

# Fundamental limits on the rate of bacterial cell division

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14

**Abstract** Recent years have seen a deluge of experiments dissecting 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  $\approx 4000$  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.

25

## 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 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. This remarkable flexibility 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*).

Jacques Monod once remarked that “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 (*Monod, 1949*) with the quantitative power of this “method” recently undergoing renaissance. 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 *E. coli*.

Several of the evergreen questions about bacterial growth and physiology that were originally raised by

42 microbiologists in the middle of the 20th century can now be reframed in light of this newly available data. For  
43 example, what biological processes are the primary determinants for how quickly bacterial cells can grow and  
44 reproduce? How do cells modulate the absolute numbers and relative ratios of their molecular constituents as  
45 a function of changes in growth rate or nutrient availability? In this paper, we begin by considering these two  
46 questions from two distinct angles. First, as a result of an array of high-quality proteome-wide measurements of *E.*  
47 *coli* under diverse growth conditions, we have a census that allows us to explore how the number of key molecular  
48 players change as a function of growth rate. Here, we have assembled a singular data set using measurements  
49 collected over the past decade via mass spectrometry (*Schmidt et al., 2016; Peebo et al., 2015; Valgepea et al.,  
50 2013*) or ribosomal profiling (*Li et al., 2014*) of the composition of the *E. coli* proteome across 36 unique growth  
51 rates (see Appendix Experimental Details Behind Proteomic Data for a further discussion of the data). Second,  
52 by compiling molecular turnover rate measurements for many of the fundamental processes associated with  
53 bacterial growth, we make quantitative estimates of key cellular processes (schematized in *Figure 1*) to determine  
54 whether our current understanding of the dynamics of these processes are sufficient to explain the magnitude of  
55 the observed protein copy numbers across conditions (see *Box 1* describing the philosophy behind this approach).  
56 The census, combined with these estimates, provide a window into the question of whether the rates of central  
57 processes such as energy generation or DNA synthesis are regulated systematically as a function of cell growth  
58 rate by altering protein copy number.

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

67 From these estimates, it emerges that translation, particularly the synthesis of ribosomal proteins, is a plausible  
68 candidate that limits the rate of cell division in *E. coli*. We reach this conclusion by considering that ribosome  
69 synthesis is 1) a rate limiting step for the *fastest* bacterial division, and 2) a major determinant of bacterial growth  
70 across the nutrient conditions we have considered under steady state, exponential growth. This enables us to  
71 suggest that the long-observed correlation between growth rate and cell size (*Schaechter et al., 1958; Si et al.,  
72 2017*) can be simply attributed to the increased absolute number of ribosomes per cell under conditions supporting  
73 extremely rapid growth. To better understand how the observed alterations in absolute protein abundances, and  
74 in particular, changes in ribosome copy number, influence growth rate across different nutrient conditions we  
75 consider a minimal model of cellular growth. Our conclusions from these analyses provide important insight  
76 into how *E. coli* regulates growth across conditions of differing nutrient availability and identifies fundamental  
77 constraints in bacterial growth more broadly.

## 118 Nutrient Transport

119 We begin by considering the critical transport processes diagrammed in *Figure 1(A)*. In order to build new cellular  
120 mass, the molecular and elemental building blocks must be scavenged from the environment in different forms.  
121 Carbon, for example, is acquired via the transport of carbohydrates and sugar alcohols with some carbon sources  
122 receiving preferential treatment in their consumption (*Monod, 1947*). Phosphorus, sulfur, and nitrogen, on the  
123 other hand, are harvested primarily in the forms of inorganic salts, namely phosphate, sulfate, and ammonia (*Jun  
124 et al., 2018; Assentoft et al., 2016; Stasi et al., 2019; Antonenko et al., 1997; Rosenberg et al., 1977; Willsky et al.,  
125 1973*). All of these compounds have different membrane permeabilities (*Phillips, 2018*) and most require some  
126 energetic investment either via ATP hydrolysis or through the proton electrochemical gradient to bring the material  
127 across the hydrophobic cell membrane.

128 The elemental composition of *E. coli* has received much quantitative attention over the past half century  
129 (*Neidhardt et al., 1991; Taymaz-Nikerel et al., 2010; Heldal et al., 1985; Bauer and Ziv, 1976*), providing us with  
130 a starting point for estimating how many atoms of each element must be scavenged from the environment. A  
131 synthesis of these studies presents an approximate dry mass composition of  $\approx 50\%$  carbon (BNID: 100649, see

79 **Box 1. The Rules of Engagement for Order-Of-Magnitude Estimates**

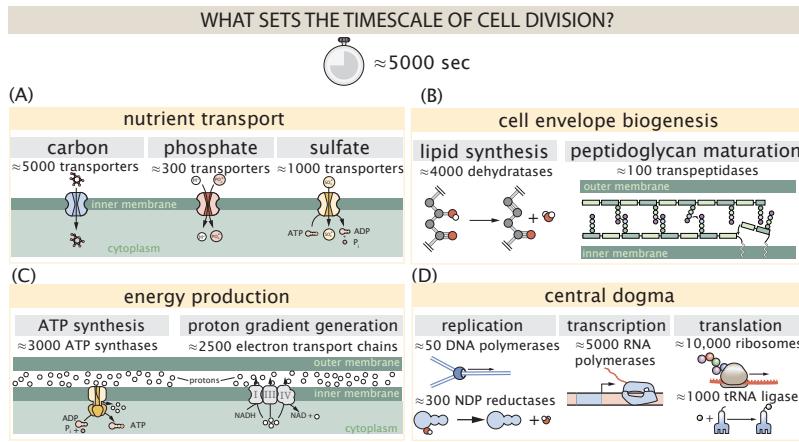
80 This work relies heavily on "back-of-the-envelope" estimates to understand the growth-rate dependent  
81 abundances of molecular complexes. This moniker arises from the limitation that any estimate should be  
82 able to fit on the back of a postage envelope. As such, we must draw a set of rules governing our precision  
83 and sources of key values.

84 **The rule of "one, few, and ten".** The philosophy behind order-of-magnitude estimates is to provide a estimate  
85 of the appropriate scale, not a prediction with many significant digits. We therefore define three different  
86 scales of precision in making estimates. The scale of "one" is reserved for values that range between 1 and 2.  
87 For example, If a particular process has been experimentally measured to transport 1.87 protons for a process  
88 to occur, we approximate this process to require 2 protons per event. The scale of "few" is reserved for values  
89 ranging between 3 and 7. For example, we will often use Avogadro's number to compute the number of  
90 molecules in a cell given a concentration and a volume. Rather than using Avogadro's number as  $6.02214 \times 10^{23}$ ,  
91 we will approximate it as  $5 \times 10^{23}$ . Finally, the scale of "ten" is reserved for values which we know within an  
92 order of magnitude. If a particular protein complex is present at 883 copies per cell, we say that it is present  
93 in approximately  $10^3$  copies per cell. These different scales will be used to arrive at simple estimates that  
94 report the expected scale of the observed data. Therefore, the estimates presented here should not be viewed  
95 as hard-and-fast predictions of precise copy numbers, but as approximate lower (or upper) bounds for the  
96 number of complexes that may be needed to satisfy some cellular requirement.

97 Furthermore, we use equality symbols (=) sparingly and frequently defer to approximation ( $\approx$ ) or scaling ( $\sim$ )  
98 symbols when reporting an estimate. When  $\approx$  is used, we are implicitly stating that we are confident in this  
99 estimate within a factor of a few. When a scaling symbol  $\sim$  is used, we are stating that we are confident in our  
100 estimate to within an order of magnitude.

101 **The BioNumbers Database as a source for values.** In making our estimates, we often require approximate  
102 values for key cellular properties, such as the elemental composition of the cell, the average dry mass, or  
103 approximate rates of synthesis. We rely heavily on the BioNumbers Database ([bionumbers.hms.harvard.edu](http://bionumbers.hms.harvard.edu),  
104 *Milo et al. (2010)*) as a repository for such information. Every value we draw from this database has an  
105 associated BioNumbers ID number, abbreviated as BNID, and we provide this reference in grey-boxes in each  
106 figure.

107 **Uncertainty in the data sets and the accuracy of an estimate.** The data sets presented in this work are the  
108 products of careful experimentation with the aim to report, to the best of their ability, the absolute copy  
109 numbers of proteins in the cell. These data, collected over the span of a few years, come from different  
110 labs and use different internal standards, controls, and even techniques (discussed further in Appendix  
111 Experimental Details Behind Proteomic Data). As a result, there is notable disagreement in the measured copy  
112 numbers for some complexes across data sets. In assessing whether our estimates could explain the observed  
113 scales and growth-rate dependencies, we also considered the degree of variation between the different data  
114 sets. For example, say a particular estimate undercuts the observed data by an order of magnitude. If all  
115 data sets agree within a factor of a few of each other, we revisit our estimate and consider what me may  
116 have missed. However, if the data sets themselves disagree by an order of magnitude, we determine that our  
117 estimate is appropriate given the variation in the data.



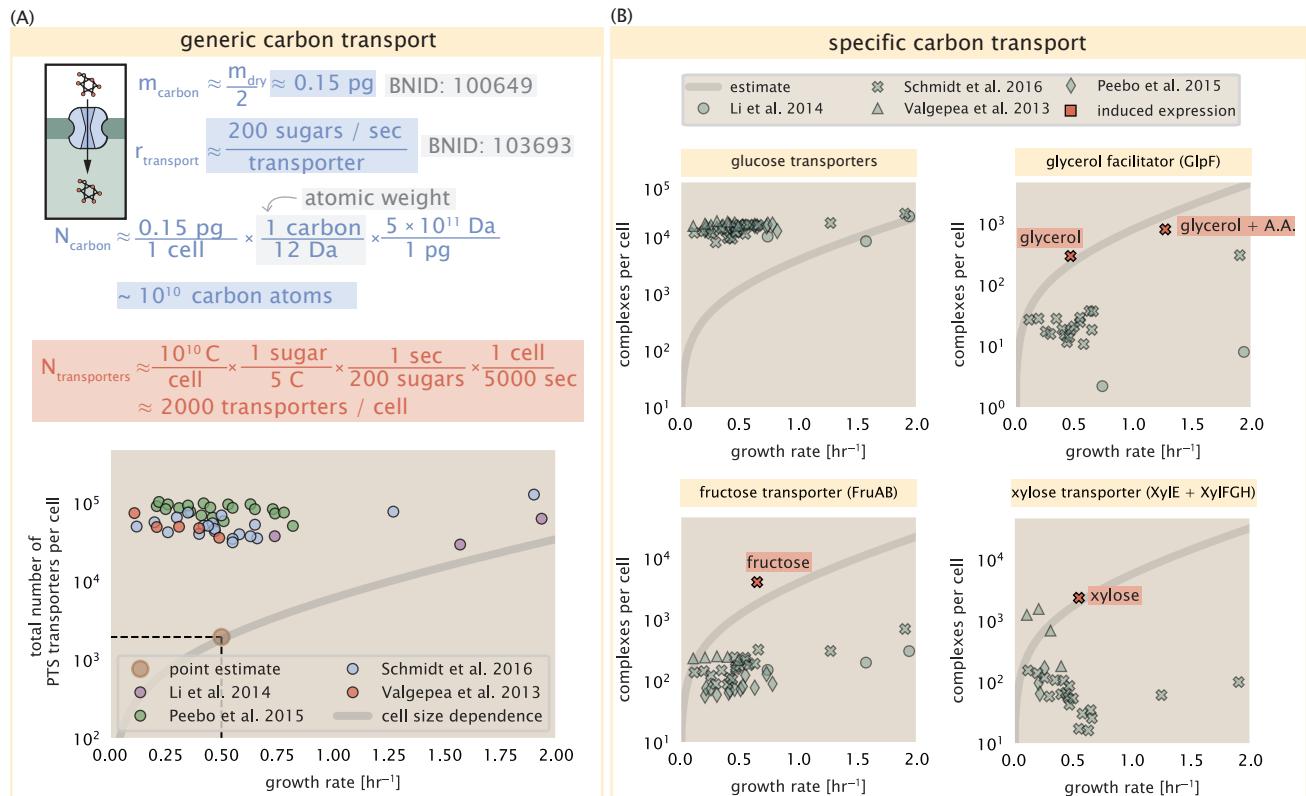
**Figure 1. Transport and synthesis processes necessary for cell division.** We consider an array of processes necessary for a cell to double its molecular components, broadly grouped into four classes. These categories are (A) nutrient transport across the cell membrane, (B) cell envelope biogenesis, (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  $0.5 \text{ hr}^{-1}$ , or a cell doubling time of  $\approx 5000$  s.

132 **Box 1**),  $\approx 15\%$  nitrogen (BNID: 106666),  $\approx 3\%$  phosphorus (BNID: 100653), and  $1\%$  sulfur (BNID: 100655) with  
 133 remainder being attributable to oxygen, hydrogen, and various transition metals. We use this stoichiometric  
 134 breakdown to estimate the abundance and growth rate dependence of a variety of transporters responsible for  
 135 carbon uptake, and provide more extensive investigation of the other critical elements – phosphorus, sulfur, and  
 136 nitrogen – in the Appendix Additional Estimates of Fundamental Biological Processes.

137 Using  $\approx 0.3 \text{ pg}$  as the typical *E. coli* dry mass at a growth rate of  $\approx 0.5 \text{ hr}^{-1}$  (BNID: 103904), coupled with an  
 138 approximation that  $\approx 50\%$  of this mass is carbon, we estimate that  $\sim 10^{10}$  carbon atoms must be brought into  
 139 the cell in order to double all of the carbon-containing molecules (**Figure 2(A)**, top). Typical laboratory growth  
 140 conditions provide carbon as a single class of sugar (such as glucose, galactose, or xylose) often transported cross  
 141 the cell membrane by a transporter complex specific to that particular sugar. One such mechanism of transport  
 142 is via the PTS system which is a highly modular system capable of transporting a diverse range of sugars with  
 143 high specificity (*Escalante et al., 2012*). The glucose-specific component of this system transports  $\approx 200$  glucose  
 144 molecules ( $\approx 1200$  carbon atoms) per second per transporter (BNID: 114686). Making the assumption that this is a  
 145 typical sugar transport rate for the PTS system, coupled with the need to transport  $\sim 10^{10}$  carbon atoms, we then  
 146 expect on the order of  $\approx 1000$  transporters must be expressed per cell in order to bring in enough carbon atoms  
 147 (**Figure 2(A)**, top).

148 However, we find this estimate to be exceeded by several fold by experimental measurements (**Figure 2(A)**,  
 149 bottom), implying that the cell is capable of transporting more carbon atoms than strictly needed for biosynthesis.  
 150 While we estimate  $\approx 1000$  transporters are needed with a 5000 second division time, we can abstract this calculation  
 151 to consider any particular growth rate given knowledge of the cell density and volume as a function of growth rate  
 152 and direct the reader to the Appendix Extending Estimates to a Continuum of Growth Rates for more information.  
 153 This abstraction, shown as a grey line in **Figure 2(A)**, reveals an excess of transporters even at faster growth rates.  
 154 This contrasts with our observations for uptake of phosphorus and sulfur, which align well with our expectations  
 155 across different growth conditions (**Figure 2–Figure Supplement 1** and discussed further in Appendix Additional  
 156 Estimates of Fundamental Biological Processes).

157 It is important to note, however, that this estimate neglects any specifics of the regulation of the carbon  
 158 transport system. Using the diverse array of growth conditions available in the data, we can explore how individual  
 159 carbon transport systems depend on specific carbon availability. In **Figure 2(B)**, we show the total number of  
 160 carbohydrate transporters specific to different carbon sources. A striking observation, shown in the top-left plot of  
 161 **Figure 2(B)**, is the constancy in the expression of the glucose-specific transport systems, an observation that stands  
 162 in contrast with other species of transporters. Additionally, we note that the total number of glucose-specific  
 163 transporters is tightly distributed at  $\approx 10^4$  per cell, the approximate number of transporters needed to sustain



**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  $\sim 10^{10}$  C are needed to complete division, each transported sugar contains  $\approx 5$  C, and each transporter conducts sugar molecules at a rate of  $\approx 200$  per second. Bottom plot shows the estimated number of transporters needed at a growth rate of  $\approx 0.5$  per hr (light-brown point and dashed lines). Colored points correspond to the mean number of complexes involved in carbohydrate import (complexes annotated with the Gene Ontology terms GO:0009401 and GO:0098704) 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. The rates of substrate transport differ between these transporter species. To compute the continuum growth rate estimate (grey line), we used the following transport rates for each transporter species: 200 glucose- $s^{-1}$  (BNID: 103693), 2000 glycerol- $s^{-1}$  (Lu et al., 2003), 200 fructose- $s^{-1}$  (assumed to be similar to PtsI, BNID: 103693), and 50 xylose- $s^{-1}$  (assumed to be comparable to LacY, BNID: 103159). Red points and highlighted text indicate conditions in which the only source of carbon in the growth medium induces expression of the transport system. Grey lines in (A) and (B) represent the estimated number of transporters per cell at a continuum of growth rates.

**Figure 2-Figure supplement 1.** Estimates and observed abundances of phosphate and sulfate transporters.

164 rapid growth of several divisions per hour. This illustrates that *E. coli* maintains a substantial number of complexes  
165 present for transporting glucose regardless of growth condition, which is known to be the preferential carbon  
166 source (*Monod, 1947; Liu et al., 2005; Aidelberg et al., 2014*).

167 Many metabolic operons are regulated with dual-input logic gates that are only expressed when glucose  
168 concentrations are low and the concentration of other carbon sources are elevated (*Gama-Castro et al., 2016;*  
169 *Zhang et al., 2014b; Gama-Castro et al., 2016; Belliveau et al., 2018; Ireland et al., 2020*). Points colored in red in  
170 *Figure 2(B)* (labeled by red text-boxes) correspond to growth conditions in which the specific carbon source (glycerol,  
171 xylose, or fructose) is present as the sole source of carbon. The grey lines in *Figure 2(B)* show the estimated number  
172 of transporters needed at each growth rate to satisfy the cellular carbon requirement, adjusted for the specific  
173 carbon source in terms of number of carbon atoms per molecule and the rate of transport for the particular  
174 transporter species. These plots show that, in the absence of the particular carbon source, expression of the  
175 transporters is maintained on the order of  $\sim 10^2$  per cell. The low but non-zero abundances may reflect the specific  
176 regulatory logic involved, requiring that cells are able to transport some minimal amount of an alternative carbon  
177 source in order to induce expression of these alternative carbon-source systems.

### 178 **Limits on Transporter Expression**

179 If acquisition of nutrients was a limiting process in cell division under the typical growth conditions explored  
180 here, the growth rate could be theoretically increased simply by expressing more transporters, but is this feasible  
181 at a physiological level? A way to approach this question is to compute the amount of space in the bacterial  
182 membrane that could be occupied by nutrient transporters. Considering a rule-of-thumb for the surface area  
183 of *E. coli* of about  $5 \mu\text{m}^2$  (BNID: 101792), we expect an areal density for 1000 transporters to be approximately  
184 200 transporters/  $\mu\text{m}^2$ . For a typical transporter occupying about  $50 \text{ nm}^2$ , this amounts to about only  $\approx 1\%$  of the  
185 total inner membrane area (*Szenk et al., 2017*). Additionally, bacterial cell membranes typically have densities  
186 of  $10^5$  proteins/ $\mu\text{m}^2$  (*Phillips, 2018*), implying that the cell could accommodate more membrane and this places  
187 additional limitations on cell size and surface area that we will consider further in the coming sections.

### 188 **Cell Envelope Biogenesis**

189 In contrast to nutrient transporters, which support the synthesis of biomolecules throughout the cell and therefore  
190 need to scale with the cell size, here we must consider the synthesis of components that will need to scale with  
191 the surface area of the cell. *E. coli* is a rod-shaped bacterium with a remarkably robust length-to-width aspect  
192 ratio of  $\approx 4:1$  (*Harris and Theriot, 2018; Ojkic et al., 2019*). At modest growth rates, the total cell surface area is  
193  $\approx 5 \mu\text{m}^2$  (BNID: 101792). Assuming this surface area is approximately the same between the inner and outer  
194 membranes of *E. coli*, and the fact that each membrane is itself a lipid bilayer, cells have a the total membrane  
195 surface area of  $\approx 20 \mu\text{m}^2$  (see Appendix Estimation of Cell Size and Surface Area for a description of the calculation  
196 of cell surface area as a function of cell size). In this section, we will estimate the number of protein complexes  
197 needed to produce this membrane surface area as well as the complexes involved in assembling the peptidoglycan  
198 scaffold it encapsulates.

### 199 **Lipid Synthesis**

200 The dense packing of the membrane with proteins means that the cell membranes are not composed entirely of  
201 lipid molecules, with only  $\approx 40\%$  of the membrane area is occupied by lipids (BNID: 100078). Using a rule-of-thumb  
202 of  $0.5 \text{ nm}^2$  as the surface area of the typical lipid (BNID: 106993), we can estimate  $\sim 2 \times 10^7$  lipids per cell, which is  
203 in close agreement with experimental measurements (BNID: 100071, 102996).

204 The membranes of *E. coli* are composed of a variety of different lipids, each of which are unique in their  
205 structures and biosynthetic pathways (*Sohlenkamp and Geiger, 2016*). Recently, a combination of stochastic kinetic  
206 modeling (*Ruppe and Fox, 2018*) and *in vitro* kinetic measurements (*Ranganathan et al., 2012; Yu et al., 2011*)  
207 have revealed remarkably slow steps in the fatty acid synthesis pathways which may serve as the rate limiting  
208 reactions for making new membrane phospholipids. One such step is the removal of hydroxyl groups from the  
209 fatty-acid chain by ACP dehydratase that leads to the formation of carbon-carbon double bonds. This reaction,  
210 catalyzed by proteins FabZ and FabA in *E. coli* (*Yu et al., 2011*), have been estimated to have kinetic turnover  
211 rates of  $\approx 1$  dehydration per second per enzyme (*Ruppe and Fox, 2018*). Thus, given this rate and the need to

212 synthesize  $\approx 2 \times 10^7$  lipids over 5000 seconds, one can estimate that a typical cell requires  $\approx 4000$  ACP dehydratases.  
213 This is in reasonable agreement with the experimentally observed copy numbers of FabZ and FabA (**Figure 3(A)**).  
214 Furthermore, we can extend this estimate to account for the change in membrane surface area as a function of the  
215 growth rate (grey line in **Figure 3(A)**), which captures the observed growth rate dependent expression of these two  
216 enzymes.

## 217 Peptidoglycan Synthesis

218 Bacterial cells demonstrate exquisite control over their cell shape. This is primarily due to the cell wall, a stiff, several  
219 nanometer thick meshwork of polymerized disaccharides. The formation of the peptidoglycan is an intricate  
220 process involving many macromolecular players (*Shi et al., 2018; Morgenstein et al., 2015*), whose coordinated  
221 action maintains cell shape and integrity even in the face of large-scale perturbations (*Harris and Theriot, 2018;*  
222 *Shi et al., 2018*). The peptidoglycan alone comprises  $\approx 3\%$  of the cellular dry mass (BNID: 1019360, making it  
223 the most massive molecule in *E. coli*. The polymerized unit of the peptidoglycan is a N-acetylglucosamine and  
224 N-acetylmuramic acid disaccharide, of which the former is functionalized with a short pentapeptide. With a mass  
225 of  $\approx 1000$  Da, this unit, which we refer to as a murein monomer, it is polymerized to form long strands in the  
226 periplasm which are then attached to each other via their peptide linkers. Together, these quantities provide an  
227 estimate of  $\approx 5 \times 10^6$  murein monomers per cell.

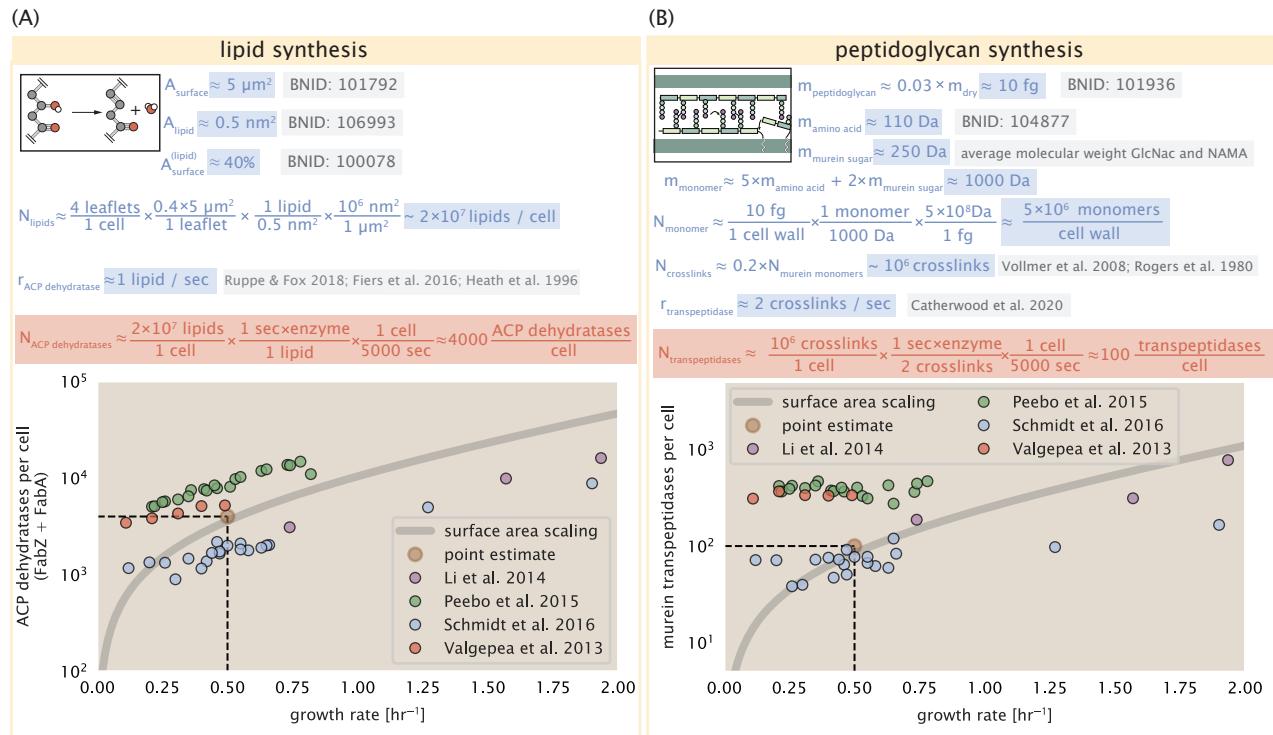
228 The crosslinking of the pentapeptides between adjacent glycan strands is responsible for maintaining the  
229 structural integrity of the cell wall and, in principle, each murein monomer can be involved in such a crosslink.  
230 In some microbes, such as in gram-positive bacterium *Staphylococcus aureus*, the extent of crosslinking can be  
231 large with  $> 90\%$  of pentapeptides forming a connection between glycan strands. In *E. coli*, however, a much  
232 smaller proportion ( $\approx 20\%$ ) of the peptides are crosslinked, resulting in a weaker and more porous cell wall *Vollmer*  
233 *et al. (2008); Rogers et al. (1980)*. The formation of these crosslinks occurs primarily during the polymerization  
234 of the murein monomers and is facilitated by a family of enzymes called transpeptidases. The four primary  
235 transpeptidases of *E. coli* have only recently been quantitatively characterized *in vivo* via liquid chromatography  
236 mass spectrometry which revealed a notably slow kinetic turnover rate of  $\approx 2$  crosslinking reactions formed per  
237 second per enzyme (*Catherwood et al., 2020*).

238 Assembling these quantities permits us to make an estimate that on the order of  $\approx 100$  transpeptidases per cell  
239 are needed for complete maturation of the peptidoglycan, given a division time of  $\approx 5000$  seconds; a value that is  
240 comparable to experimental observations (**Figure 3(B)**). Expanding this estimate to account for the changing mass  
241 of the peptidoglycan as a function of growth rate (grey line in **Figure 3(B)**) also qualitatively captures the observed  
242 dependence in the data, though systematic disagreements between the different data sets makes the comparison  
243 more difficult.

## 244 Limits on Cell Wall Biogenesis

245 While the processes we have considered represent only a small portion of proteins devoted to cell envelope  
246 biogenesis, we find it unlikely that they limit cellular growth in general. The relative amount of mass required  
247 for lipid and peptidoglycan components decrease at faster growth rates due to a decrease in their surface area  
248 to volume (S/V) ratio (*Ojkic et al., 2019*). Furthermore, despite the slow catalytic rate of FabZ and FabA in lipid  
249 synthesis, experimental data and recent computational modeling has shown that the rate of fatty-acid synthesis  
250 can be drastically increased by increasing the concentration of FabZ (*Yu et al., 2011; Ruppe and Fox, 2018*). With  
251 a proteome size of  $\approx 3 \times 10^6$  proteins, a hypothetical 10-fold increase in expression from 4000 to 40,000 ACP  
252 dehydratases would result in a paltry  $\approx 1\%$  increase in the size of the proteome. In the context of peptidoglycan  
253 synthesis, we note that our estimate considers only the transpeptidase enzymes that are involved lateral and  
254 longitudinal elongation of the peptidoglycan. This neglects the presence of other transpeptidases that are present  
255 in the periplasm and also involved in remodeling and maturation of the peptidoglycan. It is therefore possible that  
256 if this was setting the speed limit for cell division, the simple expression of more transpeptidases may be sufficient  
257 to maintain the structural integrity of the cell wall.

## CELL ENVELOPE BIOSYNTHESIS



**Figure 3.** (A) Top panel shows an estimation for the number of ACP dehydratases necessary to form functional phospholipids, which is assumed to be a rate-limiting step on lipid synthesis. The rate of ACP dehydratases was inferred from experimental measurements via a stochastic kinetic model described in [Ruppe and Fox \(2018\)](#). Bottom panel shows the experimentally observed complex copy numbers using the stoichiometries  $[\text{FabA}]_2$  and  $[\text{FabZ}]_2$ . (B) An estimate for the number of peptidoglycan transpeptidases needed to complete maturation of the peptidoglycan. The mass of the murein monomer was estimated by approximating each amino acid in the pentapeptide chain as having a mass of 110 Da and each sugar in the disaccharide having a mass of 250 Da. The *in vivo* rate of transpeptidation in *E. coli* was taken from recent analysis by [Catherwood et al. \(2020\)](#). The bottom panel shows experimental measurements of the transpeptidase complexes in *E. coli* following the stoichiometries  $[\text{MrcA}]_2$ ,  $[\text{MrcB}]_2$ ,  $[\text{MrdA}]_1$ , and  $[\text{MrdB}]_1$ . Grey curves in each plot show the estimated number of complexes needed to satisfy the synthesis requirements scaled by the surface area as a function of growth rate.

258 **Energy Production**

259 Cells consume and generate energy predominantly in the form of nucleoside triphosphates (NTPs) in order to  
260 grow. The high-energy phosphodiester bonds of (primarily) ATP power a variety of cellular processes that drive  
261 biological systems away from thermodynamic equilibrium. We next turn to the synthesis of ATP as a potential  
262 process that may limit growth, which also requires us to consider the maintenance of the electrochemical proton  
263 gradient which powers it.

264 **ATP Synthesis**

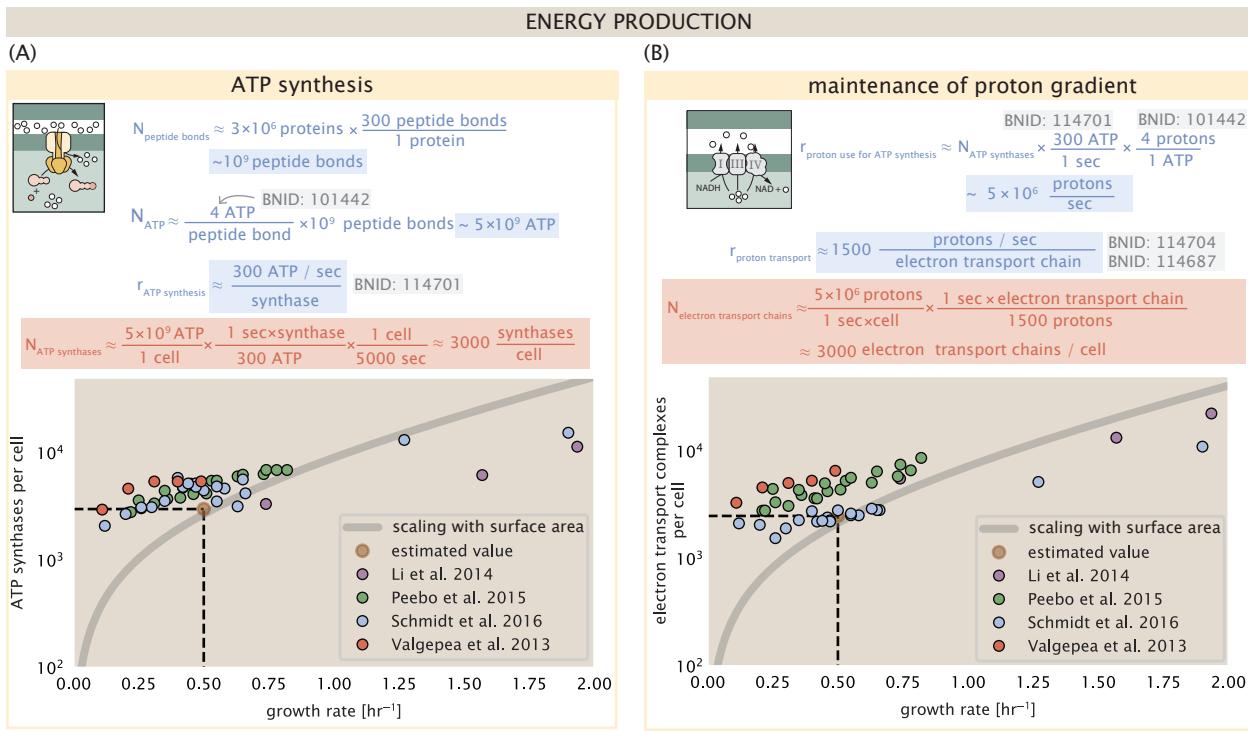
265 Hydrolysis of the terminal phosphodiester bond of ATP into ADP (or alternatively GTP and GDP) and an inorganic  
266 phosphate provides the thermodynamic driving force in a wide array of biochemical reactions. One such reaction  
267 is the formation of peptide bonds during translation, which requires  $\approx 2$  ATPs for the charging of an amino acid  
268 to the tRNA and  $\approx 2$  GTP for the formation of each peptide bond. Assuming the ATP costs associated with error  
269 correction and post-translational modifications of proteins are negligible, we can make the approximation that each  
270 peptide bond has a net cost of  $\approx 4$  ATP (BNID: 101442, *Milo et al. (2010)*). Formation of GTP from ATP is achieved  
271 via the action of nucleoside diphosphate kinase, which catalyzes this reaction without an energy investment  
272 (*Lascu and Gonin, 2000*) and therefore consider all NTP requirements of the cell to be functionally equivalent to  
273 being exclusively ATP. In total, the energetic costs of peptide bond formation consume  $\approx 80\%$  of the cells ATP  
274 budget (BNID: 107782; 106158; 101637; 111918, *Lynch and Marinov (2015); Stouthamer (1973)*). The pool of ATP is  
275 produced by the  $F_1$ - $F_0$  ATP synthase – a membrane-bound rotary motor which under ideal conditions can yield  $\approx$   
276 300 ATP per second (BNID: 114701; *Weber and Senior (2003)*).

277 To estimate the total number of ATP equivalents consumed during a cell cycle, we will make the approximation  
278 that there are  $\approx 3 \times 10^6$  proteins per cell with an average protein length of  $\approx 300$  peptide bonds (BNID: 115702;  
279 108986; 104877). Taking these values together, coupled with an estimate of  $\approx 4$  ATP equivalents per peptide bond,  
280 we find that the typical *E. coli* cell consumes  $\sim 5 \times 10^9$  ATP per cell cycle on protein synthesis alone. Assuming  
281 that each ATP synthases operates at its maximal speed (300 ATP per second per synthase),  $\approx 3000$  ATP synthases  
282 are needed to keep up with the energy demands of the cell. This estimate is comparable with the experimental  
283 observations, shown in *Figure 4* (A). We note that this estimate assumes all ATP is synthesized via ATP synthase  
284 and neglects synthesis via fermentative metabolism. This assumption may explain why at the fastest growth rates  
285 ( $\approx 2$  hr $^{-1}$ ), our continuum estimate predicts more synthase than is experimentally observed (gray line in *Figure 4*).  
286 At rapid growth rates, *E. coli* enters a type of overflow metabolism where fermentative metabolism becomes  
287 pronounced (*Szenk et al., 2017*).

288 **Generating the Proton Electrochemical Gradient**

289 In order to produce ATP, the  $F_1$ - $F_0$  ATP synthase itself must consume energy. Rather than burning through its  
290 own product (and violating thermodynamics), this intricate macromolecular machine has evolved to exploit the  
291 electrochemical potential established across the inner membrane through cellular respiration. This electrochemical  
292 gradient is manifest by the pumping of protons into the intermembrane space via the electron transport chains as  
293 they reduce NADH. In *E. coli*, this potential difference is  $\approx -200$  mV (BNID: 102120). A simple estimate of the inner  
294 membrane as a capacitor with a working voltage of -200 mV reveals that  $\approx 2 \times 10^4$  protons must be present in the  
295 intermembrane space. However, each rotation of an ATP synthase shuttles  $\approx 4$  protons into the cytosol (BNID:  
296 103390). With a few thousand ATP synthases producing ATP at their maximal rate, the potential difference would  
297 be rapidly abolished in a few milliseconds if it were not being actively maintained.

298 The electrochemistry of the electron transport complexes of *E. coli* have been the subject of intense biochemical  
299 and biophysical study (*Ingledew and Poole, 1984; Khademian and Imlay, 2017; Cox et al., 1970; Henkel et al., 2014*).  
300 A recent work (*Szenk et al., 2017*) examined the respiratory capacity of the *E. coli* electron transport complexes  
301 using structural and biochemical data, revealing that each electron transport chain rapidly pumps protons into the  
302 intermembrane space at a rate of  $\approx 1500$  protons per second (BNID: 114704; 114687). Using our estimate of the  
303 number of ATP synthases required per cell [*Figure 4(A)*], coupled with these recent measurements, we estimate  
304 that  $\approx 3000$  electron transport complexes would be necessary to facilitate the  $\sim 5 \times 10^6$  protons per second diet of  
305 the cellular ATP synthases. This estimate is in agreement with the number of complexes identified in the proteomic



**Figure 4. The abundance of F<sub>1</sub>-F<sub>0</sub> ATP synthases and electron transport chain complexes as a function of growth rate.**

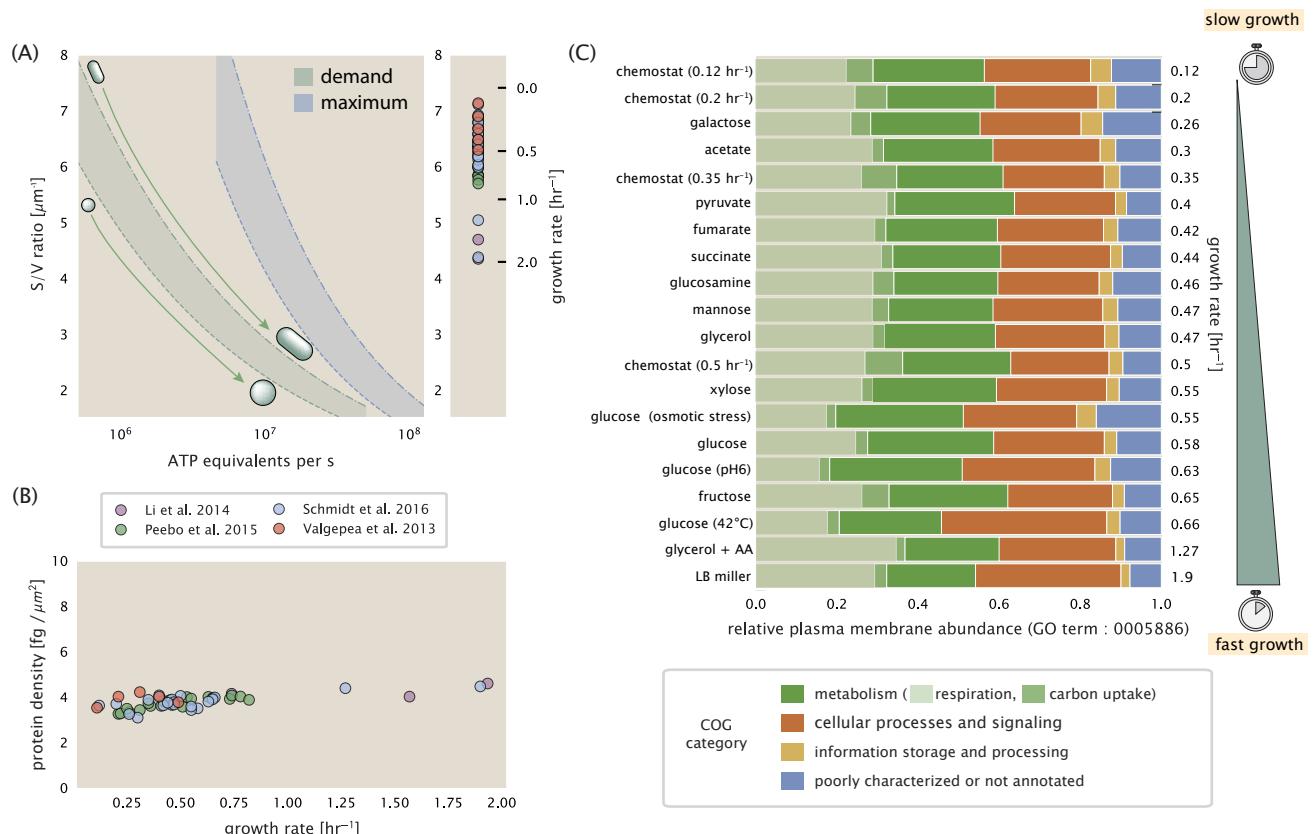
(A) Estimate of the number of F<sub>1</sub>-F<sub>0</sub> ATP synthase complexes needed to accommodate peptide bond formation and other NTP dependent processes. Points in plot correspond to the mean number of complete F<sub>1</sub>-F<sub>0</sub> ATP synthase complexes that can be formed given proteomic measurements and the subunit stoichiometry [AtpE]<sub>10</sub>[AtpF]<sub>2</sub>[AtpB][AtpC][AtpH][AtpA]<sub>3</sub>[AtpG][AtpD]<sub>3</sub>. (B) Estimate of the number of electron transport chain complexes needed to maintain a membrane potential of -200 mV given estimate of number of F<sub>1</sub>-F<sub>0</sub> ATP synthases from (A). Points in plot correspond to the average number of complexes identified as being involved in aerobic respiration by the Gene Ontology identifier GO:0019646 that could be formed given proteomic observations. These complexes include cytochromes *bd1* ([CydA][CydB][CydX][CydH]), *bdII* ([AppC][AppB]), *bo<sub>3</sub>*,([CyoD][CyoA][CyoB][CyoC]) and NADH:quinone oxioreductase I ([NuoA][NuoH][NuoJ][NuoK][NuoL][NuoM][NuoN][NuoB][NuoC][NuoE][NuoF][NuoG][NuoI]) and II ([Ndh]). Grey lines in both (A) and (B) correspond to the estimate procedure described, but applied to a continuum of growth rates. We direct the reader to the Supporting Information for a more thorough description of this approach.

306 datasets (plot in **Figure 4(B)**). This suggests that every ATP synthase must be accompanied by  $\approx 1$  functional electron  
307 transport chain.

### 308 Limits on Biosynthesis in a Crowded Membrane

309 Our estimates thus far have focused on biochemistry at the periphery of the cell and have generally been  
310 concordant with the abundances predicted by our estimates. However, as surface area and volume do not scale  
311 identically, it is necessary to consider the physical limits for transport and energy production given the S/V ratio,  
312 which as we've noted will decrease at faster growth rates.

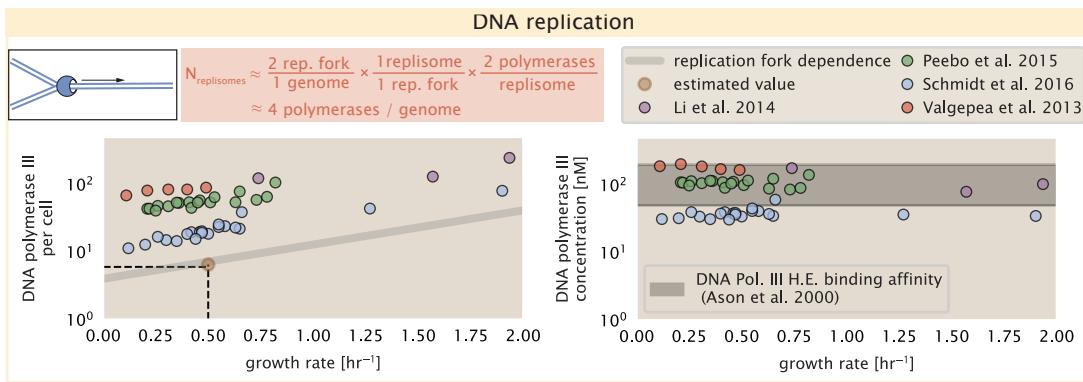
313 In our estimate of ATP production above we found that a cell demands about  $5 \times 10^9$  ATP per cell cycle or  $10^6$   
314 ATP/s. With a cell volume of roughly 1 fL (BNID: 100004), this corresponds to about  $2 \times 10^{10}$  ATP per fL of cell volume,  
315 in line with previous estimates (**Stouthamer and Bettenhausen, 1977; Szenk et al., 2017**). In **Figure 5 (A)** we plot  
316 this ATP demand as a function of the S/V ratio in green, where we have considered a range of cell shapes from  
317 spherical to rod-shaped with an aspect ratio (length/width) equal to 4. In order to consider the maximum ATP that  
318 could be produced, we consider the amount of ATP that can be generated by a membrane filled with ATP synthase  
319 and electron transport complexes, which provides a maximal production of about 3 ATP / (nm<sup>2</sup>·s) (**Szenk et al.,**  
320 **2017**). This is shown in blue in **Figure 5(A)**, which shows that at least for the growth rates observed (right column  
321 in plot), the energy demand is roughly an order of magnitude less. Interestingly, **Szenk et al. (2017)** also found  
322 that ATP production by respiration is less efficient than by fermentation per membrane area occupied due to the



**Figure 5. Influence of cell size and S/V ratio on ATP production and inner membrane composition.** (A) Scaling of ATP demand and maximum ATP production as a function of S/V ratio. Cell volumes of 0.5 fL to 50 fL were considered, with the dashed (—) line corresponding to a sphere and the dash-dot line (---) reflecting a rod-shaped bacterium like *E. coli* with a typical aspect ratio (length / width) of 4 (Shi et al., 2018). Approximately 50% of the bacterial inner membrane is assumed to be protein, with the remainder lipid. The right plot shows the measured growth rates, and their estimated S/V ratio using growth rate dependent size measurements from Si et al. (2017) (See Appendix Estimation of Cell Size and Surface Area on calculation). (B) Total protein mass per  $\mu\text{m}^2$  calculated for proteins with inner membrane annotation (GO term: 0005886). (C) Relative protein abundances by mass based on COG annotation. Metabolic proteins are further separated into respiration ( $F_1$ - $F_0$  ATP synthase, NADH dehydrogenase I, succinate:quinone oxidoreductase, cytochrome bo<sub>3</sub> ubiquinol oxidase, cytochrome bd-I ubiquinol oxidase) and carbohydrate transport (GO term: GO:0008643). Note that the elongation factor EF-Tu can also associate with the inner membrane, but was excluded in this analysis due to its high relative abundance (roughly identical to the summed protein shown in part (B)).

323 additional proteins of the electron transport chain. This suggests that, even under anaerobic growth, there will be  
324 sufficient membrane space for ATP production.

325 The analysis highlights the diminishing capacity to provide resources as the cell increases in size. However,  
326 the maximum energy production in **Figure 5(A)** does represent a somewhat unachievable limit since the inner  
327 membrane must also include other proteins including those required for lipid and membrane synthesis. To  
328 better understand the overall proteomic makeup of the inner membrane, we therefore used Gene Ontology (GO)  
329 annotations (Ashburner et al., 2000; The Gene Ontology Consortium, 2018) to identify all proteins embedded or  
330 peripheral to the inner membrane (GO term: 0005886). Those associated but not membrane-bound include  
331 proteins like MreB and FtsZ and must nonetheless be considered as a vital component occupying space on the  
332 membrane. In **Figure 5(B)**, we find that the total protein mass per  $\mu\text{m}^2$  is nearly constant across growth rates.  
333 Interestingly, when we consider the distribution of proteins grouped by their Clusters of Orthologous Groups (COG)  
334 (Tatusov et al., 2000), the relative abundance for those in metabolism (including ATP synthesis via respiration) is  
335 also relatively constant across growth rates, suggesting that no one process (energy production, nutrient uptake,  
336 etc.) is particularly dominating even at fast growth rates **Figure 5(C)**.



**Figure 6. Complex abundance estimates for dNTP synthesis and DNA replication.** An estimate for the minimum number of DNA polymerase holoenzyme complexes needed to facilitate replication of a single genome. Points in the left-hand plot correspond to the total number of DNA polymerase III holoenzyme complexes ( $[\text{DnaE}]_3[\text{DnaQ}]_3[\text{HolE}]_3[\text{DnaX}]_5[\text{HolB}][\text{HolA}][\text{DnaN}]_4[\text{HolC}]_4[\text{HolD}]_4$ ) per cell. Right-hand plot shows the effective concentration of DNA polymerase III holoenzyme (See Appendix Estimation of Cell Size and Surface Area for calculation of cell size). Grey lines in left-hand panel show the estimated number of complexes needed as a function of growth, the details of which are described in the Supplemental Information.

**Figure 6-Figure supplement 1.** Estimate and observations of the abundance of ribonucleotide reductase, a key component in dNTP synthesis.

### 337 Processes of the Central Dogma

338 Up to this point, we have considered a variety of transport and biosynthetic processes that are critical to acquiring  
 339 and generating new cell mass. While there are of course many other metabolic processes we could consider, we  
 340 now turn our focus to some of the most central processes which *must* be undertaken irrespective of the growth  
 341 conditions – those of the central dogma.

### 342 DNA Replication

343 Most bacteria (including *E. coli*) harbor a single, circular chromosome and can have extra-chromosomal plasmids  
 344 up to  $\sim 100$  kbp in length. While we consider the starting material dNTPs in **Figure 6-Figure Supplement 1** and  
 345 discussed further in Appendix Additional Process of the Central Dogma, here we focus our quantitative thinking on  
 346 the chromosome of *E. coli* which harbors  $\approx 5000$  genes and  $\approx 5 \times 10^6$  base pairs.

347 To successfully divide and produce viable progeny, this chromosome must be faithfully replicated and seg-  
 348 regated into each nascent cell. Replication is initiated at a single region of the chromosome termed the *oriC*  
 349 locus at which a pair of replisomes, each consisting of two DNA polymerase III, begin their high-fidelity replication  
 350 of the genome in opposite directions (Fijalkowska et al., 2012). *In vitro* measurements have shown that DNA  
 351 Polymerase III copies DNA at a rate of  $\approx 600$  nucleotides per second (BNID: 104120). Therefore, to replicate a single  
 352 chromosome, two replisomes moving at their maximal rate would copy the entire genome in  $\approx 4000$  s. Thus, with  
 353 a division time of 5000 s, there is sufficient time for a pair of replisomes complexes to replicate the entire genome.

354 In rapidly growing cultures, bacteria like *E. coli* can initiate as many as 10 - 12 replication forks at a given time  
 355 (Bremer and Dennis, 2008; Si et al., 2017), we expect only a few DNA polymerases ( $\approx 10$ ) are needed. However, as  
 356 shown in **Figure 6** DNA polymerase III is nearly an order of magnitude more abundant. This discrepancy can be  
 357 understood by considering its binding constant to DNA. *In vitro* characterization has quantified the  $K_D$  of DNA  
 358 polymerase III holoenzyme to single-stranded and double-stranded DNA to be 50 and 200 nM, respectively (Ason  
 359 et al., 2000). The right-hand plot in **Figure 6** shows that the concentration of DNA polymerase III across all data  
 360 sets is within this range. Thus, its copy number appears to vary such that its concentration is approximately  
 361 equal to the dissociation constant to the DNA. While the processes regulating the initiation of DNA replication are  
 362 complex and involve more than just the holoenzyme, these data indicate that the kinetics of replication rather than  
 363 the explicit copy number of the DNA polymerase III holoenzyme is the more relevant feature of DNA replication  
 364 to consider. In light of this, the data in **Figure 6** suggests that for bacteria like *E. coli*, DNA replication does not  
 365 represent a rate-limiting step in cell division. However, it is worth noting that for bacterium like *C. crescentus*

366 whose chromosomal replication is initiated only once per cell cycle (*Jensen et al., 2001*), the time to double their  
367 chromosome indeed represents an upper limit to their growth rate.

### 368 RNA Synthesis

369 We now turn our attention to the next stage of the central dogma – the transcription of DNA to form RNA. We  
370 consider three major groupings of RNA, namely the RNA associated with ribosomes (rRNA), the RNA encoding the  
371 amino-acid sequence of proteins (mRNA), and the RNA which links codon sequence to amino-acid identity during  
372 translation (tRNA).

373 rRNA serves as the catalytic and structural component of the ribosome, comprising approximately 2/3 of the  
374 total ribosomal mass, and is decorated with  $\approx$  50 ribosomal proteins. Each ribosome contains three rRNA molecules  
375 of lengths 120, 1542, and 2904 nucleotides (BNID: 108093), meaning each ribosome contains  $\approx$  4500 nucleotides  
376 overall. *In vivo* measurements of the kinetics of rRNA transcription have revealed that RNA polymerases are loaded  
377 onto the promoter of an rRNA gene at a rate of  $\approx$  1 per second (BNID: 111997, 102362). If RNA polymerases are  
378 constantly loaded at this rate, then we can assume that  $\approx$  1 functional rRNA unit is synthesized per second per  
379 rRNA operon. While *E. coli* possesses 7 of these operons per chromosome, the fact that chromosome replication  
380 can be parallelized means that the average dosage of rRNA genes can be substantially higher (up to  $\approx$  70 copies) at  
381 fast growth rates. At a growth rate of  $\approx$  0.5 hr<sup>-1</sup>, however, the average cell has  $\approx$  1 copy of its chromosome and  
382 therefore approximately  $\approx$  7 copies of the rRNA operons, therefore producing  $\approx$  7 rRNA units per second. With a  
383 5000 second division time, this means the cell is able to generate around  $3 \times 10^4$  functional rRNA units, comparable  
384 within an order of magnitude to the number of ribosomes per cell.

385 How many RNA polymerases are then needed to constantly transcribe the required rRNA? If one polymerase is  
386 loaded per second, and the transcription rate is  $\approx$  40 nucleotides per second (BNID: 101094), then the typical  
387 spacing between polymerases will be  $\approx$  40 nucleotides. However, we must note that the polymerase itself has a  
388 footprint of  $\approx$  40 nucleotides (BNID: 107873), meaning that one could expect to find one RNA polymerase per 80  
389 nucleotide stretch of an rRNA gene. With a total length of  $\approx$  4500 nucleotides per operon and 7 operons per cell,  
390 the number of RNA polymerases transcribing rRNA at any given time is then  $\approx$  500 per cell.

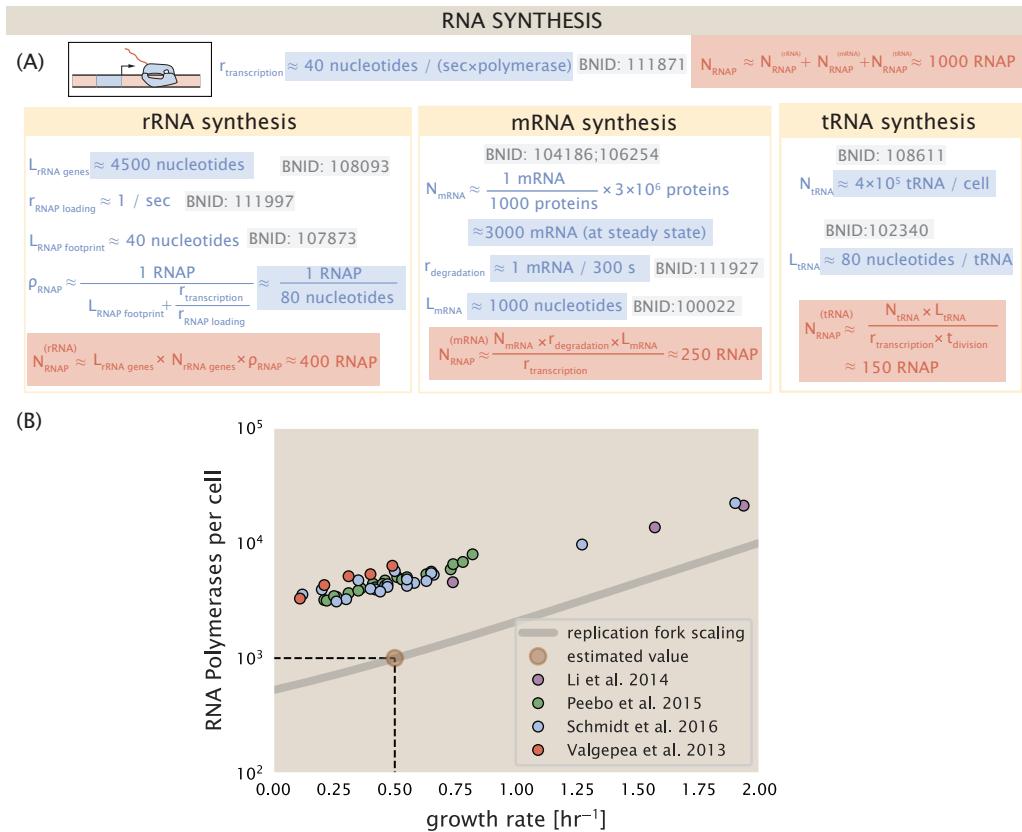
391 As outlined in *Figure 7*, and discussed further the Appendix Additional Process of the Central Dogma, synthesis  
392 of mRNA and tRNA together require on the order of  $\approx$  400 RNAP. Thus, in total, one would expect the typical  
393 cell to require  $\approx$  1000 RNAP to satisfy its transcriptional demands. As is revealed in *Figure 7(B)*, this estimate  
394 is about an order of magnitude below the observed number of RNA polymerase complexes per cell ( $\approx$  5000 -  
395 7000). The difference between the estimated number of RNA polymerase needed for transcription and these  
396 observations, however, are consistent with recent literature revealing that  $\approx$  80 % of RNA polymerases in *E. coli* are  
397 not transcriptionally active (*Patrick et al., 2015*).

398 Our estimates also neglect other mechanistic features of transcription and transcriptional initiation more  
399 broadly. For example, we acknowledge that some fraction of the RNAP pool is nonspecifically bound to DNA  
400 during its search for promoters from which to begin transcription. Furthermore, we ignore the obstacles that RNA  
401 polymerase and DNA polymerase present to each other as they move along the DNA (*Finkelstein and Greene, 2013*). Finally, we neglect the fact that RNA polymerase also require  $\sigma$ -factors for promoter recognition and  
402 transcription initiation (*Browning and Busby, 2016*).

403 While they are the machinery for transcription, RNA polymerase is not sufficient to initiate transcription.  
404 Promoter recognition and initiation of transcription is dependent on the presence of  $\sigma$ -factors, protein cofactors  
405 which bind directly to the polymerase (*Browning and Busby, 2016*). In *Figure 7-Figure Supplement 1*, we show that  
406 the predicted RNA polymerase copy number indeed is more comparable with the abundance of  $\sigma$ -70 (RpoD), the  
407 primary sigma factor in *E. coli*. There therefore remains more to be investigated as to what sets the observed  
408 abundance of RNA polymerase in these proteomic data sets. However, we conclude that the observed excess  
409 in abundance for RNA polymerase abundances are generally in excess of what appears to be needed for growth,  
410 suggesting that the abundance of RNA polymerase itself is not particularly limiting.

### 412 Protein Synthesis

413 We conclude our dialogue between back-of-the-envelope estimates and comparison with the proteomic data by  
414 examining the final process in the central dogma – translation. In doing so, we will begin with an estimate of the



**Figure 7. Estimation of the RNA polymerase demand and comparison with experimental data.** (A) Estimations for the number of RNA polymerase needed to synthesize sufficient quantities of rRNA, mRNA, and tRNA from left to right, respectively.(B) The RNA polymerase core enzyme copy number as a function of growth rate. Colored points correspond to the average number RNA polymerase core enzymes that could be formed given a subunit stoichiometry of  $[RpoA]_2[RpoC][RpoB]$ .

**Figure 7-Figure supplement 1.** Abundance and growth rate dependence of  $\sigma$ -70.

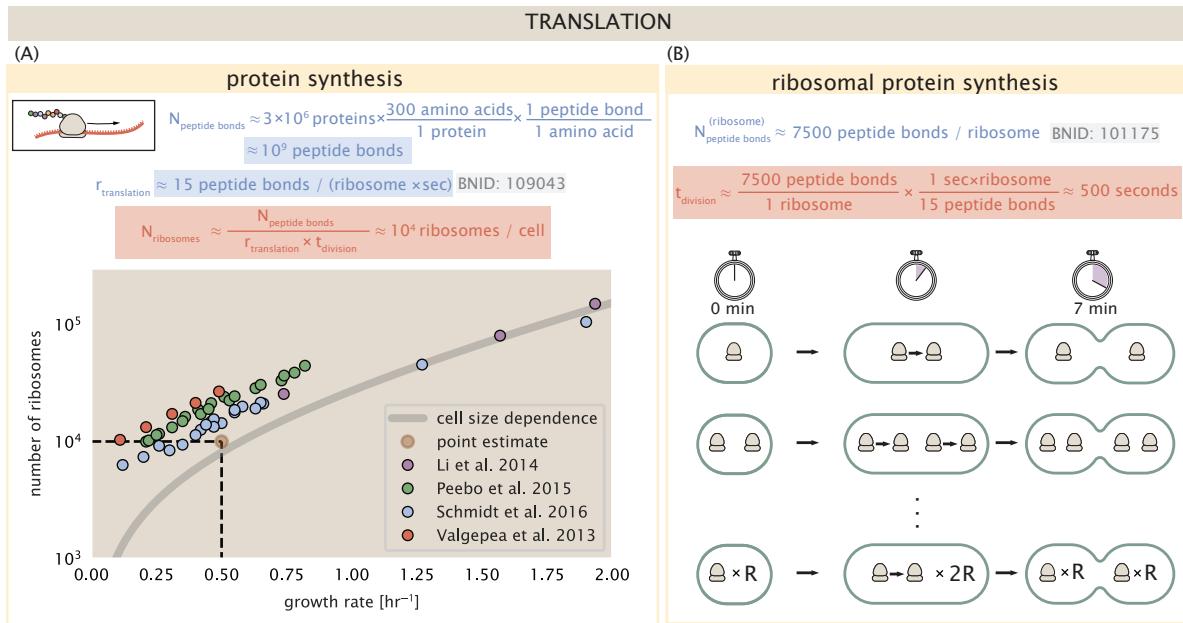
415 number of ribosomes needed to replicate the cellular proteome. While the rate at which ribosomes translate is  
416 well known to be dependent on the growth rate (*Dai et al. (2018)*, a phenomenon we consider later in this work) we  
417 will make the approximation that translation occurs at a modest rate of  $\approx$  15 amino acids per second per ribosome  
418 (BNID: 100233) Under this approximation and our previous estimate of  $10^9$  peptide bonds per cell at a growth rate  
419 of  $0.5 \text{ hr}^{-1}$ , we can easily arrive at an estimate of  $\approx 10^4$  ribosomes needed per cell to replicate the entire protein  
420 mass (*Figure 8(A)*, top). This point estimate, as well as the corresponding estimate across a continuum of growth  
421 rates, proves to be notably comparable to the experimental observations, shown in the bottom panel of *Figure 8(A)*.  
422 While the ribosome is responsible for the formation of peptide bonds, we do not diminish the importance of  
423 charging tRNAs with their appropriate amino acid, a process which occurs with remarkable fidelity. In the Appendix  
424 and in *Figure 8–Figure Supplement 1*, we consider the process of ligating tRNAs to their corresponding amino acid  
425 and again find notable accord between the data and our quantitative expectations.

426 Having completed our circuit through key processes of cellular growth outlined in *Figure 1*, we can now take stock  
427 of our understanding of the observed growth rate dependence and abundances of various protein complexes. We  
428 note that, broadly speaking, these simple estimates have been reasonably successful in quantitatively describing  
429 the observations in the proteomic data, suggesting that the proteome is tuned in composition and absolute  
430 abundance to match the growth rate requirements without any one process representing a singular bottleneck or  
431 rate limiting step in division. However, in our effort to identify key limitations on growth, there are two notable  
432 observations that we wish to emphasize.

433 The first is a recurring theme throughout our estimates. Of those investigated here, any inherent biochemical  
434 rate limitation can be overcome by expressing more proteins. We can view this as a parallelization of each  
435 biosynthesis task, which helps explain why bacteria tend to increase their protein content (and cell size) as growth  
436 rate increases (*Ojkic et al., 2019*). The second, and ultimately the most significant in defining the cellular growth  
437 rate, is that the synthesis of ribosomal proteins presents a special case where parallelization is *not* possible and  
438 thereby imposes a limit on the fastest possible growth rate. Each ribosome has  $\approx 7500$  amino acids across all of its  
439 protein components which must be strung together as peptide bonds through the action of another ribosome.  
440 Once again using a modest elongation rate of  $\approx 15$  amino acids per second, we arrive an estimate of  $\approx 500$  seconds  
441 or  $\approx 7$  minutes to replicate a single ribosome. This limit, as remarked upon by others (*Dill et al., 2011*), serves as a  
442 hard theoretical boundary for how quickly *E. coli* could replicate. As each ribosome would therefore need to copy  
443 itself, this 7 minute speed limit is independent of the number of ribosomes per cell (*Figure 8(B)*), yet assumes that  
444 the only proteins that need to be replicated for division to occur are ribosomal proteins, an unrealistic regime not  
445 met in biological reality. This poses an optimization problem for the cell – how are the translational demands of  
446 the entire proteome met without investing resources in the production of an excess of ribosomes?

447 This question, more frequently presented as a question of optimal resource allocation, has been the target of  
448 an extensive dialogue between experiment and theory over the past decade. In a now seminal work, *Scott et al.*  
449 (*2010*) present an elegant treatment of resource allocation through partitioning of the proteome into sectors – one  
450 of which being ribosome-associated proteins whose relative size ultimately defines the total cellular growth rate. In  
451 more recent years, this view has been more thoroughly dissected experimentally (*Klumpp and Hwa, 2014; Basan*  
452 *et al., 2015; Dai et al., 2018, 2016; Erickson et al., 2017*) and together have led to a paradigm-shift in how we think  
453 of cellular physiology at the proteomic-level. However, the quantitative description of these observations is often  
454 couched in terms of phenomenological constants and effective parameters with the key observable features of  
455 expression often computed in relative, rather than absolute, abundances. Furthermore, these approaches often  
456 exclude or integrate away effects of cell size and chromosome content, which we have found through our estimates  
457 to have important connections to the observed cellular growth rate.

458 In the closing sections of this work, we explore how ribosomal content, total protein abundance, and chromo-  
459 somal replication are intertwined in their control over the cellular growth rate. To do so, we take a more careful  
460 view of ribosome abundance, increasing the sophistication of our analysis by exchanging our order-of-magnitude  
461 estimates for a minimal mathematical model of growth rate control. This is defined by parameters with tangible  
462 connections to the biological processes underlying cellular growth and protein synthesis. Using this model, we  
463 interrogate how the size of the ribosome pool and its corresponding translational capacity enable cells to maintain  
464 a balance between the of amino acids via metabolism and catabolism and their consumption through the peptide  
465 bond formation required for growth.



**Figure 8. Estimation of the required number of ribosomes and the speed limit for bacterial replication.** (A) Estimation of the number of ribosomes required to synthesize  $10^9$  peptide bonds with an elongation rate of 15 peptide bonds per second. The average abundance of ribosomes is plotted as a function of growth rate. Our estimated values are shown for a growth rate of  $0.5 \text{ hr}^{-1}$ . Grey lines correspond to the estimated complex abundance calculated at different growth rates. (B) Estimation for the time to replicate a ribosome. This rate is independent of the number of ribosomes  $R$  and instead is limited by the time required to double an individual ribosome.

**Figure 8—Figure supplement 1.** Estimate and observed abundance and growth rate dependence of tRNA ligases.

#### 466 Maximum Growth Rate is Determined by the Ribosomal Mass Fraction

467 The 7 minute speed limit shown in **Figure 8(B)** assumes all proteins in the cell are ribosomes. In order to connect  
 468 this to the experimental data (and physiological reality more broadly), we first need to relax this assumption and  
 469 determine a translation-limited growth rate. Here, we will assume that the cell is composed of  $N_{\text{pep}}$  peptide bonds  
 470 and  $R$  ribosomes, whose precise values will depend on the growth rate  $\lambda$ . The protein subunits of each ribosomal  
 471 peptide sum to a total of  $\approx 7500$  amino acids as noted earlier, which we denote by  $L_R$ . With an average mass of an  
 472 amino acid of  $m_{\text{AA}} \approx 110 \text{ Da}$  (BNID: 104877), the total ribosomal mass fraction  $\Phi_R$  is given by

$$\Phi_R = \frac{m_{\text{ribosomes}}}{m_{\text{proteome}}} \approx \frac{m_{\text{AA}} \times R \times L_R}{m_{\text{AA}} \times N_{\text{pep}}} = \frac{R \times L_R}{N_{\text{pep}}}. \quad (1)$$

473 For exponentially growing cells (**Godin et al., 2010**), the rate of cellular growth will be related to the rate of protein  
 474 synthesis via

$$\lambda N_{\text{pep}} = r_t \times R \times f_a, \quad (2)$$

475 where  $r_t$  is the translation rate. Here, we've introduced a multiplicative factor  $f_a$  which represents the fraction of the  
 476 ribosomes that are actively translating. This term allows us to account for immature or non-functional ribosomes  
 477 or active sequestration of ribosomes through the action of the secondary messenger alarmone (p)ppGpp in poorer  
 478 nutrient conditions **Hauryliuk et al. (2015)**.

479 Combining **Equation 1** and **Equation 2** results in an expression for a translation-limited growth rate, which is  
 480 given by

$$\lambda_{\text{translation-limited}} = \frac{r_t \times \Phi_R \times f_a}{L_R}. \quad (3)$$

481 This result, derived in a similar manner in **Klumpp et al. (2013)**, reflects mass-balance under steady state growth  
 482 and has long provided a rationalization of the apparent linear increase in *E. coli*'s ribosomal content as a function  
 483 of growth rate (**Maaløe, 1979; Dennis et al., 2004; Scott et al., 2010**). The left-hand panel of **Figure 9(A)** shows this  
 484 growth rate plotted as a function of the ribosomal mass fraction. In the regime where all ribosomes are active

485 ( $f_a = 1$ ) and the entire proteome is composed of ribosomal proteins ( $\Phi_R = 1$ ), indeed, we arrive at the maximum  
486 theoretical growth rate of  $r_i/L_R$ , and  $\approx 7$  min for *E. coli*.

487 Connecting **Equation 3** to the proteomic data serving as the centerpiece of our work, however, requires  
488 knowledge of  $f_a$  at each growth rate as proteomic measurements only provide a measure of  $\Phi_R$ . Recently, *Dai et al.*  
489 (2016) determined  $f_a$  as a function of the growth rate (**Figure 9(A)**, right-hand panel, inset), revealing that  $f_a \approx 1$  at  
490 growth rates above  $0.75 \text{ hr}^{-1}$  and  $f_a < 1$  as the growth rate slows. Using these data, we inferred the approximate  
491 active fraction (see Appendix Calculation of active ribosomal fraction) at each growth rate and used this to compute  
492  $\Phi_R \times f_a$  (**Figure 9(A)**, colored points in right-hand panel). In general, these data skirt the translation-limited growth  
493 rate determined using **Equation 3** with  $r_i$  taken to be the maximal elongation rate of 17 amino acids per second  
494 measured by *Dai et al.* (2016). There is a notable discrepancy between the data collected in *Schmidt et al.* (2016);  
495 *Li et al.* (2014) and that collected from *Valgepea et al.* (2013); *Peebo et al.* (2015). When compared to other  
496 measurements (non-proteomic with significantly lower resolution) of the active ribosome mass fraction (**Figure 9(B)**,  
497 grey points in right-hand panel), the data from *Valgepea et al.* (2013) and *Peebo et al.* (2015) are notably aberrant,  
498 suggesting a systematic error in these data. These additional measurements come from a number of recent studies  
499 and are determined from measurements of total RNA to total protein mass ratios (**Figure 9–Figure Supplement 1**).

500 Together, these results illustrate that the growth rates observed across the amalgamated data sets are close to  
501 the translation-limited growth rate determined through their ribosomal activity, at least for the data reported in  
502 *Schmidt et al.* (2016) and *Li et al.* (2014). While this is a useful framework to consider how the relative abundance of  
503 ribosomes (compared to all other proteins) defines the growth rate, it is worth noting that as growth rate increases,  
504 so does the cell size and therefore so will the total proteomic mass (*Basan et al.*, 2015). With a handle on how  
505 elongation rate and the total number of peptide bonds per proteome is related to the growth rate, we now expand  
506 this description to account for the increasing cell size and ribosome copy number at faster growth rates, enabling  
507 us to identify a potential bottleneck in the synthesis of rRNA.

### 508 **rRNA Synthesis Presents a Potential Bottleneck During Rapid Growth**

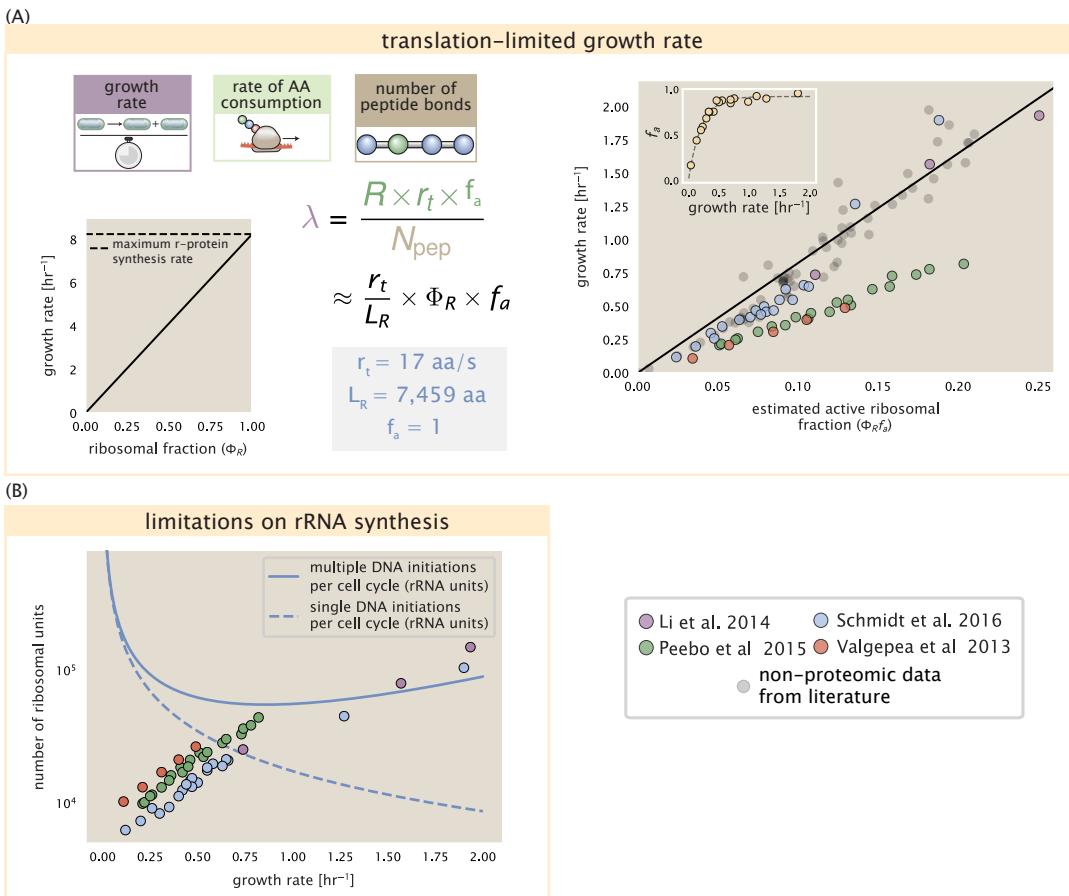
509 Even under idealized experimental conditions, *E. coli* rarely exhibits growth rates above  $2 \text{ hr}^{-1}$  (*Bremer and Dennis*,  
510 2008), which is still well-below the synthesis rate of a single ribosome, and below the maximum growth rates  
511 reported for several other bacteria (*Roller et al.*, 2016). While we have considered potential limits imposed by  
512 translation of ribosomal proteins, here we consider potential limiting regimes for the production of rRNA.

513 Due to multiple initiations of chromosomal replication per cell doubling, the effective number of rRNA operons  
514 increases with growth rate and will do so in proportion to the average number of origins per cell,  $\langle \# \text{ ori} \rangle$ . This later  
515 parameter is set by how often replication must be initiated in order to keep up with cell doubling times  $\tau$  whose  
516 time may be shorter than the cell cycle time  $t_{\text{cyc}}$  (referring to the time from replication initiation to cell division)  
517 *Dennis et al.* (2004). This is quantified by

$$\langle \# \text{ ori} \rangle = 2^{\tau_{\text{cyc}}/\tau} = 2^{\tau_{\text{cyc}}\lambda / \log(2)}. \quad (4)$$

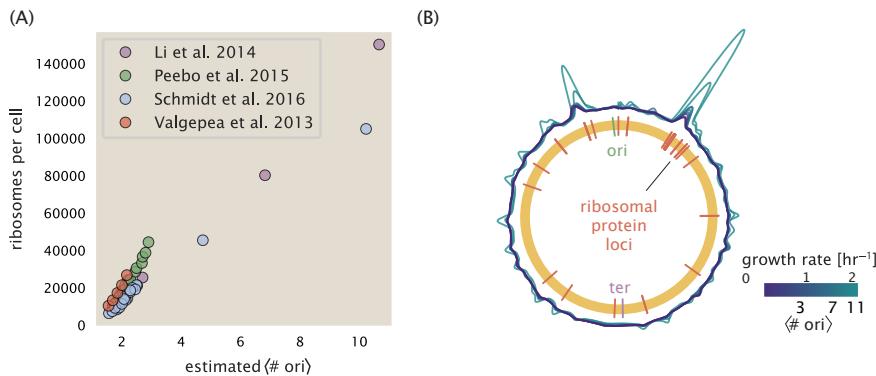
518 We used the experimental measurements of  $\tau_{\text{cyc}}$  (the timescale of chromosome replication and cell division) and  $\tau$   
519 (the timescale of a cell doubling) from *Si et al.* (2017) (**Figure 9–Figure Supplement 1(B)**) to calculate  $\langle \# \text{ ori} \rangle$  with  
520 **Equation 4** as a function of growth rates. For growth rates above about  $0.5 \text{ hr}^{-1}$ ,  $t_{\text{cyc}}$  is approximately constant  
521 at about 70 minutes, implying that  $\langle \# \text{ ori} \rangle$  will grow exponentially with growth rates beyond  $0.5 \text{ hr}^{-1}$ . As the  
522 rRNA operons are predominantly located close to origin of replication (BNID: 100352), we make the simplifying  
523 assumption that that the number of rRNA operons will be directly proportional to  $\langle \# \text{ ori} \rangle$ .

524 Returning to our rule-of-thumb of 1 functional rRNA unit per second per transcribing operon, we estimate the  
525 maximum number of ribosomes that could be made as a function of growth rate (**Figure 9(B)**, blue curve). Although  
526 we expect this estimate to significantly overestimate rRNA abundance at slower growth rates ( $\lambda < 0.5 \text{ hr}^{-1}$ ), this  
527 provides a useful reference alongside the proteomic measurements particularly in the regime of fast growth. For  
528 growth rates above about  $1 \text{ hr}^{-1}$ , for example, we find that cells will need to transcribe rRNA near their maximal  
529 rate. As a counter example, if *E. coli* did not initiate multiple rounds of replication, but managed to replicate their  
530 chromosome within the requisite time limit, they would be unable to make enough rRNA for the observed number  
531 of ribosomes (dashed blue curve in **Figure 9(C)**). The convergence between the maximum rRNA production and



**Figure 9. Translation-limited growth rate.** (A) *left*: Translation-limited growth as a function of the ribosomal fraction. The solid line is calculated for an elongation rate of 17 aa per second. The dashed line corresponds to the maximum rate of ribosomal protein synthesis ( $\approx 7$  min). *right*: Translation-limited growth rate as a function of the actively translating ribosomal fraction. The actively translating ribosomal fraction is calculated using the estimated values of  $f_a$  from [Dai et al. \(2016\)](#) (shown in inset; see Appendix Calculation of active ribosomal fraction for additional detail). Gray data points show additional measurements from literature and consider further in the supplemental figure part (A). (B) Maximum number of rRNA units that can be synthesized as a function of growth rate. Solid curve corresponding to the rRNA copy number is calculated by multiplying the number of rRNA operons by the estimated number of (# ori) at each growth rate. The quantity (# ori) was calculated using Equation 4 and the measurements from [Si et al. \(2017\)](#). The dashed line shows the maximal number of functional rRNA units produced from a single chromosomal initiation per cell cycle.

**Figure 9–Figure supplement 1.** Comparison of  $\Phi_R f_a$  with literature and estimation of (# ori).



**Figure 10. Cells increase both absolute ribosome abundance and  $\Phi_R$  with (# ori).** (A) Plot of the ribosome copy number estimated from the proteomic data against the estimated (# ori) (see Appendix Estimation of  $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$  and  $\langle \# \text{ori} \rangle$  for additional details). (B) A running Gaussian average (20 kbp st. dev.) of protein copy number is calculated for each growth condition considered by (Schmidt et al., 2016) based on each gene's transcriptional start site. Since total protein abundance increases with growth rate, protein copy numbers are median-subtracted to allow comparison between growth conditions. (# ori) are estimated using the data in (A) and Equation 4.

measured ribosome copy number suggests rRNA synthesis may begin to present a bottleneck at the fastest growth rates due to the still-limited copies of rRNA genes.

#### 534 Rapid Growth Requires *E. coli* to Increase Both Cell Size and Ribosomal Mass Fraction

535 In Figure 9(B, right-hand side) we find that above about  $0.75 \text{ hr}^{-1}$ , the growth rate is determined by the ribosomal  
536 mass fraction  $\Phi_R$ , since  $f_a$  is close to 1, and  $r_i$  is near its maximal rate (Dai et al., 2016). While  $\Phi_R$  will need to  
537 increase in order for cells to grow faster, the fractional dependence in Equation 3 gives little insight into how this is  
538 actually achieved in the cell.

539 It is now well-documented that *E. coli* cells add a constant volume per origin of replication, which is robust  
540 to a remarkable array of cellular perturbations (Si et al., 2017). Given the proteomic measurements featured in  
541 this work, it becomes apparent that the ribosome copy number is also scaled in proportion to (# ori) Figure 10(A).  
542 Importantly, however, it will only be due to an increase in  $\Phi_R$  at these moderate to fast growth rates that cells  
543 can achieve an increase in their growth rate. Indeed, we find that the deviations in protein expression with (# ori)  
544 are largely restricted to regions of ribosomal protein genes Figure 10(B). Here we have calculated the position-  
545 dependent protein expression across the chromosome by a running Gaussian average of protein copy number (20  
546 kbp st. dev. averaging window) based on each gene's transcriptional start site. These were median-subtracted to  
547 account for the change in total protein abundance with (# ori). This result suggests that  $\Phi_R$  is also being tuned in  
548 proportion to (# ori) under nutrient-limited growth, and in particular, it is through this additional dependence on  
549  $\Phi_R$  that *E. coli* exhibits an exponential increase in cell size with growth rate.

#### 550 A Minimal Model of Nutrient-Mediated Growth Rate Control

551 While the preceding subsections highlight a dominant role for ribosomes in setting the growth rate, our analysis on  
552 the whole emphasizes that the total proteomic content must also change in response to variable growth conditions  
553 and growth rate. In this final section we use a minimal model of growth rate control to better understand how this  
554 interconnection between ribosomal abundance and total protein influences the observed growth rate.

555 Here we propose that cells modulate their protein abundance in direct response to the availability of nutrients  
556 in their environment. As noted earlier, bacteria can modulate ribosomal activity through the secondary-messenger  
557 molecules like (p)ppGpp in poorer nutrient conditions (Figure 9(C) - inset; Dai et al. (2016)). Importantly, these  
558 secondary-messengers also cause global changes in transcriptional and translational activity (Hauryliuk et al.,  
559 2015; Zhu and Dai, 2019; Büke et al., 2020). In *E. coli*, amino acid starvation leads to the accumulation of de-acylated  
560 tRNAs at the ribosome's A-site and a strong increase in (p)ppGpp synthesis activity by the enzyme RelA (Hauryliuk  
561 et al., 2015). Along with this, there is increasing evidence that (p)ppGpp also acts to inhibit the initiation of DNA

replication (*Kraemer et al., 2019*), providing a potential mechanism to lower  $\langle \# \text{ ori} \rangle$  and maintain a smaller cell size in poorer growth conditions (*Fernández-Coll et al., 2020*).

To consider this quantitatively, we assume that cells modulate their proteome ( $N_{\text{pep}}$ ,  $R$ ,  $\Phi_R$ ) to better maximize their rate of peptide elongation  $r_t$ . The elongation rate  $r_t$  will depend on how quickly the ribosomes can match codons with their correct amino-acyl tRNA, along with the subsequent steps of peptide bond formation and translocation. This ultimately depends on the cellular concentration of amino acids, which we treat as a single effective species,  $[AA]_{\text{eff}}$ . In our model, we determine the the rate of peptide elongation  $r_t$  and achievable growth rate as simply depending on the supply of amino acids (and, therefore, also amino-acyl tRNAs), through a parameter  $r_{AA}$  in units of AA per second, and the rate of amino acid consumption by protein synthesis ( $r_t \times R \times f_a$ ). This is shown schematically in *Figure 11(A)* and derived in Appendix Derivation of Minimal Model for Nutrient-Mediated Growth Rate Control. Given our observation that protein synthesis and energy production are not limiting, we assume that other molecular players required by ribosomes such as elongation factors and GTP are available in sufficient abundance.

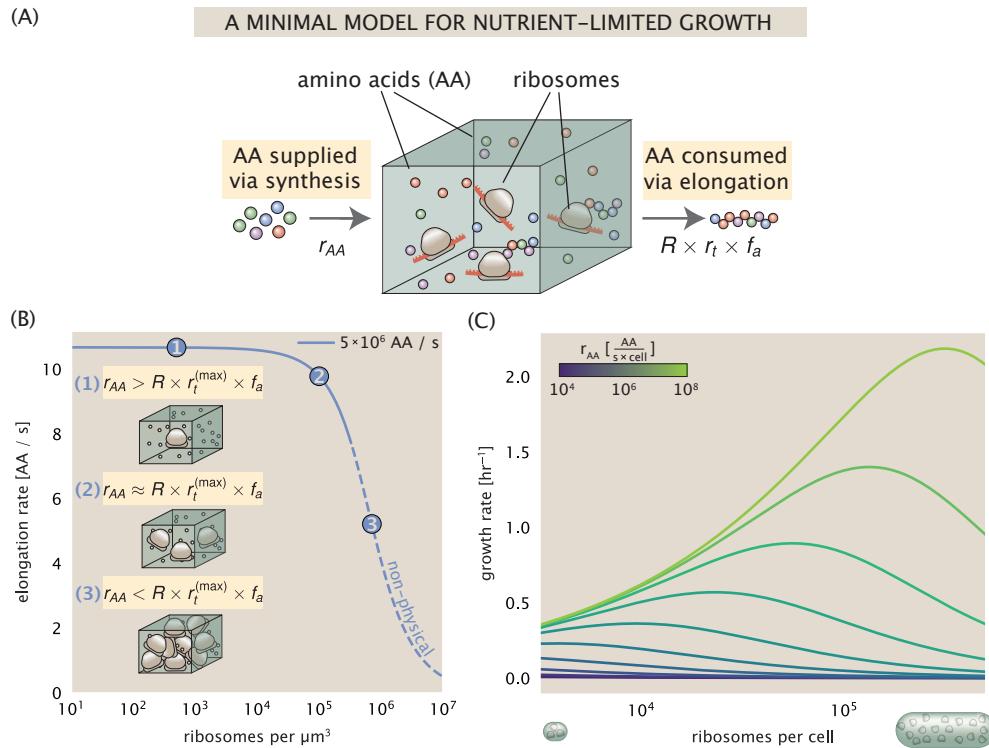
In *Figure 11(B)*, we illustrate how the elongation rate will depend on the ribosomal copy number. Here, we have considered an arbitrarily chosen  $r_{AA} = 5 \times 10^6 \text{ AA} \cdot \text{s}^{-1} \cdot \mu\text{m}^{-3}$  and  $f_a = 1$  for a unit cell volume  $V = 1\text{fL}$ . At low ribosome copy numbers, the observed elongation rate is dependent primarily on  $[AA]_{\text{eff}}$  through  $r_{AA}$  [as  $r_t^{\max} \times R \times f_a \ll r_{AA}$ , point (1) in *Figure 11(B)*]. As the ribosome copy number is increased such that the amino acid supply rate and consumption rate are nearly equal [point (2) in *Figure 11(B)*], the observed elongation rate begins to decrease sharply. When the ribosome copy number is increased even further, consumption at the maximum elongation rate exceeds the supply rate, yielding a significantly reduced elongation rate [point (3) in *Figure 11B*]. While the elongation rate will always be dominated by the amino acid supply rate at sufficiently low ribosome copy numbers, the elongation rate at larger ribosome abundances can be increased by tuning  $f_a$  such that not all ribosomes are elongating, reducing the total consumption rate.

Optimal Ribosomal Content and Cell Size Depend on Nutrient Availability and Metabolic Capacity  
To relate elongation rate to growth rate, we constrain the set of parameters based on our available proteomic measurements; namely, we restrict the values of  $R$ ,  $N_{\text{pep}}$ , and cell size to those associated with the amalgamated proteomic data (described in Appendix Estimation of Total Protein Content per Cell). We then consider how changes in the nutrient conditions, through the parameter  $r_{AA}$ , influence the maximum growth rate as determined by **Equation 3**. *Figure 11(C)* shows how the observed growth rate depends on the rate of amino acid supply  $r_{AA}$  as a function of the cellular ribosome copy number. A feature immediately apparent is the presence of a maximal growth rate whose dependence on  $R$  (and consequently, the cell size) increases with increasing  $r_{AA}$ . Importantly, however, there is an optimum set of  $R$ ,  $N_{\text{pep}}$ , and  $V$  that are strictly dependent on the value of  $r_{AA}$ . Increasing the ribosomal concentration beyond the cell's metabolic capacity has the adverse consequence of depleting the supply of amino acids and a concomitant decrease in the elongation rate  $r_t$ , [*Figure 11(B)*].

Also of note is the growth rate profiles shown for low amino acid supply rates [purple and blue lines in *Figure 11(C)*], representing growth in nutrient-poor media. In these conditions, there no longer exists a peak in growth, at least in the range of physiologically-relevant ribosome copy numbers. Instead, cells limit their pool of actively translating ribosomes by decreasing  $f_a$  (*Dai et al., 2016*), which would help maintain the pool of available amino acids  $[AA]_{\text{eff}}$  and increase the achievable elongation rate. This observation is in agreement with the central premise of the cellular resource allocation principle proposed by *Scott et al. (2010); Klumpp et al. (2009); Klumpp and Hwa (2014)* and *Hui et al. (2015)*.

## Discussion

Continued experimental and technological improvements have led to a treasure trove of quantitative biological data (*Hui et al., 2015; Schmidt et al., 2016; Si et al., 2017; Gallagher et al., 2020; Peebo et al., 2015; Valgepea et al., 2013*), and an ever advancing molecular view and mechanistic understanding of the constituents that support bacterial growth (*Taheri-Araghi et al., 2015; Morgenstein et al., 2015; Si et al., 2019; Karr et al., 2012; Kostinski and Reuveni, 2020*). In this work we have compiled what we believe to be the state-of-the-art knowledge on proteomic copy number across a broad range of growth conditions in *E. coli*. We have made this data accessible through a [GitHub repository](#), and an [interactive figure](#) that allows exploration of specific protein and protein



**Figure 11. A minimal model of growth rate control under nutrient limitation.** (A) We consider a unit volume of cellular material composed of amino acids (colored spheres) provided at a supply rate  $r_{AA}$ . These amino acids are polymerized by a pool of ribosomes (brown blobs) at a rate  $r_t \times R \times f_a$ , where  $r_t$  is the elongation rate,  $R$  is the ribosome copy number in the unit volume, and  $f_a$  is the fraction of those ribosomes actively translating. (B) The observed elongation rate is plotted as a function of ribosomes. The three points correspond to three regimes of ribosome copy numbers and are shown schematically on the left-hand side. The region of the curve shown as dashed lines represents a non-physical copy number, but is shown for illustrative purposes. This curve was generated using an amino acid supply rate of  $5 \times 10^6 \text{ AA} / \text{s}$ , a maximal elongation rate of  $17.1 \text{ AA} / \text{s}$ ,  $f_a = 1$ , and a unit cell volume of  $V = 1 \text{ fL}$ . See Appendix Derivation of Minimal Model for Nutrient-Mediated Growth Rate Control for additional model details. (C) The cellular growth rate is plotted as a function of total cellular ribosome copy number for different cellular amino acid supply rates, with blue and green curves corresponding to low and high supply rates, respectively. As the ribosome copy number is increased, so too is the cell size and total protein abundance  $N_{\text{pep}}$ . We direct the reader to the Supplemental Information for discussion on the inference of the relationship between cell size, number of peptide bonds, and ribosome copy number.

**Figure 11–Figure supplement 1.** An interactive figure for exploration of the model parameter space.

611 complex copy numbers. Through a series of order-of-magnitude estimates that traverse key steps in the bacterial  
612 cell cycle, this proteomic data has been a resource to guide our understanding of two key questions: what biological  
613 processes limit the absolute speed limit of bacterial growth, and how do cells alter their molecular constituents  
614 as a function of changes in growth rate or nutrient availability? While not exhaustive, our series of estimates  
615 provide insight on the scales of macromolecular complex abundance across four classes of cellular processes – the  
616 transport of nutrients, the production of energy, the synthesis of the membrane and cell wall, and the numerous  
617 steps of the central dogma.

618 In general, the copy numbers of the complexes involved in these processes were in reasonable agreement  
619 with our order-of-magnitude estimates. Since many of these estimates represent soft lower-bound quantities, this  
620 suggests that cells do not express proteins grossly in excess of what is needed for a particular growth rate. Several  
621 exceptions, however, also highlight the dichotomy between a proteome that appears to "optimize" expression  
622 according to growth rate and one that must be able to quickly adapt to environments of different nutritional quality.  
623 Take, for example, the expression of carbon transporters. Shown in *Figure 2(B)*, we find that cells always express a  
624 similar number of glucose transporters irrespective of growth condition. At the same time, it is interesting to note  
625 that many of the alternative carbon transporters are still expressed in low but non-zero numbers ( $\approx$  10-100 copies  
626 per cell) across growth conditions. This may relate to the regulatory configuration for many of these operons,  
627 which require the presence of a metabolite signal in order for alternative carbon utilization operons to be induced  
628 (*Monod, 1949; Laxhuber et al., 2020*). Furthermore, upon induction, these transporters are expressed and present  
629 in abundances in close agreement with a simple estimate.

630 Of the processes illustrated in *Figure 1*, we arrive at a ribosome-centric view of cellular growth rate control.  
631 This is in some sense unsurprising given the long-held observation that *E. coli* and many other organisms vary  
632 their ribosomal abundance as a function of growth conditions and growth rate (*Scott et al., 2010; Metzl-Raz et al.,  
633 2017*). However, through our dialogue with the proteomic data, two additional key points emerge. The first relates  
634 to our question of what process sets the absolute speed limit of bacterial growth. While a cell can parallelize  
635 many of its processes simply by increasing the abundance of specific proteins or firing multiple rounds of DNA  
636 replication, this is not so for synthesis of ribosomes (*Figure 9(A)*). The translation time for each ribosome [ $\approx$  7 min,  
637 *Dill et al. (2011)*] places an inherent limit on the growth rate that can only be surpassed if the cell were to increase  
638 their polypeptide elongation rate, or if they could reduce the total protein and rRNA mass of the ribosome. The  
639 second point relates to the long-observed correlations between growth rate and cell size (*Schaechter et al., 1958; Si  
640 et al., 2017*), and between growth rate and ribosomal mass fraction. While both trends have sparked tremendous  
641 curiosity and driven substantial amounts of research in their own regards, these relationships are themselves  
642 intertwined. In particular, it is the need for cells to increase their absolute number of ribosomes under conditions  
643 of rapid growth that require cells to also grow in size. Further experiments are needed to test the validity of this  
644 hypothesis. In particular, we believe that the change in growth rate in response to translation-inhibitory drugs  
645 (such as chloramphenicol) could be quantitatively predicted, given one had precision measurement of the relevant  
646 parameters, including the fraction of actively translating ribosomes  $f_a$  and changes in the metabolic capacity of the  
647 cell (i.e. the rate of amino acid supply) for a particular growth condition.

648 While the generation of new ribosomes plays a dominant role in growth rate control, there exist other physical  
649 limits to the function of cellular processes. One of the key motivations for considering energy production was  
650 the physical constraints on total volume and surface area as cells vary their size (*Harris and Theriot, 2018; Ojikic  
651 et al., 2019*). While *E. coli* get larger as it expresses more ribosomes, an additional constraint begins to arise in  
652 energy production due to a relative decrease in total surface area where ATP is predominantly produced (*Szenk  
653 et al., 2017*). Specifically, the cell interior requires an amount of energy that scales cubically with cell size, but the  
654 available surface area only grows quadratically (*Figure 5(A)*). While this threshold does not appear to be met for  
655 *E. coli* cells growing at 2 hr<sup>-1</sup> or less, it highlights an additional constraint on growth given the apparent need to  
656 increase in cell size to grow faster. This limit is relevant even to eukaryotic organisms, whose mitochondria exhibit  
657 convoluted membrane structures that nevertheless remain bacteria-sized organelles (*Guo et al., 2018*). In the  
658 context of bacterial growth and energy production more generally, we have limited our analysis to the aerobic  
659 growth conditions associated with the proteomic data and further consideration will be needed for anaerobic  
660 growth.

661 This work is by no means meant to be an exhaustive dissection of bacterial growth rate control, and there are

many aspects of the bacterial proteome and growth that we neglected to consider. For example, other recent work (*Liebermeister et al., 2014; Hui et al., 2015; Schmidt et al., 2016*) has explored how the proteome is structured and how that structure depends on growth rate. In the work of *Hui et al. (2015)*, the authors coarse-grained the proteome into six discrete categories being related to either translation, catabolism, anabolism, and others related to signaling and core metabolism. The relative mass fraction of the proteome occupied by each sector could be modulated by external application of drugs or simply by changing the nutritional content of the medium. While we have explored how the quantities of individual complexes are related to cell growth, we acknowledge that higher-order interactions between groups of complexes or metabolic networks at a systems-level may reveal additional insights into how these growth-rate dependences are mechanistically achieved. Furthermore, while we anticipate the conclusions summarized here are applicable to a wide collection of bacteria with similar lifestyles as *E. coli*, other bacteria and archaea may have evolved other strategies that were not considered. Further experiments with the level of rigor now possible in *E. coli* will need to be performed in a variety of microbial organisms to learn more about how regulation of proteomic composition and growth rate control has evolved over the past 3.5 billion years.

## Methods

### Data Analysis and Availability

All proteomic measurements come from the experimental work of *Schmidt et al. (2016); Peebo et al. (2015); Valgepea et al. (2013)* (mass spectrometry) and *Li et al. (2014)* (ribosomal profiling). Data curation and analysis was done programmatically in Python, and compiled data and analysis files are accessible through a [GitHub repository] (DOI:XXX) associated with this paper as well as on the associated [paper website](#). An interactive figure that allows exploration of specific protein and protein complex copy numbers is available at [link].

### Acknowledgements

We thank Matthias Heinemann, Alexander Schmidt, and Gene-Wei Li for additional input regarding their data. We also thank members of the Phillips, Theriot, Kondev, and Garcia labs for useful discussions. We thank Suzannah M. Beeler, Avi Flamholz, Soichi Hirokawa, and Manuel Razo-Mejia for reading and providing comments on drafts of this manuscript. R.P. is supported by La Fondation Pierre-Gilles de Gennes, the Rosen Center at Caltech, and the NIH 1R35 GM118043 (MRA). J.A.T. is supported by the Howard Hughes Medical Institute, and NIH Grant R37-AI036929. N.M.B is a HHMI Fellow of The Jane Coffin Childs Memorial Fund.

### Competing Interests

The authors declare no competing interests.

# 692 Appendix for: Fundamental limits on the 693 rate of bacterial cell division

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763 **Additional Estimates of Fundamental Biological Processes**

764 In the main text of this work, we present estimates for a significant number of fundamental biological processes  
765 that are necessary for cell division. While we believe the estimates provided in the main text provide a succinct  
766 summary of the corresponding process, we left out additional estimates of related processes for brevity. In this  
767 section of the appendix, we present these additional estimates in full.

768 **Nutrient Transport**

769 In the main text, we make passing mention that while transport carbon often comes in the form of carbohydrates  
770 and sugar alcohols while other critical elements – such as nitrogen, sulfur, and phosphorus – are transported as  
771 inorganic ions. Below, we present estimates for the transport requirements of these materials.

772 **Nitrogen**

773 We must first address which elemental sources must require active transport, meaning that the cell cannot acquire  
774 appreciable amounts simply via diffusion across the membrane. The permeability of the lipid membrane to a large  
775 number of solutes has been extensively characterized over the past century. Large, polar molecular species (such  
776 as various sugar molecules, sulfate, and phosphate) have low permeabilities while small, non-polar compounds  
777 (such as oxygen, carbon dioxide, and ammonia) can readily diffuse across the membrane. Ammonia, a primary  
778 source of nitrogen in typical laboratory conditions, has a permeability on par with water ( $\sim 10^5$  nm/s, BNID:110824).  
779 In nitrogen-poor conditions, *E. coli* expresses a transporter (AmtB) which appears to aid in nitrogen assimilation,  
780 though the mechanism and kinetic details of transport are still a matter of debate (*van Heeswijk et al., 2013*;  
781 *Khademi et al., 2004*). Beyond ammonia, another plentiful source of nitrogen come in the form of glutamate,  
782 which has its own complex metabolism and scavenging pathways. However, nitrogen is plentiful in the growth  
783 conditions examined in this work, permitting us to neglect nitrogen transport as a potential rate limiting process in  
784 cell division in typical experimental conditions.

785 **Phosphorus**

786 Phosphorus is critical to the cellular energy economy in the form of high-energy phosphodiester bonds making  
787 up DNA, RNA, and the NTP energy pool as well as playing a critical role in the post-translational modification of  
788 proteins and defining the polar-heads of lipids. In total, phosphorus makes up  $\approx 3\%$  of the cellular dry mass which  
789 in typical experimental conditions is in the form of inorganic phosphate. The cell membrane has remarkably low  
790 permeability to this highly-charged and critical molecule, therefore requiring the expression of active transport  
791 systems. In *E. coli*, the proton electrochemical gradient across the inner membrane is leveraged to transport  
792 inorganic phosphate into the cell (*Rosenberg et al., 1977*). Proton-solute symporters are widespread in *E. coli*  
793 (*Ramos and Kaback, 1977; Booth et al., 1979*) and can have rapid transport rates of 50 to 100 molecules per second  
794 for sugars and other solutes (BNID: 103159; 111777). As a more extreme example, the proton transporters in  
795 the F<sub>1</sub>-F<sub>0</sub> ATP synthase, which use the proton electrochemical gradient for rotational motion, can shuttle protons  
796 across the membrane at a rate of  $\approx 1000$  per second (BNID: 104890; 103390). In *E. coli* the PitA phosphate  
797 transport system has been shown to be very tightly coupled with the proton electrochemical gradient with a 1:1  
798 proton:phosphate stoichiometric ratio (*Harris et al., 2001; Feist et al., 2007*). Taking the geometric mean of the  
799 aforementioned estimates gives a plausible rate of phosphate transport on the order of 300 per second. Illustrated  
800 in *Figure 2–Figure Supplement 1(A)*, we can estimate that  $\approx 200$  phosphate transporters are necessary to maintain  
801 an  $\approx 3\%$  dry mass with a 5000 s division time. This estimate is consistent with observation when we examine  
802 the observed copy numbers of PitA in proteomic data sets (plot in *Figure 2–Figure Supplement 1(A)*). While our  
803 estimate is very much in line with the observed numbers, we emphasize that this is likely a slight overestimate of the  
804 number of transporters needed as there are other phosphorous scavenging systems, such as the ATP-dependent  
805 phosphate transporter Pst system which we have neglected.

806 **Sulfur**

807 Similar to phosphate, sulfate is highly-charged and not particularly membrane permeable, requiring active transport.  
808 While there exists a H<sup>+</sup>/sulfate symporter in *E. coli*, it is in relatively low abundance and is not well characterized  
809 (*Zhang et al., 2014a*). Sulfate is predominantly acquired via the ATP-dependent ABC transporter CysUWA system

810 which also plays an important role in selenium transport (*Sekowska et al., 2000; Sirko et al., 1995*). While specific  
811 kinetic details of this transport system are not readily available, generic ATP transport systems in prokaryotes  
812 transport on the order of 1 to 10 molecules per second (BNID: 109035). Combining this generic transport rate,  
813 measurement of sulfur comprising 1% of dry mass, and a 5000 second division time yields an estimate of  $\approx 1000$   
814 CysUWA complexes per cell (*Figure 2–Figure Supplement 1(B)*). Once again, this estimate is in notable agreement  
815 with proteomic data sets, suggesting that there are sufficient transporters present to acquire the necessary sulfur.  
816 In a similar spirit of our estimate of phosphorus transport, we emphasize that this is likely an overestimate of  
817 the number of necessary transporters as we have neglected other sulfur scavenging systems that are in lower  
818 abundance.

## 819 Additional Process of the Central Dogma

820 In the main text, we consider the processes underlying the backbone of the central dogma, namely DNA replication,  
821 RNA transcription, and protein translation. In this section we turn our attention to additional processes related to  
822 the central dogma, primarily dNTP synthesis for DNA replication and amino-acyl tRNA synthesis for translation.  
823 Additionally, we explore in more detail the estimates shown in *Figure 7(A)* for the RNA polymerase requirements of  
824 mRNA and tRNA synthesis.

### 825 dNTP synthesis

826 The four major dNTPs (dATP, dTTP, dCTP, and dGTP) serve as the fundamental units of the genetic code. Thus, to  
827 faithfully replicate the chromosome, the cell must be able to synthesize enough of these bases in the first place.  
828 All dNTPs are synthesized *de novo* in separate pathways, requiring different building blocks. However, a critical  
829 step present in all dNTP synthesis pathways is the conversion from ribonucleotide to deoxyribonucleotide via  
830 the removal of the 3' hydroxyl group of the ribose ring (*Rudd et al., 2016*). This reaction is mediated by a class of  
831 enzymes termed ribonucleotide reductases, of which *E. coli* possesses two aerobically active complexes (termed I  
832 and II) and a single anaerobically active enzyme. Due to their peculiar formation of a radical intermediate, these  
833 enzymes have received much biochemical, kinetic, and structural characterization. One such work (*Ge et al.,*  
834 *2003*) performed a detailed *in vitro* measurement of the steady-state kinetic rates of these complexes, revealing a  
835 turnover rate of  $\approx 10$  dNTP per second.

836 Since this reaction is central to the synthesis of all dNTPs, it is reasonable to consider the abundance of  
837 these complexes is a measure of the total dNTP production in *E. coli*. Illustrated schematically in *Figure 6 (A)*, we  
838 consider the fact that to replicate the cell's genome, on the order of  $\approx 10^7$  dNTPs must be synthesized. Assuming a  
839 production rate of 10 per second per ribonucleotide reductase complex and a cell division time of 5000 seconds,  
840 we arrive at an estimate of  $\approx 200$  complexes needed per cell. As shown in the bottom panel of *Figure Supplement 1*  
841 (A), this estimate agrees with the experimental measurements of these complexes abundances within  $\approx 1/2$  an  
842 order of magnitude. Extension of this estimate across a continuum of growth rate, including the fact that multiple  
843 chromosomes can be replicated at a given time, is shown as a grey transparent line in *Figure Supplement 1*.  
844 Similarly to our point estimate, this refinement agrees well with the data, accurately describing both the magnitude  
845 of the complex abundance and the dependence on growth rate.

846 Recent work has revealed that during replication, the ribonucleotide reductase complexes coalesce to form  
847 discrete foci colocalized with the DNA replisome complex (*Sánchez-Romero et al., 2011*). This is particularly  
848 pronounced in conditions where growth is slow, indicating that spatial organization and regulation of the activity of  
849 the complexes plays an important role.

### 850 mRNA and tRNA Synthesis

851 In *Figure 7* of the main text, we present with limited explanation estimates for the number of RNA polymerases  
852 needed to synthesize enough mRNA and tRNA molecules. Here, we present a rationalization for these estimates.

853 To form a functional protein, all protein coding genes must first be transcribed from DNA to form an mRNA  
854 molecule. While each protein requires an mRNA blueprint, many copies of the protein can be synthesized from  
855 a single mRNA. Factors such as strength of the ribosomal binding site, mRNA stability, and rare codon usage  
856 frequency dictate the number of proteins that can be made from a single mRNA, with yields ranging from  $10^1$  to  $10^4$   
857 (BNID: 104186; 100196; 106254). Computing the geometric mean of this range yields  $\approx 1000$  proteins synthesized

858 per mRNA, a value that agrees with experimental measurements of the number of proteins per cell ( $\approx 3 \times 10^6$ , BNID:  
859 100088) and total number of mRNA per cell ( $\approx 3 \times 10^3$ , BNID:100064).

860 This estimation captures the *steady-state* mRNA copy number, meaning that at any given time, there will exist  
861 approximately 3000 unique mRNA molecules. To determine the *total* number of mRNA that need to be synthesized  
862 over the cell's lifetime, we must consider degradation of the mRNA. In most bacteria, mRNAs are rather unstable  
863 with life times on the order of several minutes (BNID: 104324; 106253; 111927; 111998). For convenience, we  
864 assume that the typical mRNA in our cell of interest has a typical lifetime of  $\approx 300$  seconds. Using this value, we  
865 can determine the total mRNA production rate to maintain a steady-state copy number of 3000 mRNA per cell.  
866 While we direct the reader to the appendix for a more detailed discussion of mRNA transcriptional dynamics, we  
867 state here that the total mRNA production rate must be on the order of  $\approx 15$  mRNA per second. In *E. coli*, the  
868 average protein is  $\approx 300$  amino acids in length (BNID: 108986), meaning that the corresponding mRNA is  $\approx 900$   
869 nucleotides which we will further approximate as  $\approx 1000$  nucleotides to account for the non-protein coding regions  
870 on the 5' and 3' ends. This means that the cell must have enough RNA polymerase molecules about to sustain a  
871 transcription rate of  $\approx 1.5 \times 10^4$  nucleotides per second. Knowing that a single RNA polymerase polymerizes RNA  
872 at a clip of 40 nucleotides per second, we arrive at a comfortable estimate of  $\approx 250$  RNA polymerase complexes  
873 needed to satisfy the mRNA demands of the cell. It is worth noting that this number is approximately half of that  
874 required to synthesize enough rRNA, as we saw in the previous section. We find this to be a striking result as these  
875 250 RNA polymerase molecules are responsible for the transcription of the  $\approx 4000$  protein coding genes that are  
876 not ribosome associated.

877 We now turn our attention to the synthesis of tRNA. Unlike mRNA or rRNA, each individual tRNA is remarkably  
878 short, ranging from 70 to 95 nucleotides each (BNID: 109645; 102340). What they lack in length, they make up  
879 for in abundance, with reported values ranging from  $\approx 5 \times 10^4$  (BNID: 105280) to  $\approx 5 \times 10^5$  (BNID: 108611). To test  
880 tRNA synthesis as a possible growth-rate limiting stage, we will err towards a higher abundance of  $\approx 5 \times 10^5$  per cell.  
881 Combining the abundance and tRNA length measurements, we make the estimate that  $\approx 5 \times 10^7$  nucleotides are  
882 sequestered in tRNA per cell. Unlike mRNA, tRNA is remarkably stable with typical lifetimes *in vivo* on the order of  $\approx$   
883 48 hours (*Abelson et al., 1974; Svenningsen et al., 2017*) – well beyond the timescale of division. Once again using  
884 our rule-of-thumb for the rate of transcription to be 40 nucleotides per second and assuming a division time of  $\approx$   
885 5000 seconds, we arrive at an estimate of  $\approx 200$  RNA polymerases to synthesize enough tRNA. This requirement  
886 pales in comparison to the number of polymerases needed to generate the rRNA and mRNA pools and can be  
887 neglected as a significant transcriptional burden.

### 888 tRNA Charging

889 In the previous subsection, we focused solely on estimating the number of RNA polymerases needed for the  
890 generation of the tRNA molecule itself. We now explore the protein complex requirements for ligation of the  
891 appropriate amino acid to each tRNA. We begin by again using an estimate of  $\approx 3 \times 10^6$  proteins per cell at a 5000 s  
892 division time (BNID: 115702) and a typical protein length of  $\approx 300$  amino acids (BNID: 100017), we can estimate  
893 that a total of  $\approx 10^9$  amino acids are stitched together by peptide bonds.

894 How many tRNAs are needed to facilitate this remarkable number of amino acid delivery events to the translating  
895 ribosomes? It is important to note that tRNAs are recycled after they've passed through the ribosome and can be  
896 recharged with a new amino acid, ready for another round of peptide bond formation. While some *in vitro* data  
897 exists on the turnover of tRNA in *E. coli* for different amino acids, we can make a reasonable estimate by comparing  
898 the number of amino acids to be polymerized to cell division time. Using our stopwatch of 5000 s and  $10^9$  amino  
899 acids, we arrive at a requirement of  $\approx 2 \times 10^5$  tRNA molecules to be consumed by the ribosome per second.

900 There are many processes which go into synthesizing a tRNA and ligating it with the appropriate amino acids.  
901 As we discussed previously, there appear to be more than enough RNA polymerases per cell to synthesize the  
902 needed pool of tRNAs. Without considering the many ways in which amino acids can be scavenged or synthesized  
903 *de novo*, we can explore ligation the as a potential rate limiting step. The enzymes which link the correct amino acid  
904 to the tRNA, known as tRNA synthetases or tRNA ligases, are incredible in their proofreading of substrates with the  
905 incorrect amino acid being ligated once out of every  $10^4$  to  $10^5$  events (BNID: 103469). This is due in part to the  
906 consumption of energy as well as a multi-step pathway to ligation. While the rate at which tRNA is ligated is highly  
907 dependent on the identity of the amino acid, it is reasonable to state that the typical tRNA synthetase has charging

**Table 1.** Overview of proteomic data sets.

Author	Method	Reported Quantity
Taniguchi <i>et al.</i> (2010)	YFP-fusion, cell fluorescence	fg/copies per cell
Valgepea <i>et al.</i> (2012)	mass spectrometry	fg/copies per cell
Peebo <i>et al.</i> (2014)	mass spectrometry	fg/copies per fl
Li <i>et al.</i> (2014)	ribosomal profiling	fg/copies per cell <sup>a</sup>
Soufi <i>et al.</i> (2015)	mass spectrometry	fg/copies per cell
Schmidt <i>et al.</i> (2016)	mass spectrometry	fg/copies per cell <sup>b</sup>

a. The reported values assume that the proteins are long-lived compared to the generation time but are unable to account for post-translational modifications that may alter absolute protein abundances.

b. This mass spectrometry approach differs substantially from the others since in addition to the relative proteome-wide abundance measurements, the authors performed absolute quantification of 41 proteins across all growth conditions (see section on Additional Considerations of Schmidt *et al.* Data Set for more details on this).

908 rate of  $\approx$  20 AA per tRNA synthetase per second (BNID: 105279).

909 We can make an assumption that amino-acyl tRNAs are in steady-state where they are produced at the same  
910 rate they are consumed, meaning that  $2 \times 10^5$  tRNAs must be charged per second. Combining these estimates  
911 together, as shown schematically in **Figure 8–Figure Supplement 1**, yields an estimate of  $\sim 10^4$  tRNA synthetases  
912 per cell with a division time of 5000 s. This point estimate is in very close agreement with the observed number  
913 of synthetases (the sum of all 20 tRNA synthetases in *E. coli*). This estimation strategy seems to adequately  
914 describe the observed growth rate dependence of the tRNA synthetase copy number (shown as the grey line in  
915 **Figure 8–Figure Supplement 1**, suggesting that the copy number scales with the cell volume).

916 In total, the estimated and observed  $\sim 10^4$  tRNA synthetases occupy only a meager fraction of the total cell  
917 proteome, around 0.5% by abundance. It is reasonable to assume that if tRNA charging was a rate limiting  
918 process, cells would be able to increase their growth rate by devoting more cellular resources to making more tRNA  
919 synthetases. As the synthesis of tRNAs and the corresponding charging can be highly parallelized, we can argue that  
920 tRNA charging is not a rate limiting step in cell division, at least for the growth conditions explored in this work.

## 921 Experimental Details Behind Proteomic Data

922 Here we provide a brief summary of the experiments behind each proteomic data set considered. The purpose of  
923 this section is to identify how the authors arrived at absolute protein abundances. In the following section (see  
924 section on Summary of Proteomic Data) we will then provide a summary of the protein abundance measurements.  
925 Table 1 provides an overview of the publications we considered. These are predominately mass spectrometry-based,  
926 with the exception of the work from Li *et al.* (2014) which used ribosomal profiling, and the fluorescence-based  
927 counting done in Taniguchi *et al.* (2010). After having compiled and comparing these measurements, we noted  
928 substantial deviations in the measurements from Taniguchi *et al.* (2010) and Soufi *et al.* (2015) (shown in the  
929 following section), and decided to only use the data from Taniguchi *et al.* (2010); Li *et al.* (2014); Valgepea *et al.*  
930 (2013); Peebo *et al.* (2015) in the main text. For completeness, we include these additional datasets in our discussion  
931 of the experimental data.

## 932 Fluorescence based measurements

933 In the work of Taniguchi *et al.* (2010), the authors used a chromosomal YFP fusion library where individual strains  
934 have a specific gene tagged with a YFP-coding sequence. 1018 of their 1400 attempted strains were used in the work.  
935 A fluorescence microscope was used to collect cellular YFP intensities across all these strains. Through automated  
936 image analysis, the authors normalized intensity measurements by cell size to account for the change in size and  
937 expression variability across the cell cycle. Following correction of YFP intensities for cellular autofluorescence,  
938 final absolute protein levels were determined by a calibration curve with single-molecule fluorescence intensities.  
939 This calibration experiment was performed separately using a purified YFP solution.

940 **Ribosomal profiling measurements**

941 The work of *Li et al. (2014)* takes a sequencing based approach to estimate protein abundance. Ribosomal  
942 profiling, which refers to the deep sequencing of ribosome-protected mRNA fragments, can provide a quantitative  
943 measurement of the protein synthesis rate. As long as the protein life-time is long relative to the cell doubling time,  
944 it is possible to estimate absolute protein copy numbers. The absolute protein synthesis rate has units of proteins  
945 per generation, and for stable proteins will also correspond to the protein copy number per cell.

946 In the experiments, ribosome-protected mRNA is extracted from cell lysate and selected on a denaturing  
947 polyacrylamide gel for deep sequencing (15–45 nt long fragments collected and sequenced by using an Illumina  
948 HiSeq 2000 in *Li et al. (2014)*). Counts of ribosome footprints from the sequencing data were then corrected  
949 empirically for position-dependent biases in ribosomal density across each gene, as well as dependencies on  
950 specific sequences including the Shine-Dalgarno sequence. These data-corrected ribosome densities represent  
951 relative protein synthesis rates. Absolute protein synthesis rates are obtained by multiplying the relative rates by  
952 the total cellular protein per cell. The total protein per unit volume was determined with the Lowry method to  
953 quantify total protein, calibrated against bovine serum albumin (BSA). By counting colony-forming units following  
954 serial dilution of their cell cultures, they then calculated the total protein per cell.

955 **Mass spectrometry measurements**

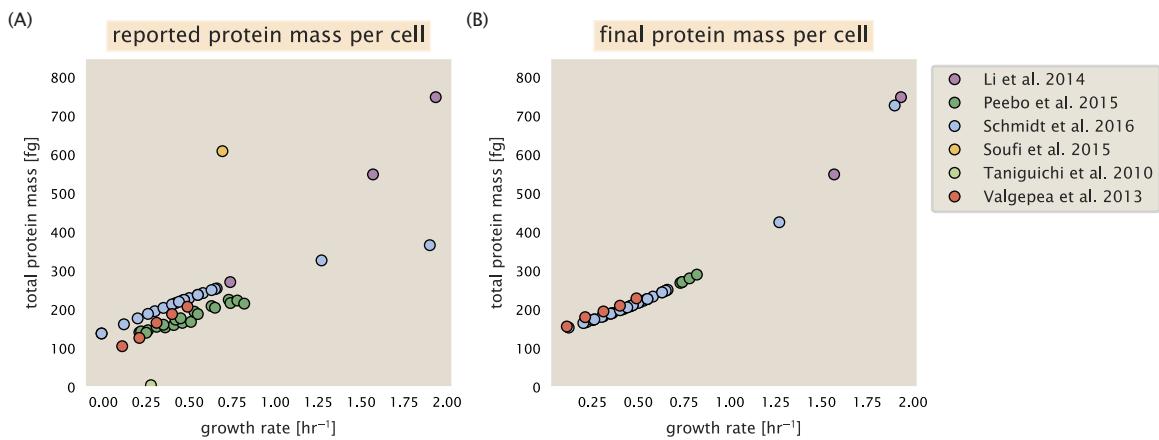
956 Perhaps not surprisingly, the data is predominantly mass spectrometry based. This is largely due to tremendous  
957 improvements in the sensitivity of mass spectrometers, as well as improvements in sample preparation and  
958 data analysis pipelines. It is now a relatively routine task to extract protein from a cell and quantify the majority  
959 of proteins present by shotgun proteomics. In general, this involves lysing cells, enzymatically digesting the  
960 proteins into short peptide fragments, and then introducing them into the mass spectrometer (e.g. with liquid  
961 chromatography and electrospray ionization), which itself can have multiple rounds of detection and further  
962 fragmentation of the peptides.

963 Most quantitative experiments rely on labeling protein with stable isotopes, which allow multiple samples  
964 to be measured together by the mass spectrometer. By measuring samples of known total protein abundance  
965 simultaneously (i.e. one sample of interest, and one reference), it is possible to determine relative protein  
966 abundances. Absolute protein abundances can be estimated following the same approach used above for  
967 ribosomal profiling, which is to multiply each relative abundance measurement by the total cellular protein per  
968 cell. This is the approach taken by *Valgepea et al. (2013)*; *Peebo et al. (2015)* and *Soufi et al. (2015)*, with relative  
969 protein abundances determined based on the relative peptide intensities (label free quantification 'LFQ' intensities).  
970 For the data of *Valgepea et al. (2013)*, total protein per cell was determined by measuring total protein by the  
971 Lowry method, and counting colony-forming units following serial dilution. For the data from *Peebo et al. (2015)*,  
972 the authors did not determine cell quantities and instead report the cellular protein abundances in protein per  
973 unit volume by assuming a mass density of 1.1 g/ml, with a 30% dry mass fraction.

974 An alternative way to arrive at absolute protein abundances is to dope in synthetic peptide fragments of known  
975 abundance. These can serve as a direct way to calibrate mass spectrometry signal intensities to absolute mass.  
976 This is the approach taken by *Schmidt et al. (2016)*. In addition to a set of shotgun proteomic measurements to  
977 determine proteome-wide relative abundances, the authors also performed absolute quantification of 41 proteins  
978 covering over four orders of magnitude in cellular abundance. Here, a synthetic peptide was generated for each of  
979 the proteins, doped into each protein sample, and used these to determine absolute protein abundances of the 41  
980 proteins. These absolute measurements, determined for every growth condition, were then used as a calibration  
981 curve to convert proteomic-wide relative abundances into absolute protein abundance per cell. A more extensive  
982 discussion of the *Schmidt et al. (2016)* data set can be found in Section Additional Considerations of Schmidt et al.  
983 Data Set.

984 **Summary of Proteomic Data**

985 In the work of the main text we only used the data from *Valgepea et al. (2013)*; *Li et al. (2014)*; *Peebo et al. (2015)*;  
986 *Schmidt et al. (2016)*. As shown in *Figure 12(A)*, the reported total protein abundances in the work of *Taniguchi*  
987 *et al. (2010)* and *Soufi et al. (2015)* differed quite substantially from the other work. For the work of *Taniguchi et al.*



**Figure 12. Summary of the growth-rate dependent total protein abundance for each data set.** (A) Total protein abundance per cell as originally reported in the data sets of *Taniguchi et al. (2010)*; *Valgepea et al. (2013)*; *Li et al. (2014)*; *Soufi et al. (2015)*; *Peebo et al. (2015)*; *Schmidt et al. (2016)*. Note that the data from *Peebo et al. (2015)* only reported protein abundances per unit volume and total protein per cell was found by multiplying these by the growth-rate dependent cell size as determined by *Si et al. (2017)*. (B) Adjusted total protein abundances across the proteomic data sets are summarized. Protein abundances were adjusted so that all data shared a common set of growth-rate dependent total protein per cell and cellular protein concentration following the cell size expectations of *Si et al. (2017)* (see section on Estimation of Cell Size and Surface Area for further details).

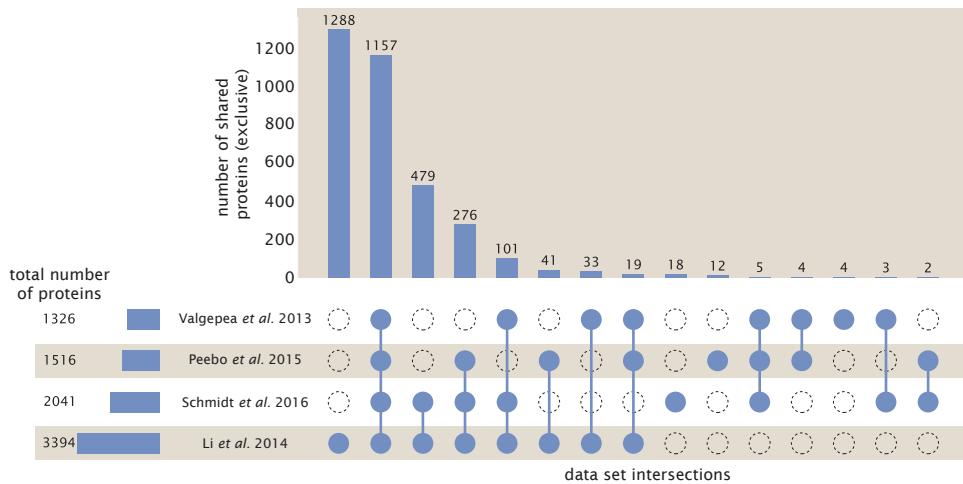
988 (2010) this is in part due to a lower coverage in total proteomic mass quantified, though we also noticed that most  
989 proteins appear undercounted when compared to the other data.

990 *Figure 12(B)* summarizes the total protein mass for each data set used in our final compiled data set. Our  
991 inclination initially was to leave reported copy numbers untouched, but a notable discrepancy between the scaling  
992 of the total protein per cell between *Schmidt et al. (2016)* and the other data sets forced us to dig deeper into  
993 those measurements (compare *Schmidt et al. (2016)* and *Li et al. (2014)* data in *Figure 12(A)*). The particular trend  
994 in *Schmidt et al. (2016)* appears to be due to assumptions made about cell size and we provide a more extensive  
995 discussion and analysis of their data in Additional Considerations of Schmidt et al. Data Set. As a compromise, and  
996 in an effort to treat all data equally, we instead applied a correction factor to all protein abundance values based  
997 on a data-driven estimate of total protein per cell. Here we used cell size measurements from *Si et al. (2017, 2019)*,  
998 and an estimate of total protein content through expected dry mass. Total protein per cell was then determined  
999 using available data on total DNA, RNA, and protein from *Basan et al. (2015)*; *Dai et al. (2016)*, which account for  
1000 the majority of dry mass in the cell. We describe these details further in sections on Estimation of Cell Size and  
1001 Surface Area and Estimation of Total Protein Content per Cell that follows.

1002 Lastly, in *Figure 13* we show the total proteomic coverage and overlap of proteins quantified across each data  
1003 set. Here we have used an UpSet diagram (*Lex et al., 2014*) to compare the data. Overall, the overlap in quantified  
1004 proteins is quite high, with 1157 proteins quantified across all data sets. The sequencing based approach of *Li*  
1005 *et al. (2014)* has substantially higher coverage compared to the mass spectrometry data sets (3394 genes versus  
1006 the 2041 genes quantified in the work of *Schmidt et al. (2016)*). However, in terms of total protein mass, the data  
1007 from *Li et al. (2014)*; *Schmidt et al. (2016)*; *Peebo et al. (2015)* each quantify roughly equivalent total protein mass.  
1008 An exception to this is in the data from *Valgepea et al. (2013)*, where we find that the total protein quantified in  
1009 *Valgepea et al. (2013)* is 90-95 % of the total protein mass (when using the data from *Schmidt et al. (2016)* as a  
1010 reference).

### 1011 Estimation of Cell Size and Surface Area

1012 Since most of the proteomic data sets lack cell size measurements, we chose instead to use a common estimate of  
1013 size for any analysis requiring cell size or surface area. Since each of the data sets used either K-12 MG1655 or  
1014 its derivative, BW25113 (from the lab of Barry L. Wanner; the parent strain of the Keio collection (*Datsenko and*  
1015 *Wanner, 2000; Baba et al., 2006*)), below we fit the MG1655 cell size data from the supplemental material of *Si*



**Figure 13. Comparison of proteomic coverage across different data sets.** An UpSet diagram (Lex et al., 2014) summarizes the total number of protein coding genes whose protein abundance was reported in the data sets of Valgepea et al. (2013); Li et al. (2014); Schmidt et al. (2016); Peebo et al. (2015). Bar plot on bottom left indicates the total number of genes reported in each individual data. The main bar plot summarizes the number of unique proteins identified across overlapping subsets of the data. For example, in the first column only the data from Li et al. (2014) is considered (indicated by solid blue circle) and 1288 proteins are identified as exclusive to the data set. In the second column, the intersection of all four data sets is considered, with 1157 proteins quantified across them. This follows for each additional column in the plot, with the subset under consideration denoted by the solid blue circles.

et al. (2017, 2019) using the optimize.curve\_fit function from the Scipy python package (Virtanen et al., 2020). A quick comment on nomenclature: throughout the text, we usually refer to cell size, in units of  $\mu\text{m}^3$ ; however, on occasion we will mention size as a volume in units of fL.

The average size measurements from each of their experiments are shown in Figure 14, with cell length and width shown in (A) and (B), respectively. The length data was well described by the exponential function  $0.5 e^{1.09 \cdot \lambda} + 1.76 \mu\text{m}$ , while the width data was well described by  $0.64 e^{0.24 \cdot \lambda} \mu\text{m}$ . In order to estimate cell size we take the cell as a cylinder with two hemispherical ends (Si et al., 2017; Basan et al., 2015). Specifically, cell size is estimated from,

$$V = \pi \cdot r^2 \cdot (l - 2r/3), \quad (5)$$

where  $r$  is half the cell width. A best fit to the data is described by  $0.533 e^{1.037 \cdot \lambda} \mu\text{m}^3$ . Calculation of the cell surface area is given by,

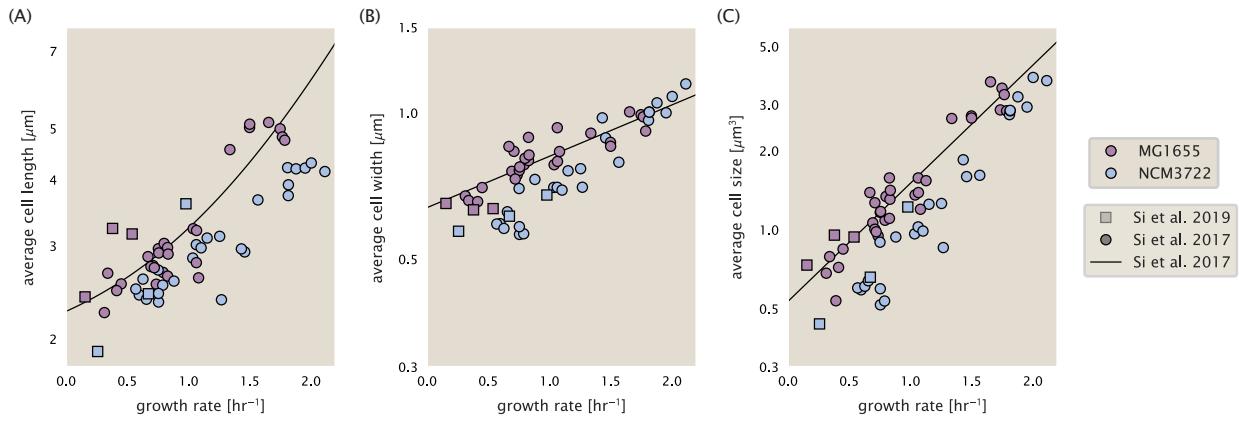
$$S = \eta \cdot \pi \left( \frac{\eta \cdot \pi}{4} - \frac{\pi}{12} \right)^{-2/3} V^{2/3}, \quad (6)$$

where  $\eta$  is the aspect ratio ( $\eta = l/w$ ) (Ojkic et al., 2019).

## Estimation of Total Protein Content per Cell

In order to estimate total protein per cell for a particular growth rate, we begin by estimating the cell size from the fit shown in Figure 14(C) (cell size =  $0.533 e^{1.037 \cdot \lambda} \mu\text{m}^3$ , as noted in the previous section). We then estimate the total protein content from the total dry mass of the cell. Here we begin by noting that for almost the entire range of growth rates considered here, protein, DNA, and RNA were reported to account for at least 90 % of the dry mass (Basan et al. (2015)). The authors also found that the total dry mass concentration was roughly constant across growth conditions. Under such a scenario, we can calculate the total dry mass concentration for protein, DNA, and RNA, which is given by  $1.1 \text{ g/ml} \times 30 \% \times 90 \% \text{ or about } [M_p] = 300 \text{ fg per fL}$ . Multiplying this by our prediction of cell size gives the total dry mass per cell.

However, even if dry mass concentration is relatively constant across growth conditions, it is not obvious how protein concentration might vary due to the substantial increase in rRNA at faster growth rates (Dai et al. (2016)). The increase in rRNA increases from the linear increase in ribosomal content with faster growth rate (Scott et al.



**Figure 14. Summary of size measurements from Si et al. 2017, 2019.** Cell lengths and widths were measured from cell contours obtained from phase contrast images, and refer to the long and short axis respectively. (A) Cell lengths and (B) cell widths show the mean measurements reported (they report 140-300 images and 5,000-30,000 for each set of samples; which likely means about 1,000-5,000 measurements per mean value reported here since they considered about 6 conditions at a time). Fits were made to the MG1655 strain data; length:  $0.5 e^{1.09 \cdot \lambda} + 1.76 \mu\text{m}$ , width:  $0.64 e^{0.24 \cdot \lambda} \mu\text{m}$ . (C) Cell size was calculated as cylinders with two hemispherical ends (Equation 5). The MG1655 strain data gave a best fit of  $0.533 e^{1.037 \cdot \lambda} \mu\text{m}^3$ .

1038 (2010)), since it makes up about about 2/3 or the ribosomal mass. To proceed we therefore relied on experimental  
 1039 measurements of total DNA content per cell from Basan et al. (2015), and RNA to protein ratios that were measured  
 1040 in Dai et al. (and cover the entire range of growth conditions considered here). These are reproduced in Figure 15(A)  
 1041 and (B), respectively.

1042 Assuming that the protein, DNA, and RNA account for 90 % of the total dry mass, the protein mass can then  
 1043 determined by first subtracting the experimentally measured DNA mass, and then using the experimental estimate  
 1044 of the RNA to protein ratio. The total protein per cell is will be related to the summed RNA and protein mass by,

$$M_P = \frac{[M_P + M_{RNA}]}{1 + (RP_{ratio})}. \quad (7)$$

1045 ( $RP_{ratio}$  refers to the RNA to protein ratio as measured by Dai et al.. In Figure 15(C) we plot the estimated cellular  
 1046 concentrations for protein, DNA, and RNA from these calculations, and in Figure 15(D) we plot their total expected  
 1047 mass per cell. This later quantity is the growth rate-dependent total protein mass that was used to estimate total  
 1048 protein abundance across all data sets (and summarized in Figure 12(B)).

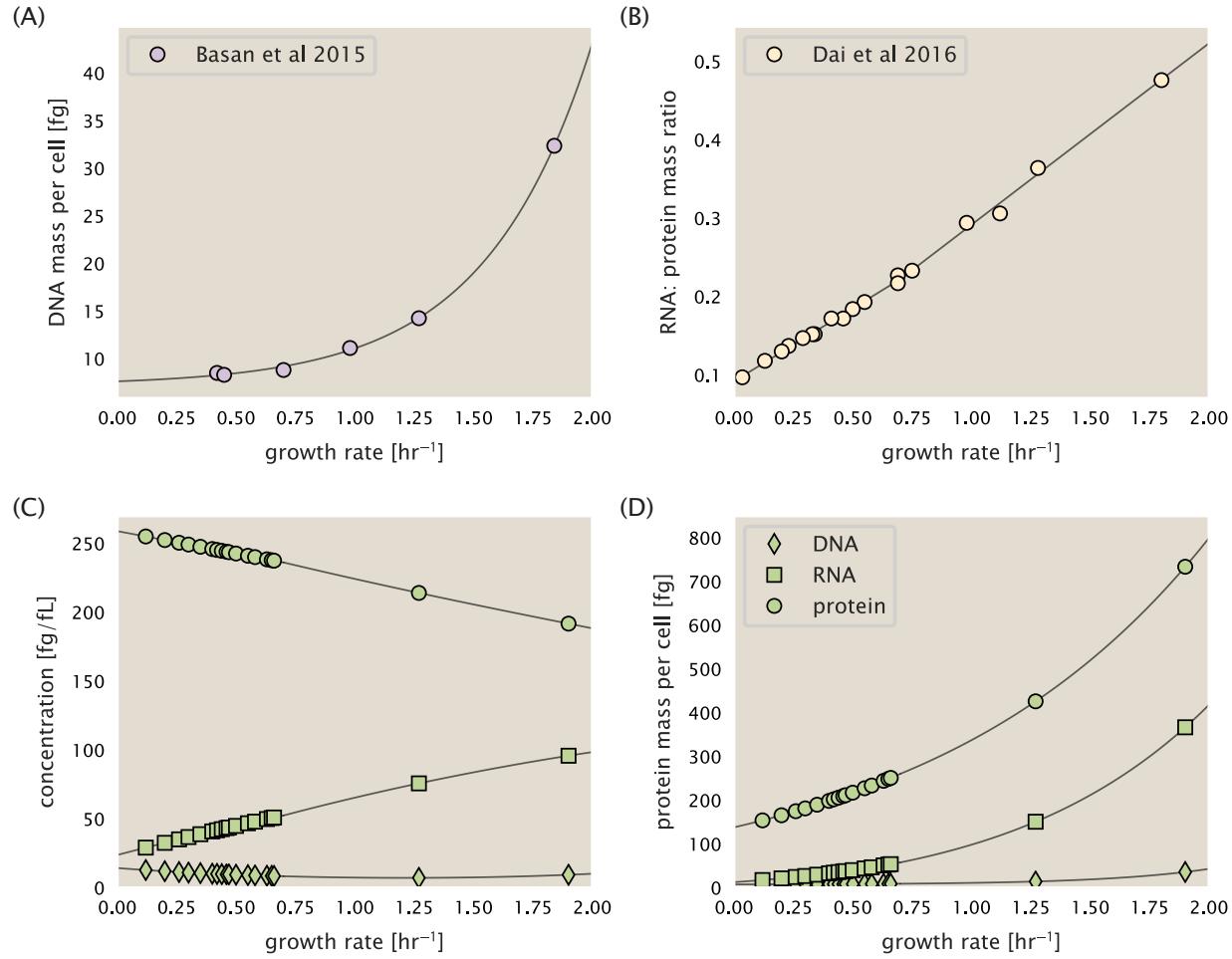
#### 1049 Estimating Volume and Number of Amino Acids from Ribosome Copy Number

1050 Towards the end of the main text, we examine a coarse-grained model of nutrient-limited growth. A key point  
 1051 in our analysis was to consider how elongation rate  $r_i$  and growth rate  $\lambda$  vary with respect to the experimentally  
 1052 observed changes in cell size, total number of peptide bonds per cell  $N_{pep}$ , and ribosomal content. In order to  
 1053 restrict parameters to those observed experimentally, but otherwise allow us to explore the model, we performed  
 1054 a phenomenological fit of  $N_{pep}$  and  $V$  as a function of the measured ribosomal copy number  $R$ . As has been  
 1055 described in the preceding sections of this supplement, we estimate cell volume for each growth condition using  
 1056 the size measurements from Si et al. (2017, 2019), and  $N_{pep}$  is approximated by taking the total protein mass and  
 1057 dividing this number by the average mass of an amino acid, 110 Da (BNID: 104877).

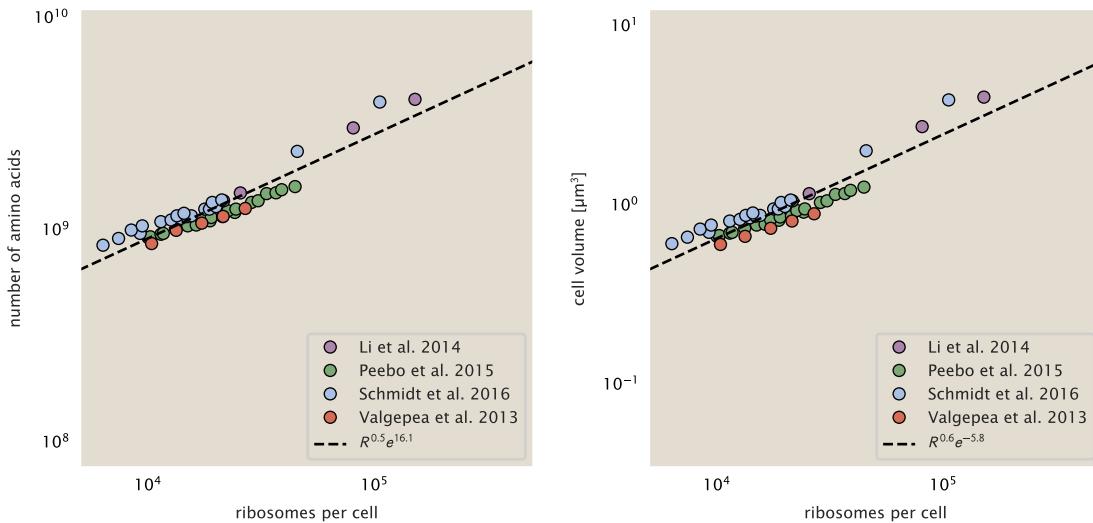
1058 Given the exponential scaling of  $V$  and  $N_{pep}$  with growth rate, we performed a linear regression of the log  
 1059 transform of these parameters as a function of the log transform of the ribosome copy number. Using optimization  
 1060 by minimization, we estimated the best-fit values of the intercept and slope for each regression. Figure 16 shows  
 1061 the result of each regression as a dashed line.

#### 1062 Additional Considerations of Schmidt et al. Data Set

1063 While the data set from Schmidt et al. (2016) remains a heroic effort that our labs continue to return to as a resource,  
 1064 there were steps taken in their calculation of protein copy number that we felt needed further consideration. In



**Figure 15. Empirical estimate of cellular protein, DNA, and RNA as a function of growth rate.** (A) Measured DNA mass per cell as a function of growth rate, reproduced from Basan *et al.* 2015. The data was fit to an exponential curve (DNA mass in fg per cell is given by  $0.42 e^{2.23 \cdot \lambda} + 7.2$  fg per cell, where  $\lambda$  is the growth rate in  $\text{hr}^{-1}$ ). (B) RNA to protein measurements as a function of growth rate. The data was fit to two lines (shown in black) due to the change in slope at slower growth rates *Neidhardt et al.* (1991); *Dai et al.* (2016). For growth rates below  $0.7 \text{ hr}^{-1}$ , the RNA/protein ratio is  $0.18 \cdot \lambda + 0.093$ , while for growth rates faster than  $0.7 \text{ hr}^{-1}$  the RNA/protein ratio is given by  $0.25 \cdot \lambda + 0.035$ . For (A) and (B) cells are grown under varying levels of nutrient limitation, with cells grown in minimal media with different carbon sources for the slowest growth conditions, and rich-defined media for fast growth rates. (C) Estimation of cellular protein, DNA, and RNA concentration. (D) Total cellular mass estimated for protein, DNA, and RNA using the cell size calculated in *Estimation of Cell Size and Surface Area*. Symbols (diamond: DNA, square: RNA, circle: protein) show estimated values of mass concentration and mass per cell for the specific growth rates in *Schmidt et al.* (2016).

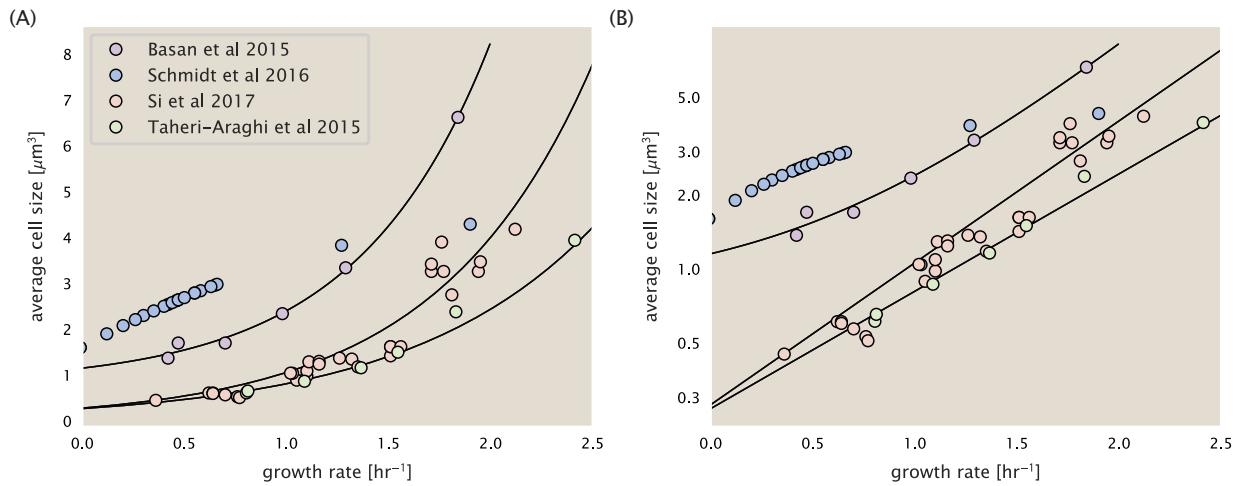


**Figure 16. Phenomenological regression of cell volume and number of amino acids per cell as a function of the ribosome copy number.** Colored points correspond to the measured value (or calculated value in the case of the cell volume) with colors denoting different data sets. The dashed black line shows the result of the fit, with the functional form of the equation given in the legend with  $R$  representing the ribosome copy number.

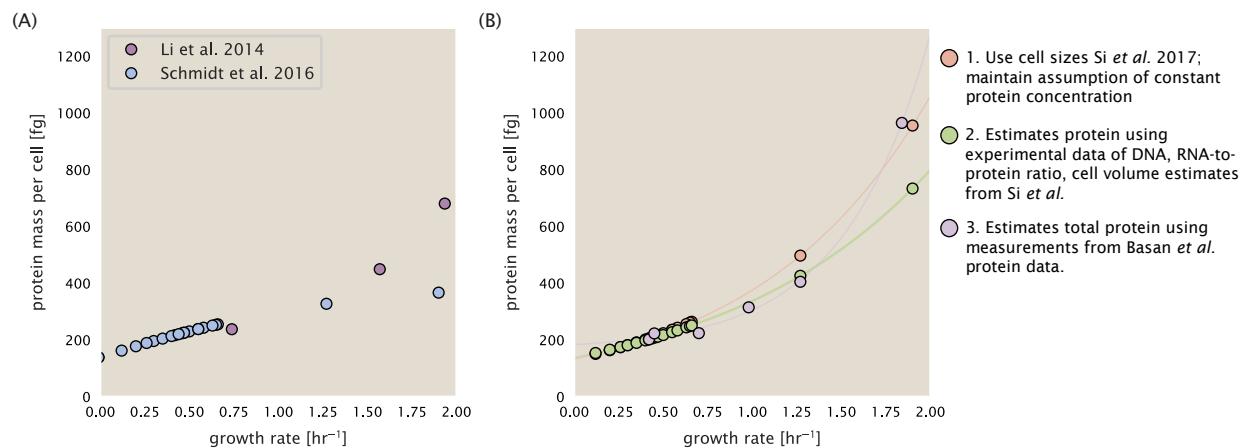
particular, the authors made an assumption of constant cellular protein concentration across all growth conditions and used measurements of cell volume that appear inconsistent with an expected exponential scaling of cell size with growth rate that is well-documented in *E. coli* (*Schaechter et al. (1958); Taheri-Araghi et al. (2015); Si et al. (2017)*).

We begin by looking at their cell volume measurements, which are shown in blue in Figure *Figure 17*. As a comparison, we also plot cell sizes reported in three other recent papers: measurements from Taheri-Araghi *et al.* and Si *et al.* come from the lab of Suckjoon Jun, while those from Basan *et al.* come from the lab of Terence Hwa. Each set of measurements used microscopy and cell segmentation to determine the length and width, and then calculated cell size by treating the cell as a cylinder with two hemispherical ends, as we considered in the previous section. While there is notable discrepancy between the two research groups, which are both using strain NCM3722, Basan *et al.* found that this came specifically from uncertainty in determining the cell width. This is prone to inaccuracy given the small cell size and optical resolution limits (further described in their supplemental text). Perhaps the more concerning point is that while each of these alternative measurements show an exponential increase in cell size at faster growth rates, the measurements used by Schmidt *et al.* appear to plateau. This resulted in an analogous trend in their final reported total cellular protein per cell as shown in *Figure 18* (purple data points), and is in disagreement with other measurements of total protein at these growth rates (*Basan et al., 2015*).

Since it is not obvious how measurements of cell size influenced their reported protein abundances, in the following subsections we begin by considering how the authors determined total protein mass per cell. We then consider three different approaches to estimate the growth-rate dependent total protein mass and compare these estimates with those reported by Schmidt *et al. (2016)*. Those results are summarized in *Figure 17(B)*, with the original values from both Schmidt *et al. (2016)* and Li *et al. (2014)* shown in *Figure 17(A)* for reference. For most growth conditions, we find reasonable agreement between our estimates and the reported total protein per cell. However, for the fastest growth conditions, with glycerol + supplemented amino acids, and LB media, all estimates are substantially higher than those originally reported. This is the main reason why we chose to readjust protein abundance as shown in *Figure 12(B)* (with the calculation described in section Estimation of Total Protein Content per Cell).



**Figure 17. Measurements of cell size as a function of growth rate.** (A) Plot of the reported cell sizes from several recent papers. The data in blue come from Volkmer and Heinemann, 2011 (*Volkmer and Heinemann (2011)*) and were used in the work of Schmidt *et al.*. Data from the lab of Terence Hwa are shown in purple (*Basan et al. (2015)*), while the two data sets shown in green and light red come from the lab of Suckjoon Jun (*Taheri-Araghi et al. (2015); Si et al. (2017)*). (B) Same as in (A) but with the data plotted on a logarithmic y-axis to highlight the exponential scaling that is expected for *E. coli*.



**Figure 18. Alternative estimates of total cellular protein for the growth conditions considered in Schmidt *et al.*** (A) The original protein mass from Schmidt *et al.* and Li *et al.* are shown in purple and blue, respectively. (B) Three alternative estimates of total protein per cell. 1. light red: Rescaling of total protein mass assuming a growth rate independent protein concentration and cell volumes estimated from Si *et al.* 2017. 2. light green: Rescaling of total protein mass using estimates of growth rate-dependent protein concentrations and cell volumes estimated from Si *et al.* 2017. Total protein per cell is calculated by assuming a 1.1 g/ml cellular mass density, 30% dry mass, with 90% of the dry mass corresponding to DNA, RNA, and protein (*Basan et al., 2015*). See Estimation of Total Protein Content per Cell for details on calculation. 3. light purple: Rescaling of total protein mass using the experimental measurements from Basan *et al.* 2015.

1092 **Effect of cell volume on reported absolute protein abundances**

1093 As noted in Experimental Details Behind Proteomic Data, the authors from the work in *Schmidt et al. (2016)*  
1094 calculated proteome-wide protein abundances by first determining absolute abundances of 41 pre-selected  
1095 proteins, which relied on adding synthetic heavy reference peptides into their protein samples at known abundance.  
1096 This absolute quantitation was performed in replicate for each growth condition. Separately, the authors also  
1097 performed a more conventional mass spectrometry measurement for samples from each growth condition,  
1098 which attempted to maximize the number of quantified proteins but only provided relative abundances based  
1099 on peptide intensities. Finally, using their 41 proteins with absolute abundances already determined, they then  
1100 created calibration curves with which to relate their relative intensity to absolute protein abundance for each  
1101 growth condition. This allowed them to estimate absolute protein abundance for all proteins detected in their  
1102 proteome-wide data set. Combined with their flow cytometry cell counts, they were then able to determine  
1103 absolute abundance of each protein detected on a per cell basis.

1104 While this approach provided absolute abundances, another necessary step to arrive at total cellular protein  
1105 was to account for any protein loss during their various protein extraction steps. Here the authors attempted  
1106 to determine total protein separately using a BCA protein assay. In personal communications, it was noted that  
1107 determining reasonable total protein abundances by BCA across their array of growth conditions was particularly  
1108 troublesome. Instead, they noted confidence in their total protein measurements for cells grown in M9 minimal  
1109 media + glucose and used this as a reference point with which to estimate the total protein for all other growth  
1110 conditions.

1111 For cells grown in M9 minimal media + glucose an average total mass of  $M_p = 240$  fg per cell was measured.  
1112 Using their reported cell volume, reported as  $V_{orig} = 2.84$  fl, a cellular protein concentration of  $[M_p]_{orig} = M_p/V_{orig} =$   
1113  $85$  fg/fl. Now, taking the assumption that cellular protein concentration is relatively independent of growth rate,  
1114 they could then estimate the total protein mass for all other growth conditions from,

$$M_{p\_i} = [M_p]_{orig} \cdot V_i \quad (8)$$

1115 where  $M_{p_i}$  represents the total protein mass per cell and  $V_i$  is the cell volume for each growth condition  $i$  as  
1116 measured in Volkmer and Heinemann, 2011. Here the thinking is that the values of  $M_{p_i}$  reflects the total cellular  
1117 protein for growth condition  $i$ , where any discrepancy from their absolute protein abundance is assumed to be due  
1118 to protein loss during sample preparation. The protein abundances from their absolute abundance measurements  
1119 noted above were therefore scaled to their estimates and are shown in Figure *Figure 18* (purple data points).

1120 If we instead consider the cell volumes predicted in the work of Si *et al.*, we again need to take growth in M9  
1121 minimal media + glucose as a reference with known total mass, but we can follow a similar approach to estimate  
1122 total protein mass for all other growth conditions. Letting  $V_{Si\_glu} = 0.6$  fl be the predicted cell volume, the cellular  
1123 protein concentration becomes  $[M_p]_{Si} = M_p/V_{Si\_glu} = 400$  fg/fl. The new total protein mass per cell can then be  
1124 calculated from,

$$M'_{p\_i} = [M_p]_{Si} \cdot V_{Si\_i} \quad (9)$$

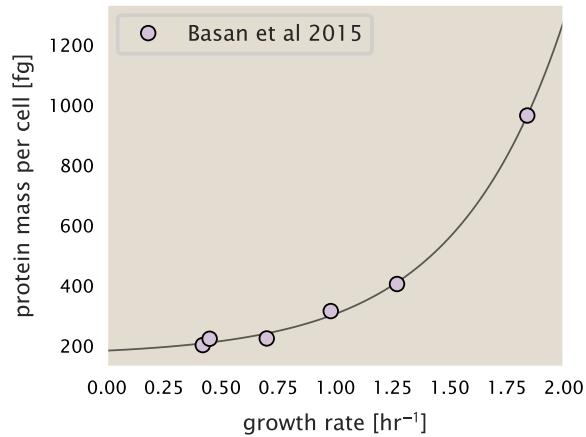
1125 where  $M'_{p_i}$  is the new protein mass prediction, and  $V_{Si\_i}$  refers to the new volume prediction for each condition  $i$ ,  
1126 These are shown as red data points in Figure *Figure 18(B)*.

1127 **Relaxing assumption of constant protein concentration across growth conditions**

1128 We next relax the assumption that cellular protein concentration is constant and instead, attempt to estimate  
1129 it using experimental data. Here we use the estimation of total protein mass per cell detailed in Estimation of  
1130 Total Protein Content per Cell for all data points in the *Schmidt et al. (2016)* data set. The green data points in  
1131 *Figure 18(B)* show this prediction, and this represents the approach used to estimate total protein per cell for all  
1132 data sets.

1133 **Comparison with total protein measurements from Basan *et al.* 2015.**

1134 One of the challenges in our estimates in the preceding sections is the need to estimate protein concentration and  
1135 cell volumes. These are inherently difficult to measure accurately due to the small size of *E. coli*. Indeed, for all



**Figure 19. Total cellular protein reported in Basan *et al.* 2015.** Measured protein mass as a function of growth rate as reproduced from Basan *et al.* 2015, with cells grown under different levels of nutrient limitation. The data was fit to an exponential curve where protein mass in fg per cell is given by  $14.65 e^{2.180 \cdot \lambda} + 172$  fg per cell, where  $\lambda$  is the growth rate in hr<sup>-1</sup>.

the additional measurements of cell volume included in Figure *Figure 17*, no measurements were performed for cells growing at rates below 0.5 hr<sup>-1</sup>. It therefore remains to be determined whether our extrapolated cell volume estimates are appropriate, with the possibility that the logarithmic scaling of cell size might break down for slower growth.

In our last approach we therefore attempt to estimate total protein using experimental data that required no estimates of concentration or cell volume. Specifically, in the work of Basan *et al.*, the authors measured total protein per cell for a broad range of growth rates (reproduced in Figure *Figure 19*). These were determined by first measuring bulk protein from cell lysate, measured by the colorimetric Biuret method (*You et al. (2013)*), and then abundance per cell was calculated from cell counts from either plating cells or a Coulter counter. While it is unclear why Schmidt *et al.* was unable to take a similar approach, the results from Basan *et al* appear more consistent with our expectation that cell mass will increase exponentially with faster growth rates. In addition, although they do not consider growth rates below about 0.5 hr<sup>-1</sup>, it is interesting to note that the protein mass per cell appears to plateau to a minimum value at slow growth. In contrast, our estimates using cell volume so far have predicted that total protein mass should continue to decrease slightly for slower growing cells. By fitting this data to an exponential function dependent on growth rate, we could then estimate the total protein per cell for each growth condition considered by Schmidt *et al.* (2016). These are plotted as red data points in *Figure 18(B)*.

## Calculation of Complex Abundance

All protein data quantified the abundance of individual proteins per cell. However, this work requires estimates on the abundance of individual protein *complexes*, rather than the copy number of individual proteins. In our analysis of the protein copy number data, it became clear that the reported copy numbers do not always align with those based on reported stoichiometry. As one example of this, the F-O subunit of ATP synthase consists of three protein subunits with a stoichiometry of [AtpB][AtpF]<sub>2</sub>[AtpE]<sub>10</sub> (also referred to as subunits a, b, and c, respectively). In the experimental data of Schmidt *et al.* (2016), the values deviate from this quite substantially, with approximately 1000 AtpB, 9000 AtpF, and 300 AtpE reported per cell (minimal media + glucose growth condition). This highlights the technical challenges that still remain in our ability to quantify cellular composition, particularly for membrane-bound proteins like the ATP synthase complex considered here. In this section, we outline the approach we used to annotate proteins as part of each macromolecular complex and how we used averaging across the individual protein measurements to estimate an absolute complex abundances per cell.

Protein complexes, and proteins individually, often have a variety of names, both longform and shorthand. As individual proteins can have a variety of different synonyms, we sought to ensure that each protein annotated in the data sets used the same synonym. To do use, we relied heavily on the EcoCyc Genomic Database (*Keseler*

1167 **et al., 2017**). Each protein in available data sets included an annotation of one of the gene name synonyms as  
1168 well as an accession ID – either a UniProt or Blattner "b-number". We programmatically matched up individual  
1169 accession IDs between the proteins in different data sets. In cases where accession IDs matched but the gene  
1170 names were different, we manually verified that the gene product was the same between the datasets and chose a  
1171 single synonym. All code used in the data cleaning and unification procedures can be found on the associated  
1172 [GitHub repository] (DOI:XXX) associated with this paper as well as on the associated [paper website](#).

1173 With each protein conforming to a single identification scheme, we then needed to identify the molecular  
1174 complexes each protein was a member of. Additionally, we needed to identify how many copies of each protein  
1175 were present in each complex (i.e. the subunit copy number) and compute the estimated abundance complex that  
1176 accounted for fluctuations in subunit stoichiometry. To map proteins to complexes, we accessed the EcoCyc *E.*  
1177 *coli* database **Keseler et al. (2017)** using PathwayTools version 23.0 **Karp et al. (2019)**. With a license for PathWay  
1178 Tools, we mapped each unique protein to its annotated complexes via the BioCyc Python package. As we mapped  
1179 each protein with *all* of its complex annotations, there was redundancy in the dataset. For example, ribosomal  
1180 protein L20 (RplT) is annotated to be a component of the 50S ribosome (EcoCyc complex CPLX-03962) as well as a  
1181 component of the mature 70S ribosome (EcoCyc complex CPLX-03964).

1182 In addition to the annotated complex, we collected information on the stoichiometry of each macromolecular  
1183 complex. For a complex with  $N_{\text{subunits}}$  protein species, for each protein subunit  $i$  we first calculate the number of  
1184 complexes that *could* be formed given the measured protein copy numbers per cell,

$$N_{\text{complex}}(\text{subunit } i) = \frac{P_{\text{subunit } i}^{(\text{measured})}}{m_{\text{subunit } i}}. \quad (10)$$

1185 Here,  $P_{\text{subunit } i}^{(\text{measured})}$  refers to the measured protein copy number of species  $i$ , and  $m$  refers to the number of monomers  
1186 present for that protein in the complex. For example, the 70S mature ribosome complex has 55 protein components,  
1187 all of which are present in a single copy except L4 (RplL), which is present in 4 copies ( $m = 4$ ). For each ribosomal  
1188 protein, we then calculate the maximum number of complexes that could be formed using **Equation 10**. This  
1189 example, along with example from 5 other macromolecular complexes, can be seen in **Figure 20**.

1190 It is important to note that measurement noise, efficiency of protein extraction, and differences in protein  
1191 stability will mean that the precise value of each calculation will be different for each component of a given complex.  
1192 Thus, to report the total complex abundance, we use the arithmetic mean of across all subunits in the complex,

$$\langle N_{\text{complex}} \rangle = \frac{1}{N_{\text{subunits}}} \sum_i^{N_{\text{subunits}}} \frac{P_i^{(\text{measured})}}{m_{\text{subunit } i}}. \quad (11)$$

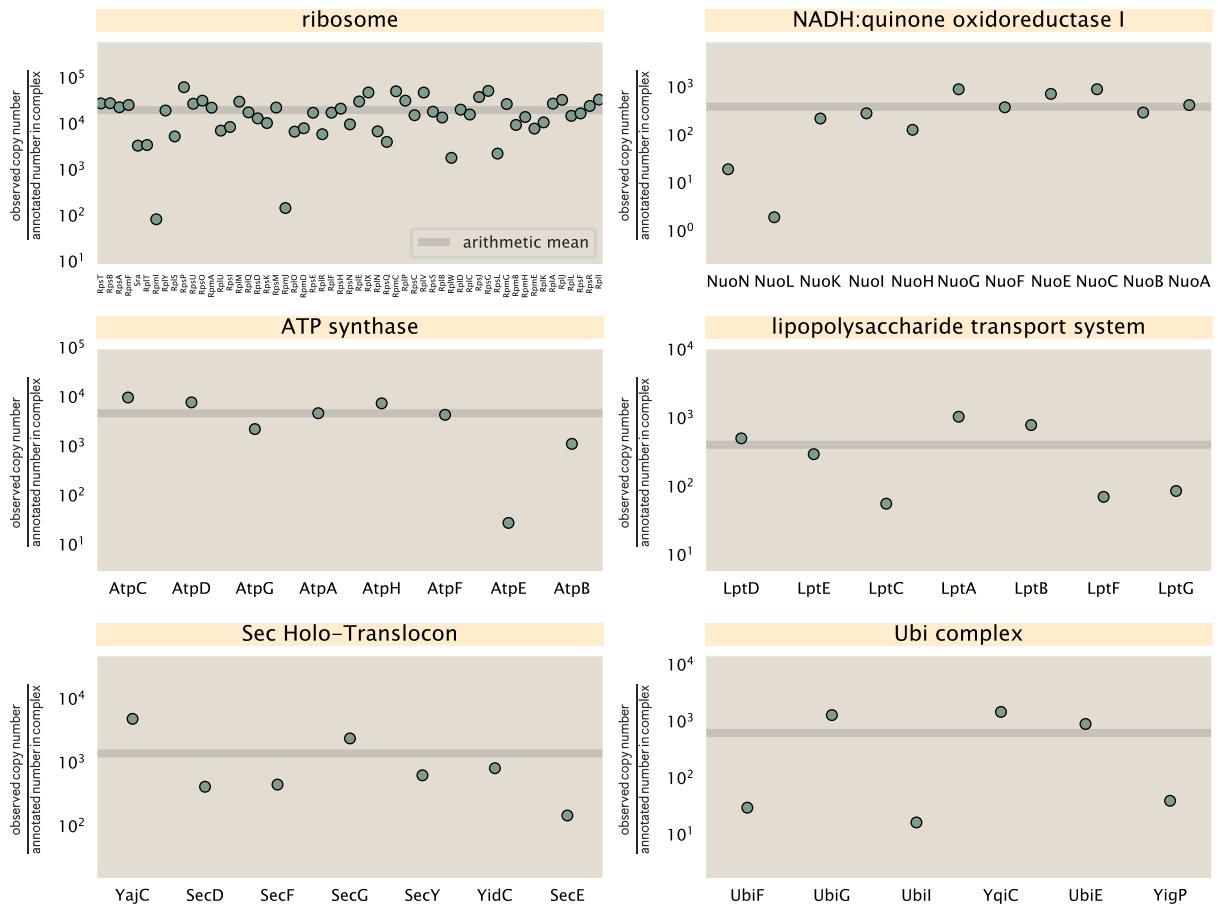
1193 in **Figure 20**, we show this mean value as a grey line for a variety of different complexes. Additionally, we have built  
1194 an interactive figure accessible on the [paper website](#) where the validity of this approach can be examined for any  
1195 complex with more than two subunits (thus, excluding monomers and dimers).

## 1196 Extending Estimates to a Continuum of Growth Rates

1197 In the main text, we considered a standard stopwatch of 5000 s to estimate the abundance of the various protein  
1198 complexes considered. In addition to point estimates, we also showed the estimate as a function of growth rate as  
1199 transparent grey curves. In this section, we elaborate on this continuum estimate, giving examples of estimates  
1200 that scale with either cell volume, cell surface area, or number of origins of replication.

## 1201 Estimation of the total cell mass

1202 For many of the processes estimated in the main text we relied on a cellular dry mass of  $\approx 300$  fg from which we  
1203 computed elemental and protein fractions using knowledge of fractional composition of the dry mass. At modest  
1204 growth rates, such as the 5000 s doubling time used in the main text, this is a reasonable number to use as the  
1205 typical cell mass is  $\approx 1$  pg and *E. coli* cells can approximated as 70% water by volume. However, as we have shown  
1206 in the preceding sections, the cell size is highly dependent on the growth rate. This means that a dry mass of 300 fg  
1207 cannot be used reliably across all growth rates.



**Figure 20. Calculation of the mean complex abundance from measurements of single subunits.** Six of the largest complexes (by number of subunits) in *E. coli*. Points correspond to the maximum number of complexes that can be formed given measurement of that individual protein. Solid grey line corresponds to the arithmetic mean across all subunits. These data correspond to measurements from Schmidt *et al.* (2016) in a glucose-supplemented minimal growth medium.

1208 Rather, using the phenomenological description of cell volume scaling exponentially with growth rate, and  
 1209 using a rule-of-thumb of a cell buoyant density of  $\approx 1.1 \text{ pg} / \text{fL}$  (BNID: 103875), we can calculate the cell dry mass  
 1210 across a range of physiological growth rates as

$$m_{\text{cell}} \approx \rho V(\lambda) \approx \rho a e^{\lambda * b} \quad (12)$$

1211 where  $a$  and  $b$  are constants with units of  $\mu\text{m}^3$  and hr, respectively. The value of these constants can be estimated  
 1212 from the careful volume measurements performed by *Si et al. (2017,?)*, as considered in Appendix Estimation of  
 1213 Cell Size and Surface Area earlier.

### 1214 Complex Abundance Scaling With Cell Volume

1215 Several of the estimates performed in the main text are implicitly dependent on the cell volume. This includes  
 1216 processes such as ATP utilization and, most prominently, the transport of nutrients, whose demand will be  
 1217 proportional to the volume of the cell. Of the latter, we estimated the number of transporters that would be  
 1218 needed to shuttle enough carbon, phosphorus, and sulfur across the membrane to build new cell mass. To do so,  
 1219 we used elemental composition measurements combined with a 300 fg cell dry mass to make the point estimate.  
 1220 As we now have a means to estimate the total cell mass as a function of volume, we can generalize these estimates  
 1221 across growth rates.

1222 Rather than discussing the particular details of each transport system, we will derive this scaling expression in  
 1223 very general terms. Consider that we wish to estimate the number of transporters for some substance  $X$ , which  
 1224 has been measured to be made up some fraction of the dry mass,  $\theta_X$ . If we assume that, irrespective of growth  
 1225 rate, the cell dry mass is relatively constant (*Basan et al., 2015*) and  $\approx 30\%$  of the total cell mass, we can state that  
 1226 the total mass of substance  $X$  as a function of growth rate is

$$m_X \approx 0.3 \times \rho V(\lambda) \theta_X, \quad (13)$$

1227 where we have used  $\rho V(\lambda)$  as an estimate of the total cell mass, defined in *Equation 12*. To convert this to the  
 1228 number of units  $N_X$  of substance  $X$  in the cell, we can use the formula weight  $w_X$  of a single unit of  $X$  in conjunction  
 1229 with *Equation 13*,

$$N_X \approx \frac{m_X}{w_X}. \quad (14)$$

1230 To estimate the number of transporters needed, we make the approximation that loss of units of  $X$  via diffusion  
 1231 through porins or due to the permeability of the membrane is negligible and that a single transporter complex  
 1232 can transport substance  $X$  at a rate  $r_X$ . As this rate  $r_X$  is in units of  $X$  per time per transporter, we must provide  
 1233 a time window over which the transport process can occur. This is related to the cell doubling time  $\tau$ , which can  
 1234 be calculated from the the growth rate  $\lambda$  as  $\tau = \log(2)/\lambda$ . Putting everything together, we arrive at a generalized  
 1235 transport scaling relation of

$$N_{\text{transporters}}(\lambda) = \frac{0.3 \times \rho V(\lambda) \theta_X}{w_X r_X \tau}. \quad (15)$$

1236 This function is used to draw the continuum estimates for the number of transporters seen in Figures 2 and  
 1237 3 as transparent grey curves. Occasionally, this continuum scaling relationship will not precisely agree with the  
 1238 point estimate outlined in the main text. This is due to the choice of  $\approx 300 \text{ fg}$  total dry mass per cell for the point  
 1239 estimate, whereas we considered more precise values of cell mass in the continuum estimate. We note, however,  
 1240 that both this scaling relation and the point estimates are meant to describe the order-of-magnitude observed,  
 1241 and not the predict the exact values of the abundances.

1242 *Equation 15* is a very general relation for processes where the cell volume is the "natural variable" of the  
 1243 problem. This means that, as the cell increases in volume, the requirements for substance  $X$  also scale with  
 1244 volume rather than scaling with surface area, for example. So long as the rate of the process, the fraction of the  
 1245 dry mass attributable to the substance, and the formula mass of the substance is known, *Equation 15* can be used  
 1246 to compute the number of complexes needed. For example, to compute the number of ATP synthases per cell,  
 1247 *Equation 15* can be slightly modified to the form

$$N_{\text{ATP synthases}}(\lambda) = \frac{0.3 \times \rho V(\lambda) \theta_{\text{protein}} N_{\text{ATP}}}{w_{AA} r_{\text{ATP}} \tau}, \quad (16)$$

1248 where we have included the term  $N_{ATP}$  to account for the number of ATP equivalents needed per amino acid for  
 1249 translation ( $\approx 4$ , BNID: 114971), and  $w_{AA}$  is the average mass of an amino acid. The grey curves in Figure 4 of the  
 1250 main text were made using this type of expression.

## 1251 A Relation for Complex Abundance Scaling With Surface Area

1252 In our estimation for the number of complexes needed for lipid synthesis and peptidoglycan maturation, we used  
 1253 a particular estimate for the cell surface area ( $\approx 5 \mu m$ , BNID: 101792) and the fraction of dry mass attributable to  
 1254 peptidoglycan ( $\approx 3\%$ , BNID: 101936). Both of these values come from glucose-fed *E. coli* in balanced growth. As we  
 1255 are interested in describing the scaling as a function of the growth rate, we must also consider how these values  
 1256 scale with cell surface area, which is the natural variable for these types of processes. In the coming paragraphs,  
 1257 we highlight how we incorporate a condition-dependent surface area into our calculation of the number of lipids  
 1258 and murein monomers that need to be synthesized and crosslinked, respectively.

### 1259 Number of Lipids

1260 To compute the number of lipids as a function of growth rate, we make the assumption that some features, such as  
 1261 the surface area of a single lipid ( $A_{lipid} \approx 0.5 \text{ nm}^2$ , BNID: 106993) and the total fraction of the membrane composed  
 1262 of lipids ( $\approx 40\%$ , BNID: 100078) are independent of the growth rate. Using these approximations combined with  
 1263 **Equation 6**, and recognizing that each membrane is composed of two leaflets, we can compute the number of  
 1264 lipids as a function of growth rate as

$$N_{lipids}(\lambda) \approx \frac{4 \text{ leaflets} \times 0.4 \times \eta \pi \left( \frac{\eta \pi}{4} - \frac{\pi}{12} \right)^{-2/3} V(\lambda)^{2/3}}{A_{lipid}} \quad (17)$$

1265 where  $\eta$  is the length-to-width aspect ratio and  $V$  is the cell volume.

### 1266 Number of Murein Monomers

1267 In calculation of the number of transpeptidases needed for maturation of the peptidoglycan, we used an empirical  
 1268 measurement that  $\approx 3\%$  of the dry mass is attributable to peptidoglycan and that a single murien monomer is  
 1269  $m_{murein} \approx 1000 \text{ Da}$ . While the latter is independent of growth rate, the former is not. As the peptidoglycan exists as  
 1270 a thin shell with a width of  $w \approx 10 \text{ nm}$  encapsulating the cell, one would expect the number of murein monomers  
 1271 scales with the surface area of this shell. In a similar spirit to our calculation of the number of lipids, the total  
 1272 number of murein monomers as a function of growth rate can be calculated as

$$N_{murein \text{ monomers}}(\lambda) \approx \frac{\rho_{pg} w \eta \pi \left( \frac{\eta \pi}{4} - \frac{\pi}{12} \right)^{-2/3} V(\lambda)^{2/3}}{m_{murein}}, \quad (18)$$

1273 where  $\rho_{pg}$  is the density of peptidoglycan.

## 1274 Complex Abundance Scaling With Number of Origins, and rRNA Synthesis

1275 While the majority of our estimates hinge on the total cell volume or surface area, processes related to the central  
 1276 dogma, namely DNA replication and synthesis of rRNA, depend on the number of chromosomes present in the  
 1277 cell. As discussed in the main text, the ability of *E. coli* to parallelize the replication of its chromosome by having  
 1278 multiple active origins of replication is critical to synthesize enough rRNA, especially at fast growth rates. Derived in  
 1279 *Si et al. (2017)* and reproduced in the main text and Appendix Estimation of  $\langle \#ori \rangle / \langle \#ter \rangle$  and  $\langle \#ori \rangle$  below, the  
 1280 average number of origins of replication at a given growth rate can be calculated as

$$\langle \#ori \rangle \approx 2^{t_{cyc} \lambda / \ln 2} \quad (19)$$

1281 where  $t_{cyc}$  is the total time of replication and division. We can make the approximation that  $t_{cyc} \approx 70 \text{ min}$ , which is  
 1282 the time from the initiation of chromosomal replication until division. This time corresponds to the sum of the  
 1283 so-called C and D periods of the cell cycle, which correspond to the time it takes to replicate the entire chromosome  
 1284 (C period) and the time from completion to eventual division (D period) *Helmstetter and Cooper (1968)*.

1285 In the case of rRNA synthesis, the majority of the rRNA operons are surrounding the origin of replication. Thus,  
1286 at a given growth rate  $\lambda$ , the average dosage of rRNA operons per cell  $D_{\text{rRNA}}$  is

$$D_{\text{rRNA}}(\lambda) \approx N_{\text{rRNA operons}} \times 2^{t_{\text{cyc}}\lambda/\ln 2}. \quad (20)$$

1287 This makes the approximation that *all* rRNA operons are localized around the origin. In reality, the operons are  
1288 some distance away from the origin, making **Equation 20** an approximation (*Dennis et al., 2004*).

1289 In the main text, we stated that at a growth rate of  $0.5 \text{ hr}^{-1}$ , there is  $\approx 1$  chromosome per cell. While a fair  
1290 approximation, **Equation 19** illustrates that is not precisely true, even at slow growth rates. In estimating the  
1291 number of RNA polymerases as a function of growth rate, we consider that regardless of the number of rRNA  
1292 operons, they are all sufficiently loaded with RNA polymerase such that each operon produces one rRNA per  
1293 second. Thus, the total number of RNA polymerase as a function of the growth rate can be calculated as

$$N_{\text{RNA polymerase}}(\lambda) \approx L_{\text{operon}} D_{\text{rRNA}} \rho_{\text{RNA polymerase}}, \quad (21)$$

1294 where  $L_{\text{operon}}$  is the total length of an rRNA operon ( $\approx 4500 \text{ bp}$ ) and  $\rho_{\text{RNA polymerase}}$  is packing density of RNA  
1295 polymerase on a given operon, taken to be 1 RNA polymerase per 80 nucleotides.

### 1296 Calculation of active ribosomal fraction.

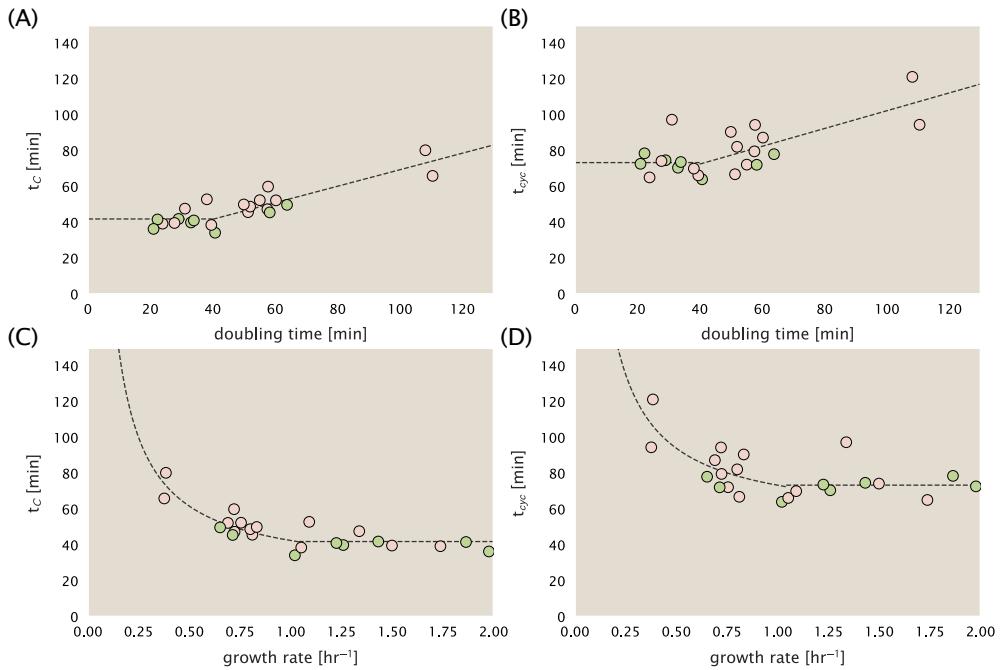
1297 In the main text we used the active ribosomal fraction  $f_a$  that was reported in the work of *Dai et al. (2016)* to  
1298 estimate the active ribosomal mass fraction  $\Phi_R \times f_a$  across growth conditions. We lacked any specific model to  
1299 consider how  $f_a$  should vary with growth rate, and instead find that the data is well-approximated by fitting to an  
1300 exponential curve ( $f_a = -0.889 e^{4.6 \cdot \lambda} + 0.922$ ; dashed line in inset of **Figure 9(C)**). We use this function to estimate  $f_a$   
1301 for each of the data points shown in **Figure 9(C)**.

### 1302 Estimation of $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$ and $\langle \# \text{ori} \rangle$ .

1303 *E. coli* shows robust scaling of cell size with the average number of origins per cell,  $\langle \# \text{ori} \rangle$  (*Si et al., 2017*). Since  
1304 protein makes up a majority of the cell's dry mass, the change in cell size is also a reflection of the changes in  
1305 proteomic composition and total abundance across growth conditions. Given the potential constraints on rRNA  
1306 synthesis and changes in ribosomal copy number with  $\langle \# \text{ori} \rangle$ , it becomes important to also consider how protein  
1307 copy numbers vary with the state of chromosomal replication. This is particularly true when trying to make sense of  
1308 the changes in ribosomal fraction and growth-rate dependent changes in proteomic composition at a mechanistic  
1309 level. As considered in the main text, it is becoming increasingly apparent that regulation through the secondary  
1310 messengers (p)ppGpp may act to limit DNA replication and also reduce ribosomal activity in poorer nutrient  
1311 conditions. In this context, both  $\langle \# \text{ori} \rangle$ , as well as the  $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$  ratio become important parameters to consider  
1312 and keep track of. An increase in  $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$  ratio in particular, causes a relatively higher gene dosage in rRNA  
1313 and r-protein genes due to skew in genes near the origin, where the majority of these are located

1314 In the main text we estimated the change in  $\langle \# \text{ori} \rangle$  with growth rate using the nutrient-limited wild-type cell  
1315 data from *Si et al. (2017)*. We consider their measurements of DNA replication time ( $t_C$ , 'C' period of cell division),  
1316 total cell cycle time ( $t_{\text{cyc}}$ , 'C' + 'D' period of cell division), and doubling time  $\tau$  from wild-type *E. coli* growing across  
1317 a range of growth conditions. Here we show how we estimate this parameter, as well as the  $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$  ratio  
1318 from their data. We begin by considering  $\langle \# \text{ori} \rangle$ . If the cell cycle time takes longer than the time of cell division, the  
1319 cell will need to initiate DNA replication more often than its rate of division,  $2^{\lambda t} = 2^{\ln(2) \cdot t / \tau}$  to maintain steady state  
1320 growth. Cells will need to do this in proportion to the ratio  $\lambda_{\text{cyc}} / \lambda = t_{\text{cyc}} / \tau$ , and the number of origins per cell (on  
1321 average) is then given by  $2^{t_{\text{cyc}} / \tau}$ . The average number of termini will in contrast depend on the lag time between  
1322 DNA replication and cell division,  $t_D$ , with  $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$  ratio =  $2^{t_{\text{cyc}} / \tau - t_D / \tau} = 2^{t_C / \tau}$ .

1323 In Figure 21(A) and (B) we plot the measured  $t_C$  and  $t_{\text{cyc}}$  values versus the doubling time from *Si et al. (2017)*.  
1324 The authors estimated  $t_C$  by marker frequency analysis using qPCR, while  $t_{\text{cyc}} = t_C + t_D$  were inferred from  $t_C$  and  
1325  $\tau$ . In the plots we see that both  $t_C$  and  $t_{\text{cyc}}$  reach a minimum at around 40 and 75 minutes, respectively. For a C  
1326 period of 40 minutes, this would correspond to a maximum rate of elongation of about 1,000 bp/sec. Since we  
1327 lacked a specific model to describe how each of these parameters vary with growth condition, we assumed that  
1328 they were linearly dependent on the doubling time. For each parameter,  $t_C$  and  $t_{\text{cyc}}$ , we split them up into two



**Figure 21. Estimation of  $\langle \#ori \rangle / \langle \#ter \rangle$  and  $\langle \#ori \rangle$  using data from Si et al. (2017).** (A) and (B) plot the reported  $t_C$  and  $t_{cyc}$  as a function of cell doubling time  $\tau$ , respectively. The dashed lines show a piecewise fit to the data. For short doubling times (rich media),  $t_C$  and  $t_{cyc}$  are assumed constant ( $t_C = 42$  minutes,  $t_{cyc} = 73$  minutes). At the transition, taken to occur at 40 minutes, the dashed line corresponds to an assumed proportional increase in each parameter as a function of the doubling time ( $t_C = 0.46\tau + 23.3$  minutes,  $t_{cyc} = 0.50\tau + 52.7$  minutes). (C) and (D) plot the same data as in (A) and (B), but as a function of growth rate, given by  $\lambda = \ln(2)/\tau$ .

domains corresponding to poorer nutrient conditions and rich nutrient conditions (cut off at  $\tau \approx 40$  minutes where chromosomal replication becomes nearly constant). The fit lines are shown as solid black lines. In Figure 21(C) and (D) we also show  $t_C$  and  $t_{cyc}$  as a function of growth rate  $\lambda$  along with our piecewise linear fits, which match the plots in the main text.

### Derivation of Minimal Model for Nutrient-Mediated Growth Rate Control

Here we provide a derivation of the minimal model for growth rate control under nutrient-limited growth. By growth rate control, we are specifically referring to the ability of bacteria to modulate their proteome ( $N_{pep}$ ,  $R$ ,  $\Phi_R$ ) and cell size as nutrient conditions change, with slower growing cells generally being smaller in size (Ojkic et al., 2019). This capability provides bacteria with a particular benefit when nutrients are more scarce since it will mean there is a smaller net demand on carbon, phosphorus, sulfur, and nitrogen. The specific goal of developing this model is to help us better explore the overall constraints on growth that follow from 1) our observation that many of the cellular processes we've considered require increased protein abundance at faster growth rates, and 2) a strict limit on growth rate that is governed by the ribosomal synthesis rate and ribosomal mass fraction  $\Phi_R$ .

In Figure 11(A) of the main text we provide a schematic of the model, where we consider growth as simply governed by the rate of protein synthesis ( $r_t \times R \times f_a$ ). In order to grow rapidly, at least to the extent possible, these three parameters need to be maximized (with  $r_t \leq 17$  amino acids per second, and  $f_a \leq 1$  reported in the work of Dai et al. (2016)). The elongation rate  $r_t$  will depend on how quickly ribosomes can match codons with their correct amino-acyl tRNA, along with the subsequent steps of peptide bond formation and translocation. This ultimately depends on the cellular concentration amino acids, which we treat as a single effective species,  $[AA]_{eff}$ .

In our model, we need to determine the rate of peptide elongation  $r_t$ , which we consider as simply depending on the supply of amino acids (and, therefore, also amino-acyl tRNAs) through a parameter  $r_{AA}$  in units of AA per second, and the rate of amino acid consumption by protein synthesis ( $r_t \times R \times f_a$ ). The balance between these two rates will determine the effective amino acid concentration in the cell  $[AA]_{eff}$ . An important premise for this formulation

1352 is growing evidence that cells are able to modulate their biosynthesis activity according to nutrient availability  
 1353 (i.e. extent of chromosomal replication, transcriptional, and translation activity) through secondary-messenger  
 1354 molecules like (p)ppGpp (*Hauryliuk et al., 2015; Zhu and Dai, 2019; Kraemer et al., 2019; Fernández-Coll et al., 2020; Büke et al., 2020*). Given our observation that protein synthesis and energy production are not limiting,  
 1356 we assume that other molecular players required by ribosomes like elongation factors and GTP are available in  
 1357 sufficient abundance. In addition, experimentally, the relative number of tRNA and elongation factor EF-Tu per  
 1358 ribosome have been found to increase in poorer nutrient conditions *Pedersen (1978); Dong et al. (1996); Klumpp*  
 1359 *et al. (2013)*.

1360 We begin by considering a coarse-grained description of peptide elongation, which includes 1) the time required  
 1361 to find and bind each correct amino-acyl tRNA, and 2) the remaining steps in peptide elongation that will not  
 1362 depend on the amino acid availability. These time scales will be related to the inverse of the elongation rate  $r_t$ ,

$$\frac{1}{r_t} = \frac{1}{k_{on}\alpha[AA]_{eff}} + \frac{1}{r_t^{\max}}. \quad (22)$$

1363 where we have assumed that the rate of binding by amino-acyl tRNA  $k_{on}$  is proportional to  $[AA]_{eff}$  by a constant  $\alpha$ .  
 1364  $r_t^{\max}$  refers to the maximum elongation rate. This leads to a Michaelis-Menten dependence of the elongation rate  $r_t$   
 1365 on the effective amino acid concentration  $[AA]_{eff}$  (*Klumpp et al., 2013; Dai et al., 2016*). We can re-write this more  
 1366 succinctly in terms of an effective dissociation constant,

$$K_D = \frac{r_t^{\max}}{\alpha k_{on}}, \quad (23)$$

1367 where the elongation rate  $r_t$  is now given by

$$r_t = \frac{r_t^{\max}}{1 + K_D/[AA]_{eff}}. \quad (24)$$

1368 The rate of amino acid supply  $r_{AA}$  will vary with changing nutrient conditions and the cell can maintain  $[AA]_{eff}$  by  
 1369 tuning the rate of amino acid consumption,  $r_t \times R \times f_a$ . Thus,  $[AA]_{eff}$  is determined by the difference in the rate of  
 1370 amino acid synthesis (or import, for rich media) and/or tRNA charging,  $r_{AA}$ , and the rate of consumption,  $r_t \times R \times f_a$ .  
 1371 Over an arbitrary length of time  $t$  of cellular growth, the cell will grow in volume, requiring us to consider these  
 1372 rates in terms of concentration rather than absolute numbers, with  $[AA]_{eff}$  given by,

$$\int_0^t \frac{d[AA]_{eff}}{dt} dt = \int_0^t ([r_{AA}] - [r_t \times R \times f_a]) dt. \quad (25)$$

1373 This considers the net change in amino acid concentration over a time from 0 to  $t$ , with the square brackets  
 1374 indicating concentrations per unit time. Integrating **Equation 25** yields.

$$[AA]_{eff} = t([r_{AA}] - [r_t \times R \times f_a]). \quad (26)$$

1375 Alternatively, to connect to the experimental data in terms of absolute ribosome copy number  $R$  we can  
 1376 consider a unit volume  $V$ ,

$$[AA]_{eff} = \frac{t(r_{AA} - r_t \times R \times f_a)}{V \times N_A}, \quad (27)$$

1377 where  $r_{AA}$  is in units of AA per unit time and  $r_t$  is in units of AA per unit time per ribosome.  $N_A$  refers to Avogadro's  
 1378 number and is needed to convert between concentration and absolute numbers per cell. With an expression for  
 1379  $[AA]_{eff}$  in hand, we can now solve **Equation 24** for  $r_t$ , which is a quadratic function with a physically-meaningful root  
 1380 of

$$r_t = \frac{t(r_{AA} + r_t^{\max} R f_a) + K_D V N_A - \sqrt{(r_{AA} + r_t^{\max} R f_a)^2 - 4(R f_a t)(r_t^{\max} r_{AA} t)}}{2R f_a t}. \quad (28)$$

1381 This is the key equation that allows us to calculate growth rate for any combination of  $N_{pep}$ ,  $R$ ,  $f_a$ , and cell size  $V$   
 1382 as a function of amino acid supply  $r_{AA}$  (**Equation 3** of the main text). We refer the reader to A Minimal Model of  
 1383 Nutrient-Mediated Growth Rate Control of the main text for our exploration of this model in the context of the  
 1384 proteomic data.

1385 We end this section by noting several distinctions of this formulation with previous work. The first, as noted  
1386 in the main text, relates to the now seminal work of *Scott et al. (2010)*, which provides a treatment of resource  
1387 allocation that partitions of the proteome into sectors – including one for ribosome-associated proteins and one  
1388 for metabolic proteins. As cells grow faster, there is a notable change in the mass fraction of these sectors, with  
1389 an increase in ribosomal content that is predominantly achieved at the expense of a decrease in the metabolic  
1390 sector. By including an additional constraint through the phenomenological parameter  $\nu$ , which characterizes the  
1391 quality of the growth medium *Scott et al. (2010); Klumpp et al. (2013); Klumpp and Hwa (2014)*, the authors derive  
1392 a model of growth rate, dependent on optimal resource allocation. Here we have developed a model that considers  
1393 the effect of changes in absolute protein abundance and ribosomal content, and consider how these influence the  
1394 achievable growth rate. In addition, by accounting for the metabolic supply of amino acids directly through their  
1395 availability in the cell (i.e.  $[AA]_{\text{eff}}$ ), we are able to consider how the balance between translation-specific metabolic  
1396 capacity and translational capacity influences both the elongation rate  $r_e$  and growth rate  $\lambda$ .

1397 The second and last point we note is that the recent works from *Dai et al. (2016)* and *Klumpp et al. (2013)* also  
1398 employ a similar coarse-graining of translation elongation as we've considered above. Here, however, a notable  
1399 distinction is that the authors consider the entire ternary complex (i.e. the complex of amino-acyl tRNA, EF-Tu,  
1400 and GTP) as rate limiting. Further, through an assumed proportionality between ternary complex and ribosome  
1401 abundance, they arrive at a formulation of elongation rate  $r_e$  that exhibits a Michaelis-Menten dependence on the  
1402 ribosomal fraction  $\Phi_R$ . They demonstrate that all their measurements of elongation rate, even upon addition of  
1403 sublethal doses of chloramphenicol (which cause an increase in both  $r_e$  and  $\Phi_R$ ), can be collapsed onto a single  
1404 curve described by this Michaelis-Menten dependence. There is always a benefit to increase their ribosomal  
1405 fraction  $\Phi_R$  on growth rate when nutrient conditions allow (see Maximum Growth Rate is Determined by the  
1406 Ribosomal Mass Fraction), and this trend in the data in part follows from the tendency for cells to increase  $\Phi_R$   
1407 and better maximize  $r_e$  as nutrient conditions improve. In addition, it does not account for the decrease in the  
1408 fraction of actively translating ribosome  $f_a$  that was strikingly apparent at slow growth rates or in sublethal doses of  
1409 chloramphenicol in the work of *Dai et al. (2016)*. Through **Equation 28** we also account for changes in the fraction  
1410 of actively translating ribosomes. Ultimately, we find that cells are able to maximize both  $\Phi_R$ ,  $r_e$ , and their growth  
1411 rate only to the extent allowed by the nutrient conditions (i.e. via  $r_{AA}$ ) and through the maintenance of the cellular  
1412 pool of amino acids  $[AA]_{\text{eff}}$ , amino-acyl tRNA, GTP, as well as the synthesis of other key molecular constituents like  
1413 EF-Tu.

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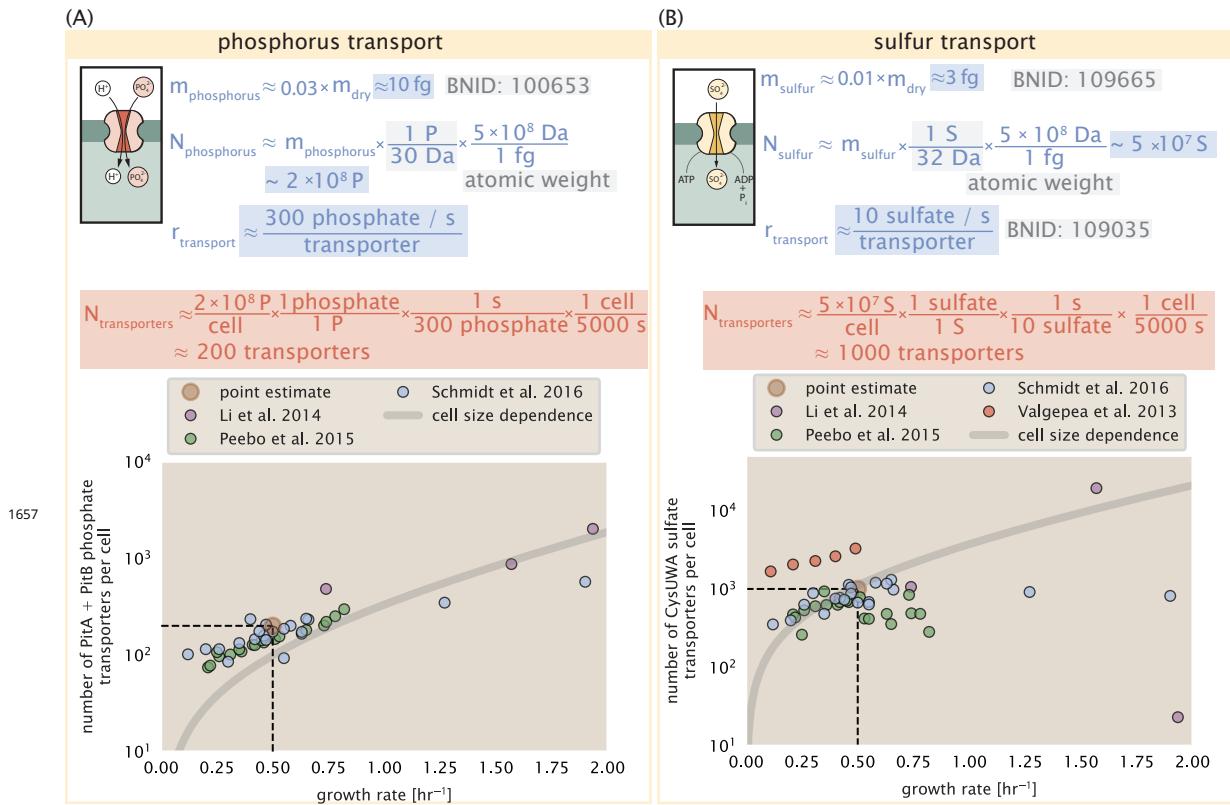
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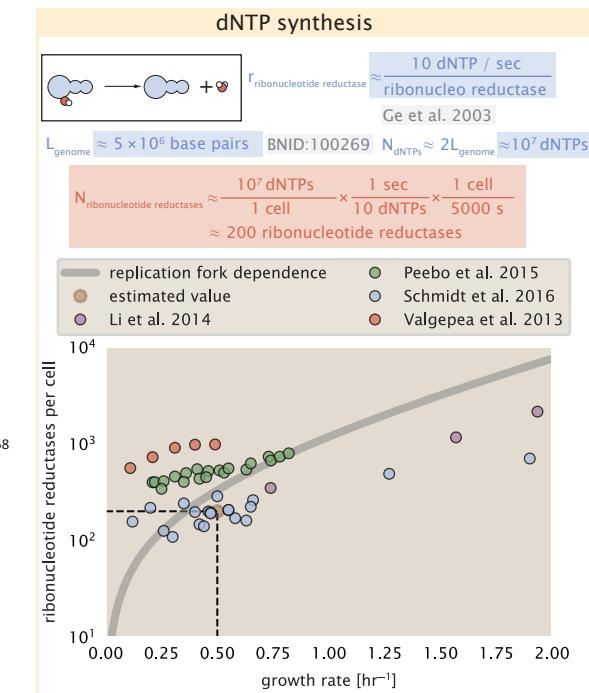
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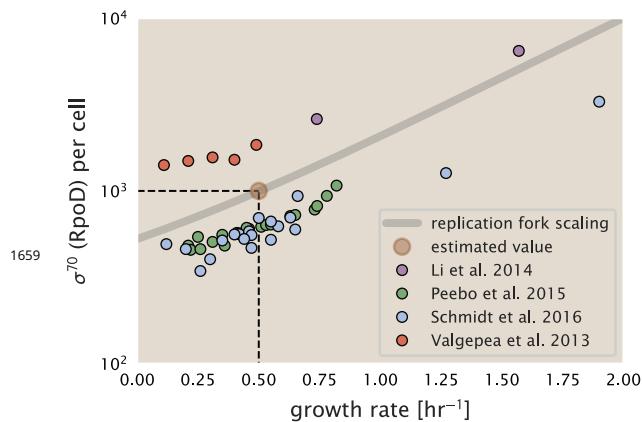
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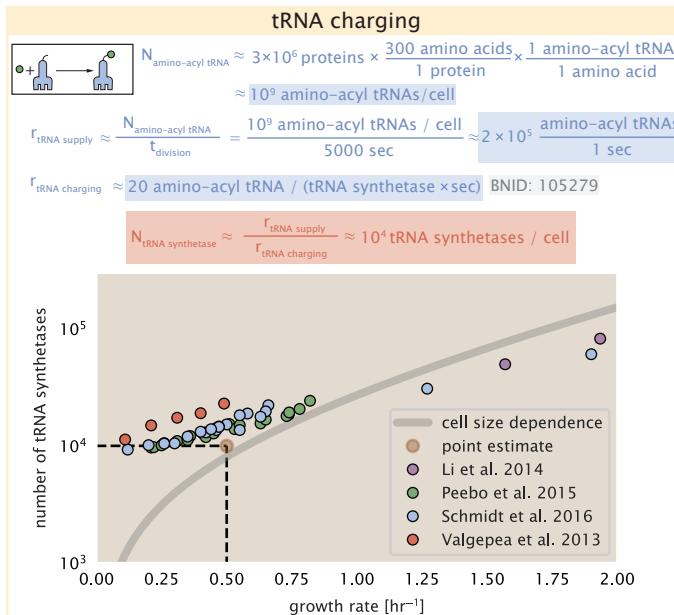
**Figure 2-Figure supplement 1.** (A) Estimate for the number of PitA phosphate transport systems needed to maintain a 3% phosphorus *E. coli* dry mass. Points in plot correspond to the total number of PitA transporters per cell. (B) Estimate of the number of CysUWA complexes necessary to maintain a 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  $[CysA]_2[CysU][CysW][Sbp/CysP]$ . Grey line in (A) and (B) represents the estimated number of transporters per cell at a continuum of growth rates.



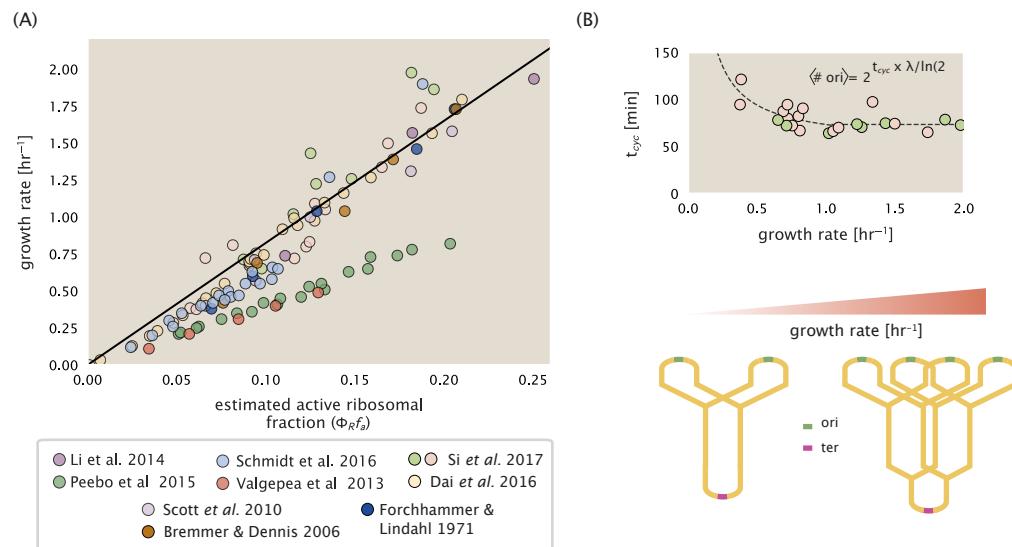
**Figure 6–Figure supplement 1.** Estimate of the number of ribonucleotide reductase enzymes needed to facilitate the synthesis of  $\approx 10^7$  dNTPs over the course of a 5000 second generation time. Points in the plot correspond to the total number of ribonucleotide reductase I ( $[\text{NrdA}]_2[\text{NrdB}]_2$ ) and ribonucleotide reductase II ( $[\text{NrdE}]_2[\text{NrdF}]_2$ ) complexes. Grey lines in top panel show the estimated number of complexes needed as a function of growth, the details of which are described in the Appendix.



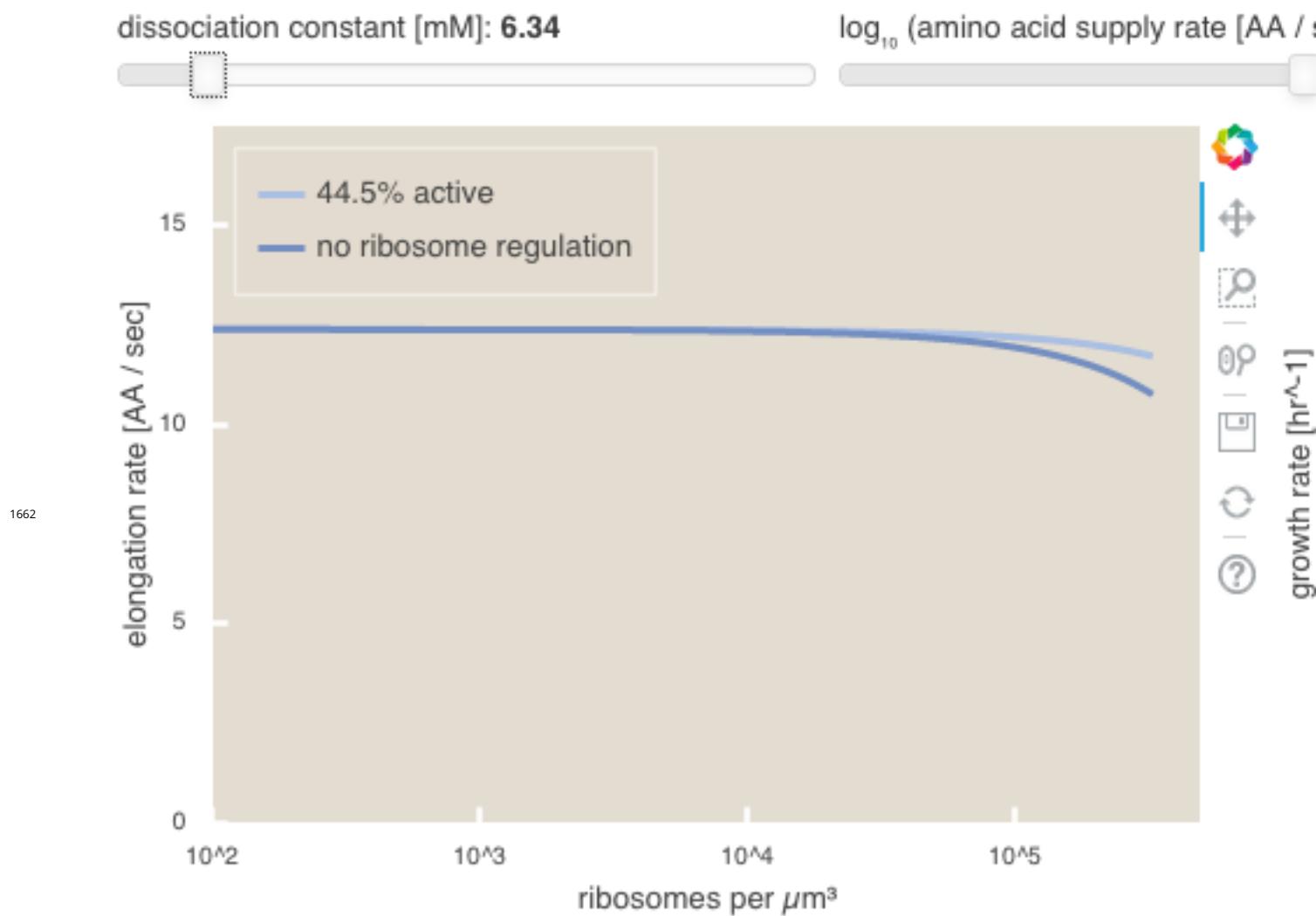
**Figure 7–Figure supplement 1.** The abundance of  $\sigma^{70}$  as a function of growth rate. Estimated value for the number of RNAP is shown as a translucent brown point and grey line.



**Figure 8-Figure supplement 1.** Estimation for the number of tRNA synthetases that will supply the required amino acid demand. The sum of all tRNA synthetases copy numbers are plotted as a function of growth rate ([ArgS], [CysS], [GlnS], [GltX], [IleS], [LeuS], [ValS], [AlaS]<sub>2</sub>, [AsnS]<sub>2</sub>, [AspS]<sub>2</sub>, [TyrS]<sub>2</sub>, [TrpS]<sub>2</sub>, [ThrS]<sub>2</sub>, [SerS]<sub>2</sub>, [ProS]<sub>2</sub>, [PheS]<sub>2</sub>[PheT]<sub>2</sub>, [MetG]<sub>2</sub>, [lysS]<sub>2</sub>, [HisS]<sub>2</sub>, [GlyS]<sub>2</sub>[GlyQ]<sub>2</sub>).



**Figure 9-Figure supplement 1.** (A) Actively translating ribosomal fraction versus growth rate. The actively translating ribosomal fraction is calculated using the estimated values of  $f_a$  from **Dai et al. (2016)** (shown in inset; see Appendix Calculation of active ribosomal fraction for additional detail). Additional measurements in addition to the proteomic measurements are based on measurements of cellular RNA to protein ratio, with  $\Phi_R \approx$  the cellular RNA to protein ratio divided by 2.1 (**Dai et al., 2016**). (B) Experimental measurements of the cell doubling time  $\tau$  and cell cycle time  $t_{\text{cyc}}$  from **Si et al. (2017)**. Dashed line shows fit to the data, which were used to estimate  $\langle \# \text{ori} \rangle$ .  $t_{\text{cyc}}$  was assumed to vary in proportion to  $\tau$  for doubling times greater than 40 minutes, and reach a minimum value of 73 minutes. See Appendix Estimation of  $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$  and  $\langle \# \text{ori} \rangle$  for additional details exact estimation of rRNA copy number. Red data points correspond to measurements in strain MG1655, while light green points are for strain NCM3722. Schematic shows the expected increase in replication forks (or number of ori regions) as *E. coli* cells grow faster.



**Figure 11–Figure supplement 1.** An interactive version of parts (B) and (C) of **Figure 11** which permit the user to modulate the rate of amino acid supply, the dissociation constant of amino acids to the ribosome, and the fraction of the ribosome pool that is actively translating. This interactive figure, and the code used to generate it, is available on the [paper website](#).