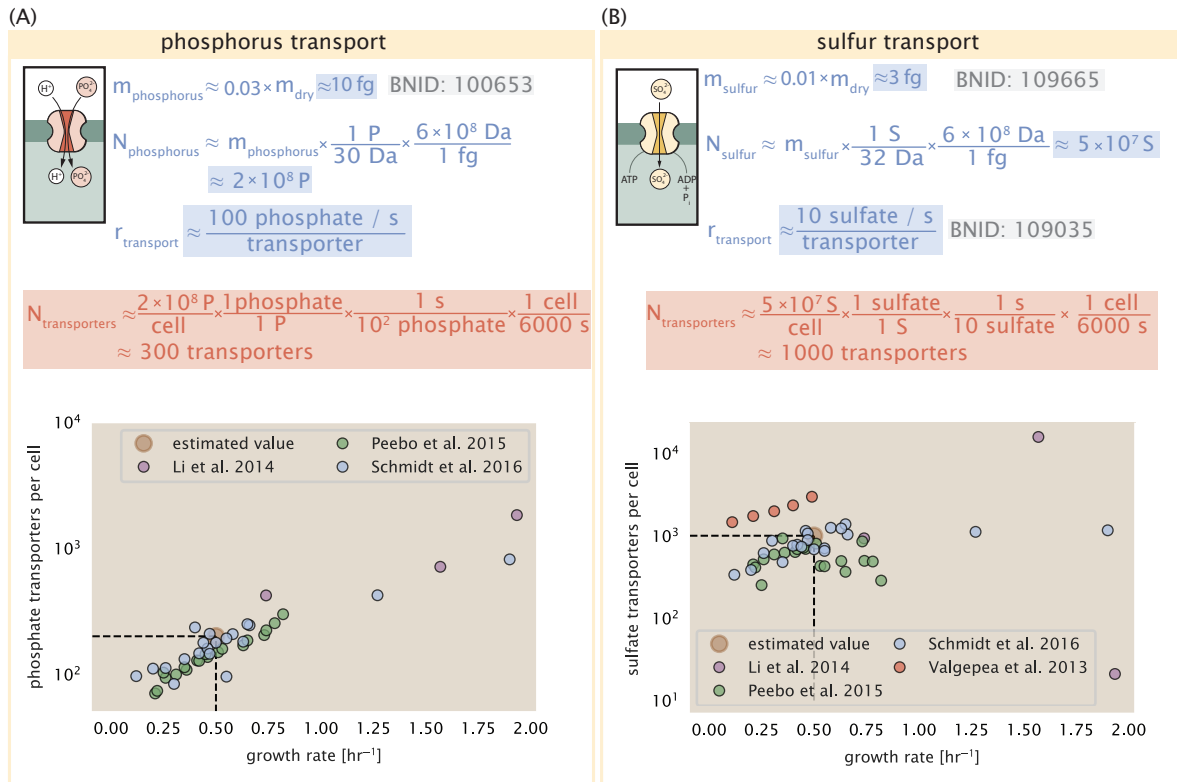


Figure 3. Estimates and measurements of phosphate and sulfate transport systems as a function of growth rate. (A) Estimate for the number of PitA phosphate transport systems needed to maintain a 3% phosphorus *E. coli* dry mass. Points in plot correspond to the the total number of PitA transporters per 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].

porter (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 is in the form of glutamate, which has its 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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