

Fundamental limits on the rate of bacterial cell division

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¹⁴

¹⁵ **Abstract** This will be written next

¹⁶

¹⁷ Introduction

¹⁸ The range of bacterial growth rates is enormously diverse. In natural environments, some micro-
¹⁹ bial organisms might double only once per year while in comfortable laboratory conditions, growth
²⁰ can be rapid with several divisions per hour. This six order of magnitude difference illustrates the
²¹ intimate relationship between environmental conditions and the rates at which cells convert nu-
²² trients into new cellular material – a relationship that has remained a major topic of inquiry in
²³ bacterial physiology for over a century (*Jun et al., 2018*). As was noted by Jacques Monod, “the
²⁴ study of the growth of bacterial cultures does not constitute a specialized subject or branch of re-
²⁵ search, it is the basic method of Microbiology.” Those words ring as true today as they did when
²⁶ they were written 70 years ago. Indeed, the study of bacterial growth has undergone a molecular
²⁷ resurgence since many of the key questions addressed by the pioneering efforts in the middle of
²⁸ the last century can be revisited by examining them through the lens of the increasingly refined
²⁹ molecular census that is available for bacteria such as the microbial workhorse *Escherichia coli*. Sev-
³⁰ eral of the outstanding questions that can now be studied about bacterial growth include: what
³¹ sets the fastest time scale that bacteria can divide, and how is growth rate tied to the quality of the
³² carbon source. In this paper, we address these two questions from two distinct angles. First, as
³³ a result of an array of high-quality proteome-wide measurements of the *E. coli* proteome under a
³⁴ myriad of different growth conditions, we have a census that allows us to explore how the num-
³⁵ ber of key molecular players change as a function of growth rate. This census provides a window
³⁶ onto whether the processes they mediate such as molecular transport into the cells and molecular
³⁷ synthesis within cells can run faster. Second, because of our understanding of the molecular path-
³⁸ ways responsible for many of the steps in bacterial growth, we can also make order of magnitude
³⁹ estimates to infer the copy numbers that would be needed to achieve a given growth rate. In this
⁴⁰ paper, we pass back and forth between the analysis of a variety of different proteomic datasets and
⁴¹ order-of-magnitude estimations to determine possible molecular bottlenecks that limit bacterial

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

43 Specifically, we leverage a combination of *E. coli* proteomic data sets collected over the past
 44 decade using either mass spectrometry (*Schmidt et al., 2016; Peebo et al., 2015; Valgepea et al.,
 45 2013*) or ribosomal profiling (*Li et al., 2014*) across 31 unique growth conditions. Broadly speaking,
 46 we entertain several classes of hypotheses as are illustrated in **Figure 1**. First, we consider potential
 47 limits on the transport of nutrients into the cell. We address this hypothesis by performing an
 48 order-of-magnitude estimate for how many carbon, phosphorous, and sulfur atoms are needed to
 49 facilitate this requirement given a 5000 second division time. As a second hypothesis, we consider
 50 the possibility that there exists a fundamental limit on how quickly the cell can generate ATP. We
 51 approach this hypothesis from two angles, considering how many ATP synthase complexes must
 52 be needed to churn out enough ATP to power protein translation followed by an estimation of
 53 how many electron transport complexes must be present to maintain the proton motive force.
 54 A third class of estimates considers the need to maintain the size and shape of the cell through
 55 the construction of new lipids for the cell membranes as well as the glycan polymers which make
 56 up the rigid peptidoglycan. Our final class of hypotheses centers on the synthesis of a variety of
 57 biomolecules. Our focus is primarily on the stages of the central dogma as we estimate the number
 58 of protein complexes needed for DNA replication, transcription, and protein translation.

59 In broad terms, we find that the majority of these estimates are in close agreement with the
 60 experimental observations, with protein copy numbers apparently well-tuned for the task of cell
 61 doubling. This allows us to systematically scratch off the hypotheses diagrammed in **Figure 1** as
 62 setting possible speed limits. Ultimately, we find that protein translation (particularly the genera-
 63 tion of new ribosomes) acts as 1) a rate limiting step for the *fastest* bacterial division, and 2) the
 64 major determinant of bacterial growth across all nutrient conditions we have considered under
 65 steady state, exponential growth. This perspective is in line with the linear correlation observed
 66 between growth rate and ribosomal content (typically quantified through the ratio of RNA to pro-
 67 tein) for fast growing cells (*Scott et al., 2010*), but suggests a more prominent role for ribosomes
 68 in setting the doubling time across all conditions of nutrient limitation. Here we again leverage the
 69 quantitative nature of this data set and present a quantitative model of the relationship between
 70 the fraction of the proteome devoted to ribosomes and the speed limit of translation, revealing
 71 a fundamental tradeoff between the translation capacity of the ribosome pool and the maximal
 72 growth rate.

73 Uptake of Nutrients

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

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

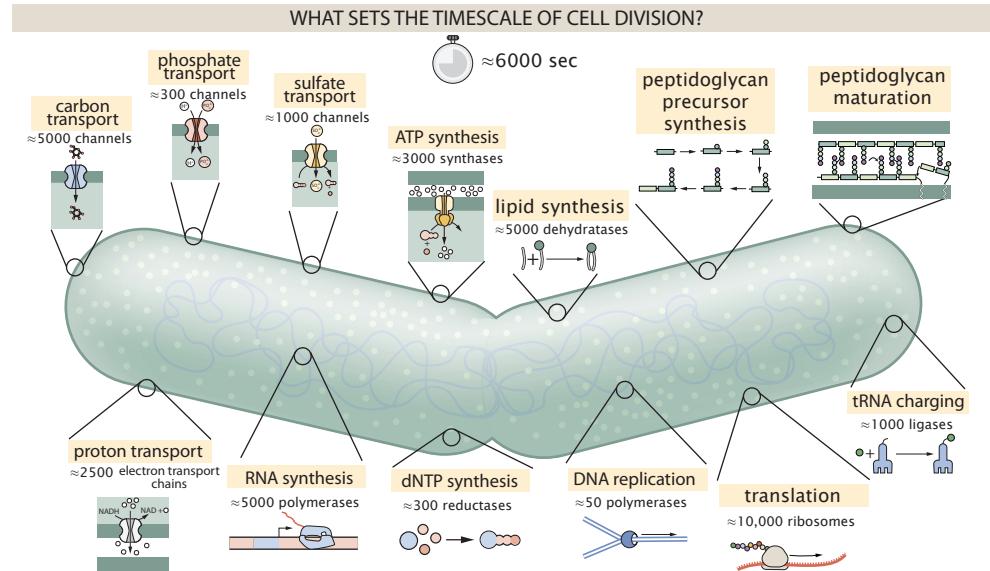


Figure 1. Transport and synthesis processes necessary for cell division. We consider an array of processes necessary for a cell to double its molecular components. Such processes include the transport of carbon across the cell membrane, the production of ATP, and fundamental processes of the central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

92 100653, *Milo et al. (2010)*), and 1% sulfur (BNID: 100655, *Milo et al. (2010)*). In the coming para-
 93 graphs, we will engage in a dialogue between back-of-the-envelope estimates for the numbers of
 94 transporters needed to facilitate these chemical stoichiometries and the experimental proteomic
 95 measurements of the biological reality. Such an approach provides the opportunity to test if our
 96 biological knowledge is sufficient to understand the scale at which these complexes are produced.
 97 Specifically, we will make these estimates considering a modest doubling time of 5000 s, a growth
 98 rate of $\approx 0.5 \text{ hr}^{-1}$, the range in which the majority of the experimental measurements reside.

99 Nitrogen Transport

100 Before we begin our back-of-the-envelope estimations, we must address which elemental sources
 101 must require proteinaceous transport, meaning that the cell cannot acquire appreciable amounts
 102 simply via diffusion from the membrane. The permeability of the lipid membrane to a large num-
 103 ber of solutes has been extensively characterized over the past century. Large, polar molecular
 104 species (such as various sugar molecules, sulfate, and phosphate) have low permeabilities while
 105 small, non-polar compounds (such as oxygen, carbon dioxide, and ammonia) can readily diffuse
 106 across the membrane. Ammonia, a primary source of nitrogen in typical laboratory conditions,
 107 has a permeability on par with water ($\approx 10^5 \text{ nm/s}$, BNID:110824 *Milo et al. (2010)*). In particularly
 108 nitrogen-poor conditions, *E. coli* expresses a transporter (AmtB) which appears to aid in nitrogen
 109 assimilation, though the mechanism and kinetic details of transport is still a matter of debate (*van*
 110 *Heeswijk et al., 2013; Khademi et al., 2004*). Beyond ammonia, another plentiful source of nitrogen
 111 come in the form of glutamate, which has its own complex metabolism and scavenging pathways.
 112 However, nitrogen is plentiful in the growth conditions examined in this work, permitting us to ne-
 113 glect nitrogen transport as a potential rate limiting process in cell division in typical experimental
 114 conditions. We direct the reader to the supplemental information for a more in-depth discussion of
 115 permeabilities and a series of calculations revealing that active nitrogen transport can be neglected
 116 for the purposes of this article.

117 Carbon Transport

118 We begin our estimations with the most abundant element in *E. coli* by mass, carbon. Using ≈ 0.3
119 pg as the typical *E. coli* dry mass (BNID: 103904, *Milo et al. (2010)*), we estimate that $\approx 10^{10}$ carbon
120 atoms must be brought into the cell in order to double all of the carbon-containing molecules (*Fig-*
121 *ure 2(A, top)*). Typical laboratory growth conditions, such as those explored in the aforementioned
122 proteomic data sets, provide carbon as a single class of sugar such as glucose, galactose, or xylose
123 to name a few. *E. coli* has evolved myriad mechanisms by which these sugars can be transported
124 across the cell membrane. One such mechanism of transport is via the PTS system which is a
125 highly modular system capable of transporting a diverse range of sugars (*Escalante et al., 2012*).
126 The glucose-specific component of this system transports ≈ 200 glucose molecules per second per
127 transporter (BNID: 114686, *Milo et al. (2010)*). Making the assumption that this is a typical sugar
128 transport rate, coupled with the need to transport 10^{10} carbon atoms, we arrive at the conclusion
129 that on the order of 1,000 transporters must be expressed in order to bring in enough carbon
130 atoms to divide in 5000 s, diagrammed in the top panel of *Figure 2(A)*. This estimate, along with
131 the observed average number of the PTS system carbohydrate transporters present in the pro-
132 teomic data sets (*Schmidt et al., 2016; Peebo et al., 2015; Valgepea et al., 2013; Li et al., 2014*), is
133 shown in *Figure 2(A)*. While we estimate 1500 transporters are needed with a 5000 s division time,
134 we can abstract this calculation to consider any particular growth rate given knowledge of the cell
135 density and volume as a function of growth rate and direct the reader to the SI for more informa-
136 tion. As revealed in *Figure 2(A)*, experimental measurements exceed the estimate by several fold,
137 illustrating that transport of carbon in to the cell is not rate limiting for cell division.

138 The estimate presented in *Figure 2(A)* neglects any specifics of the regulation of carbon trans-
139 port system and presents a data-averaged view of how many carbohydrate transporters are present
140 on average. Using the diverse array of growth conditions explored in the proteomic data sets, we
141 can explore how individual carbon transport systems depend on the population growth rate. In
142 *Figure 2(B)*, we show the total number of carbohydrate transporters specific to different carbon
143 sources. A striking observation, shown in the top-left plot of *Figure 2(B)*, is the constancy in the
144 expression of the glucose-specific transport systems. Additionally, we note that the total number
145 of glucose-specific transporters is tightly distributed $\approx 10^4$ per cell, the approximate number of
146 transporters needed to sustain rapid growth of several divisions per hour, as indicated by the grey
147 shaded line. This illustrates that *E. coli* maintains a substantial number of complexes present for
148 transporting glucose which is known to be the preferential carbon source (*Monod, 1947; Liu et al.,*
149 *2005; Aidelberg et al., 2014*).

150 It is now understood that a large number of metabolic operons are regulated with dual-input
151 logic gates that are only expressed when glucose concentrations are low (mediated by cyclic-AMP
152 receptor protein CRP) and the concentration of other carbon sources are elevated (*Gama-Castro*
153 *et al., 2016; Zhang et al., 2014b*). A famed example of such dual-input regulatory logic is in the regu-
154 lation of the *lac* operon which is only natively activated in the absence of glucose and the presence
155 of allolactose, an intermediate in lactose metabolism (*Jacob and Monod, 1961*), though we now
156 know of many other such examples (*Ireland et al., 2020; Gama-Castro et al., 2016; Belliveau et al.,*
157 *2018*). This illustrates that once glucose is depleted from the environment, cells have a means to
158 dramatically increase the abundance of the specific transporter needed to digest the next sugar
159 that is present. Several examples of induced expression of specific carbon-source transporters are
160 shown in *Figure 2(B)*. Points colored in red (labeled by red text-boxes) correspond to growth condi-
161 tions in which the specific carbon source (glycerol, xylose, or fructose) is present. These plots show
162 that, in the absence of the particular carbon source, expression of the transporters is maintained
163 on the order of $\sim 10^2$ per cell. However, when induced, the transporters become highly-expressed
164 and fall close to the predicted number of transporters needed to facilitate growth on that sub-
165 strate alone, shown as a transparent grey line. Together, this generic estimation and the specific
166 examples of induced expression suggest that transport of carbon across the cell membrane, while

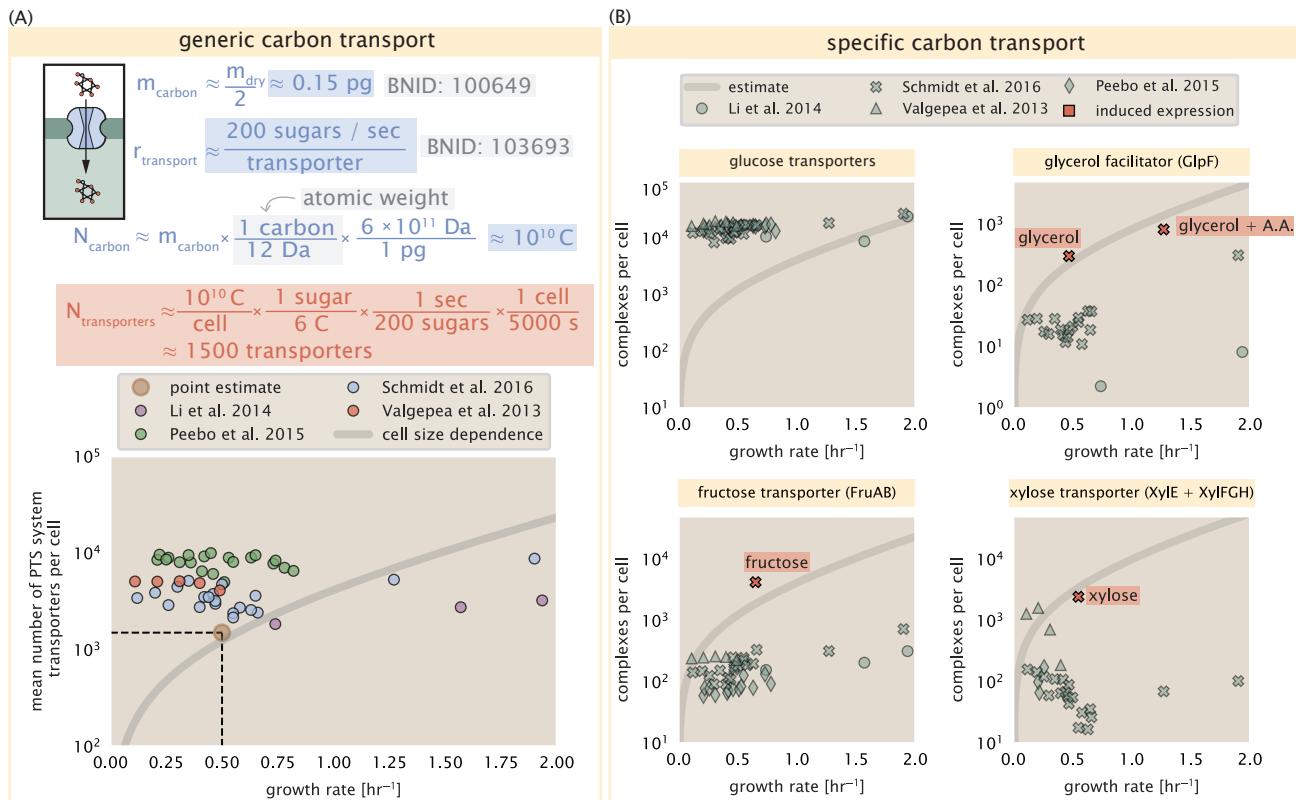


Figure 2. The abundance of carbon transport systems across growth rates. (A) A simple estimate for the minimum number of generic carbohydrate transport systems (top) assumes $\approx 10^{10}$ C are needed to complete division, each transported sugar contains ≈ 6 C, and each transporter conducts sugar molecules at a rate of ≈ 200 per second. Bottom plot shows the estimated number of transporters needed at a growth rate of ≈ 0.5 per hr (light-brown point and dashed lines). Colored points correspond to the mean number of PTS system sugar transporters (complexes annotated with the Gene Ontology term GO:0009401) for different growth conditions across different published datasets. (B) The abundance of various specific carbon transport systems plotted as a function of the population growth rate. Red points and red-highlighted text indicate conditions in which the only source of carbon in the growth medium induces expression of the transport system. Grey line in (A) and (B) represents the estimated number of transporters per cell at a continuum of growth rates.

167 critical for growth, is not the rate-limiting step of cell division.

168 **Phosphorus and Sulfur Transport**

169 We now turn our attention towards other essential elements, namely phosphorus and sulfur. Phos-
 170 phorus is critical to the cellular energy economy in the form of high-energy phosphodiester bonds
 171 making up DNA, RNA, and the NTP energy pool as well as playing a critical role in the post-translational
 172 modification of proteins and defining the polar-heads of lipids. In total, phosphorus makes up
 173 ≈3% of the cellular dry mass which in typical experimental conditions is in the form of inorganic
 174 phosphate. The cell membrane has remarkably low permeability to this highly-charged and critical
 175 molecule, therefore requiring the expression of active transport systems. In *E. coli*, the proton elec-
 176 trochemical gradient across the inner membrane is leveraged to transport inorganic phosphate
 177 into the cell (*Rosenberg et al., 1977*). Proton-solute symporters are widespread in *E. coli* (*Ramos*
 178 and *Kaback, 1977; Booth et al., 1979*) and can have rapid transport rates of 50 to 100 molecules
 179 per second for sugars and other solutes (BNID: 103159; 111777, *Milo et al. (2010)*). As a more
 180 extreme example, the proton transporters in the F₁-F₀ ATP synthase, which leverage the proton
 181 electrochemical gradient for rotational motion, can shuttle protons across the membrane at a rate
 182 of ≈ 1000 per second (BNID: 104890; 103390, *(Milo et al., 2010)*). In *E. coli* the PitA phosphate trans-
 183 port system has been shown to be very tightly coupled with the proton electrochemical gradient
 184 with a 1:1 proton:phosphate stoichiometric ratio (*Harris et al., 2001; Feist et al., 2007*). Taking the
 185 geometric mean of the aforementioned estimates gives a plausible rate of phosphate transport
 186 on the order of 300 per second. Illustrated in *Figure 3(A)*, we can estimate that ≈ 150 phosphate
 187 transporters are necessary to maintain an ≈ 3% dry mass with a 5000 s division time. This estimate
 188 is again satisfied when we examine the observed copy numbers of PitA in proteomic data sets (plot
 189 in *Figure 3(A)*). While our estimate is very much in line with the observed numbers, we emphasize
 190 that this is likely a slight overestimate of the number of transporters needed as there are other
 191 phosphorous scavenging systems, such as the ATP-dependent phosphate transporter Pst system
 192 which we have neglected.

193 Satisfied that there are a sufficient number of phosphate transporters present in the cell, we
 194 now turn to sulfur transport as another potentially rate limiting process. Similar to phosphate,
 195 sulfate is highly-charged and not particularly membrane permeable, requiring active transport.
 196 While there exists a H+/sulfate symporter in *E. coli*, it is in relatively low abundance and is not well
 197 characterized (*Zhang et al., 2014a*). Sulfate is predominantly acquired via the ATP-dependent ABC
 198 transporter CysUWA system which also plays an important role in selenium transport (*Sekowska*
 199 *et al., 2000; Sirko et al., 1995*). While specific kinetic details of this transport system are not readily
 200 available, generic ATP transport systems in prokaryotes transport on the order of 1 to 10 molecules
 201 per second (BNID: 109035, *Milo et al. (2010)*). Combining this generic transport rate, measurement
 202 of sulfur comprising 1% of dry mass, and a 5000 second division time yields an estimate of ≈ 1000
 203 CysUWA complexes per cell (*Figure 3(B)*). Once again, this estimate is in notable agreement with
 204 proteomic data sets, suggesting that there are sufficient transporters present to acquire the nec-
 205 essary sulfur. In a similar spirit of our estimate of phosphorus transport, we emphasize that this is
 206 likely an overestimate of the number of necessary transporters as we have neglected other sulfur
 207 scavenging systems that are in lower abundance.

208 **Limits on Transporter Expression**

209 So which, if any, of these processes may be rate limiting for growth? As suggested by *Figure 2*
 210 (B), induced expression can lead to an order-of-magnitude (or more) increase in the amount of
 211 transporters needed to facilitate transport. Thus, if acquisition of nutrients was the limiting state
 212 in cell division, could expression simply be increased to accommodate faster growth? A way to
 213 approach this question is to compute the amount of space in the bacterial membrane that could
 214 be occupied by nutrient transporters. Considering a rule-of-thumb for the surface area of *E. coli* of
 215 about 6 μm^2 (BNID: 101792, *Milo et al. (2010)*), we expect an areal density for 1000 transporters to

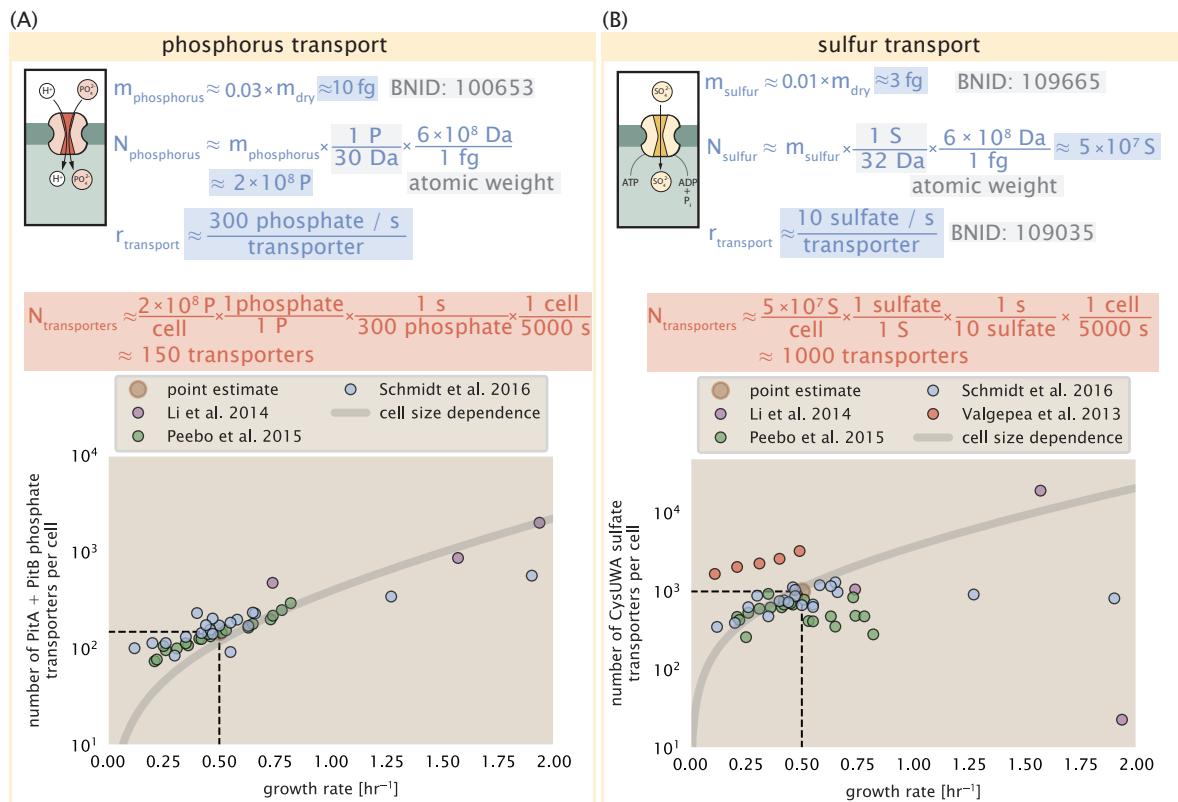


Figure 3. Estimates and measurements of phosphate and sulfate transport systems as a function of growth rate. (A) Estimate for the number of PitA phosphate transport systems needed to maintain a 3% phosphorus *E. coli* dry mass. Points in plot correspond to the total number of PitA transporters per cell.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

216 be approximately 200 transporters/ μm^2 . For a typical transporter occupying about 50 nm 2 /dimer,
 217 this amounts to about only 1 percent of the total inner membrane (*Szenk et al., 2017*). In addition,
 218 bacterial cell membranes typically have densities of 10⁵ proteins/ μm^2 (*Phillips, 2018*), implying that
 219 the cell could accommodate more transporters of a variety of species if it were rate limiting. As we
 220 will see in the next section, however, occupancy of the membrane can impose other limits on the
 221 rate of energy production.

222 Energy Production

223 While the transport of nutrients is required to build new cell mass, the metabolic pathways in-
 224 volved in assimilation both consumes and generates energy in the form of NTPs. The high-energy
 225 phosphodiester bonds of (primarily) ATP power a variety of cellular processes that drive biological
 226 systems away from thermodynamic equilibrium. Our next class of estimates consider the energy
 227 budget of a dividing cell in terms of the synthesis of ATP from ADP and inorganic phosphate as well
 228 as maintenance of the electrochemical proton gradient which powers it.

229 ATP Synthesis

230 Hydrolysis of the terminal phosphodiester bond of ATP forming ADP and an inorganic phosphate is
 231 a kinetic driving force in a wide array of biochemical reactions. One such reaction is the formation
 232 of peptide bonds during translation which requires \approx 2 ATPs for the charging of an amino acid
 233 to the tRNA and \approx 2 ATP equivalents for the formation of the peptide bond between amino acids.
 234 Together, these energetic costs consume \approx 80% of the cells ATP budget (BNID: 107782; 106158;
 235 101637; 111918, *Milo et al. (2010)*). The pool of ATP is produced by the F₁-F₀ ATP synthase – a
 236 membrane-bound rotary motor which under ideal conditions can yield \approx 300 ATP per second (BNID:
 237 114701; *Milo et al. (2010); Weber and Senior (2003)*).

238 To estimate the total number of ATP equivalents consumed during a cell cycle, we will make
 239 the approximation that there are \approx 3 \times 10⁶ proteins per cell with an average protein length of \approx 300
 240 peptide bonds (BNID: 115702; 108986; 104877, *Milo et al. (2010)*). Taking these values together,
 241 we estimate that the typical *E. coli* cell consumes \approx 5 \times 10⁹ ATP per cell cycle on protein synthesis
 242 alone and \approx 6 \times 10⁹ ATP in total. Assuming that the ATP synthases are operating at their fastest
 243 possible rate, \approx 3000 ATP synthases are needed to keep up with the energy demands of the cell.
 244 This estimate and a comparison with the data are shown in *Figure 4* (A). Despite our assumption
 245 of maximal ATP production rate per synthase and approximation of all NTP consuming reactions
 246 being the same as ATP, we find that an estimate of a few thousand complete synthases per cell to
 247 agree well with the experimental data.

248 Generating the Proton Electrochemical Gradient

249 In order to produce ATP, the F₁-F₀ ATP synthase itself must consume energy. Rather than burning
 250 through its own product, this intricate macromolecular machine has evolved to exploit the elec-
 251 trochemical potential established across the inner membrane through cellular respiration. This
 252 electrochemical gradient is manifest by the pumping of protons into the intermembrane space via
 253 the electron transport chains as they reduce NADH. In *E. coli*, this potential difference is \approx -200
 254 mV (BNID: 102120, *Milo et al. (2010)*). As estimated in the supporting information, this potential
 255 difference is generated by maintaining \approx 2 \times 10⁴ protons in the intermembrane space.

256 However, the constant rotation of the ATP synthases would rapidly abolish this potential differ-
 257 ence if it were not being actively maintained. To undergo a complete rotation (and produce a single
 258 ATP), the F₁-F₀ ATP synthase must shuttle \approx 4 protons across the membrane into the cytosol (BNID:
 259 103390, *Milo et al. (2010)*). With \approx 3000 ATP synthases each generating 300 ATP per second, the
 260 2 \times 10⁴ protons establishing the 200 mV potential would be consumed in only a few milliseconds. This
 261 brings us to our next estimate: how many electron transport complexes are needed to support
 262 the consumption rate of the ATP synthases?

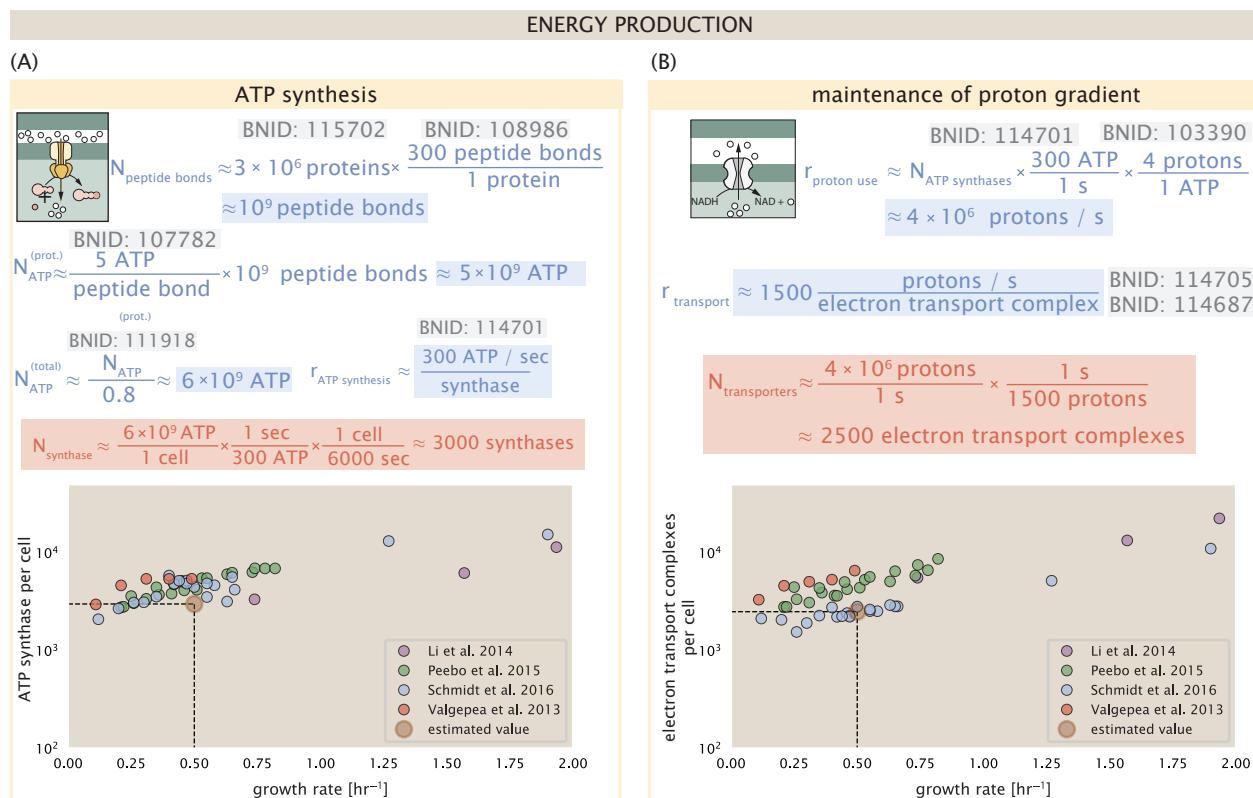


Figure 4. The abundance of F₁-F₀ ATP synthases and electron transport chain complexes as a function of growth rate. (A) Estimate of the number of F₁-F₀ ATP synthase complexes needed accommodate peptide bond formation and other NTP dependent processes. Points in plot correspond to the mean number of complete F₁-F₀ ATP synthase complexes that can be formed given proteomic measurements and the subunit stoichiometry [AtpE]₁₀[AtpF]₂[AtpB][AtpC][AtpH][AtpA]₃[AtpG][AtpD]₃. (B) Estimate of the number of electron transport chain complexes needed to maintain a membrane potential of ~ -200 mV given estimate of number of F₁-F₀ 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]), bo₃,([CyoD][CyoA][CyoB][CyoC]) and NADH:quinone oxidoreductase I ([NuoA][NuoH][NuoJ][NuoK][NuoL][NuoM][NuoN][NuoB][NuoC][NuoE][NuoF][NuoG][NuoI]) and II ([Ndh]).

263 The electrochemistry of the electron transport complexes of *E. coli* have been the subject of
 264 intense biochemical and biophysical study over the past half century (*Ingle dew and Poole, 1984;*
 265 *Khademian and Imlay, 2017; Cox et al., 1970; Henkel et al., 2014*). A recent work (*Szenk et al.,*
 266 *2017*) examined the respiratory capacity of the *E. coli* electron transport complexes using structural
 267 and biochemical data, revealing that each electron transport chain rapidly pumps protons into the
 268 intermembrane space at a clip of ≈ 1500 protons per second (BIND: 114704; 114687, *Milo et al.*
 269 *(2010)*). Using our estimate of the number of ATP synthases required per cell (*Figure 4(A)*), coupled
 270 with these recent measurements, we estimate that $\approx 4 \times 10^6$ protons per second diet of the cellular ATP synthases. This
 271 estimate is in agreement with the number of complexes identified in the proteomic datasets (plot
 272 in *Figure 4(B)*).

274 Energy Production in a Crowded Membrane.

275 For each protein considered so far, the data shows that in general their numbers increase with
 276 growth rate. This is in part a consequence of the increase in cell length and width at that is common
 277 to many rod-shaped bacteria at faster growth rates (*Ojic et al., 2019; Harris and Theriot, 2018*).
 278 For the particular case of *E. coli*, the total cellular protein and cell size increase logarithmically

279 with growth rate (*Schaechter et al., 1958; Si et al., 2017*). Indeed, this is one reason why we have
 280 considered only a single, common growth condition across all our estimates so far. Such a scaling
 281 will require that the total number of proteins and net demand on resources also grow in proportion
 282 to the increase in cell size divided by the cell's doubling time. Recall however that each transport
 283 process, as well as the ATP production via respiration, is performed at the bacterial membrane.
 284 This means that their maximum productivity can only increase in proportion to the cell's surface
 285 area divided by the cell doubling time. This difference in scaling would vary in proportion to the
 286 surface area-to-volume (S/V) ratio.

287 While we found that there was more than sufficient membrane real estate for carbon intake in
 288 our earlier estimate, the total number of ATP synthases and electron chain transport complexes
 289 both exhibit a clear increase in copy number with growth rate, reaching in excess of 10^4 copies per
 290 cell (*Figure 4*). Here we consider the consequences of this S/V ratio scaling in more detail.

291 In our estimate of ATP production above we found that a cell demands about 6×10^9 ATP or
 292 10^6 ATP/s. With a cell volume of roughly 1 fL, this corresponds to about 2×10^{10} ATP per fL of cell
 293 volume, in line with previous estimates (*Stouthamer and Bettenhausen, 1977; Szenk et al., 2017*).
 294 In *Figure 5* (A) we plot this ATP demand as a function of the S/V ratio in green, where we have
 295 considered a range of cell shapes from spherical to rod-shaped with an aspect ratio (length/width)
 296 equal to 4 (See appendix for calculations of cell volume and surface area). In order to consider the
 297 maximum power that could be produced, we consider the amount of ATP that can be generated by a
 298 membrane filled with ATP synthase and electron transport complexes, which provides a maximal
 299 production of about 3 ATP / (nm²·s) (*Szenk et al., 2017*). This is shown in red in *Figure 5*(A), which
 300 shows that at least for the growth rates observed, the energy demand is roughly an order of mag-
 301 nitude less. Interestingly, *Szenk et al. (2017)* also found that ATP production by respiration is less
 302 efficient than by fermentation per membrane area occupied due to the additional proteins of the
 303 electron transport chain. This suggests that even under anaerobic growth, there will be sufficient
 304 membrane space for ATP production in general.

305 While this serves to highlight the diminishing capacity to provide resources to grow if the cell
 306 increases in size (and its S/V decreases), the blue region in *Figure 5*(A) represents a somewhat
 307 unachievable limit since the inner membrane must also include other proteins such as those re-
 308 quired for lipid and membrane synthesis. To gain insight, we used the proteomic data to look at
 309 the distribution of proteins on the inner membrane. Here we use Gene Ontology (GO) annotations
 310 (*Ashburner et al., 2000; The Gene Ontology Consortium, 2018*) to identify all proteins embedded
 311 or peripheral to the inner membrane (GO term: 0005886). Those associated but not membrane-
 312 bound include proteins like MreB and FtsZ, that traverse the inner membrane by treadmilling and
 313 must nonetheless be considered as a vital component occupying space on the membrane. In *Fig-*
314 ure 5 (B), we find that the total protein mass per μm^2 is relatively constant with growth rate. Inter-
 315 estingly, when we consider the distribution of proteins grouped by their Clusters of Orthologous
 316 Groups (COG) (*Tatusov et al., 2000*), the relative abundance for those in metabolism (including ATP
 317 synthesis via respiration) is also relatively constant.

318 Function of the Central Dogma

319 Up to this point, we have considered a variety of transport and biosynthetic processes that are
 320 critical to acquiring and generating new cell mass. While there are of course many other metabolic
 321 processes we could consider and perform estimates of (such as the components of fermentative
 322 versus aerobic respiration), we now turn our focus to some of the most central processes which
 323 must be undertaken irrespective of the growth conditions – the processes of the central dogma.

324 DNA

325 Most bacteria (including *E. coli*) harbor a single, circular chromosome and can have extra-chromosomal
 326 plasmids up to ~ 100 kbp in length. We will focus our quantitative thinking solely on the chromo-
 327 some of *E. coli* which harbors ≈ 5000 genes and $\approx 5 \times 10^6$ base pairs. To successfully divide and

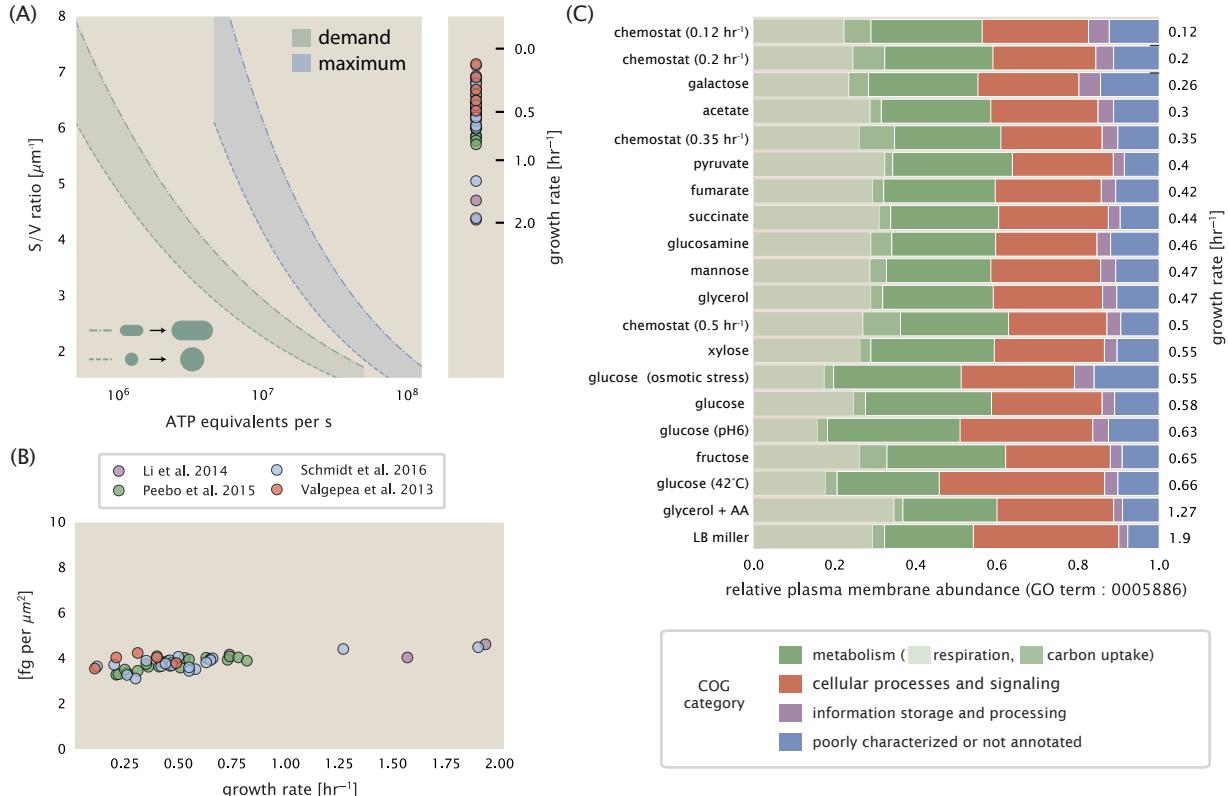


Figure 5. Influence of cell size and S/V ratio on ATP production and inner membrane composition. (A) Scaling of ATP demand and maximum ATP production as a function of S/V ratio. Cell volumes of 0.5 fL to 50 fL were considered, with the dashed (—) line corresponding to a sphere and the dash-dot line (---) reflecting a rod-shaped bacterium like *E. coli* with aspect ratio (length / width) of 0.4. Approximately 50% of the bacterial inner membrane is assumed to be protein, with the remainder lipid. (B) Total protein mass 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 b_{o3} 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 total mass shown in part (B)).

328 produce viable progeny, this chromosome must be faithfully replicated and segregated into each
 329 nascent cell. We again rely on the near century of literature in molecular biology to provide some
 330 insight on the rates and mechanics of the replicative feat as well as the production of the required
 331 starting materials, dNTPs.

332 dNTP synthesis

333 We begin our exploration DNA replication by examining the production of the deoxyribonucleotide
 334 triphosphates (dNTPs). The four major dNTPs (dATP, dTTP, dCTP, and dGTP) are synthesized *de*
 335 *novo* in separate pathways, requiring different building blocks. However, a critical step present in
 336 all dNTP synthesis pathways is the conversion from ribonucleotide to deoxyribonucleotide via the
 337 removal of the 3' hydroxyl group of the ribose ring (Rudd *et al.*, 2016). This reaction is mediated
 338 by a class of enzymes termed ribonucleotide reductases, of which *E. coli* possesses two aerobically
 339 active complexes (termed I and II) and a single anaerobically active enzyme. Due to their peculiar
 340 formation of a radical intermediate, these enzymes have received much biochemical, kinetic, and
 341 structural characterization. One such work (Ge *et al.*, 2003) performed a detailed *in vitro* measure-
 342 ment of the steady-state kinetic rates of these complexes, revealing a turnover rate of ≈ 10 dNTP
 343 per second.

344 Since this reaction is central to the synthesis of all dNTPs, it is reasonable to consider the abun-
 345 dance of these complexes is a measure of the total dNTP production in *E. coli*. Illustrated schemati-
 346 cally in **Figure 6** (A), we consider the fact that to replicate the cell's genome, on the order of $\approx 10^7$
 347 dNTPs must be synthesized. Assuming a production rate of 10 per second per ribonucleotide
 348 reductase complex and a cell division time of 6000 seconds, we arrive at an estimate of ≈ 150 com-
 349 plexes needed per cell. As shown in the bottom panel of **Figure 6** (A), this estimate agrees with the
 350 experimental measurements of these complexes abundances within $\approx 1/2$ an order of magnitude.

351 Recent work has revealed that during replication, the ribonucleotide reductase complexes co-
 352 alesce to form discrete foci colocalized with the DNA replisome complex (Sánchez-Romero *et al.*,
 353 2011). This is particularly pronounced in conditions where growth is slow, indicating that spatial
 354 organization and regulation of the activity of the complexes plays an important role.

355 DNA Replication

356 We now turn our focus to the integration of these dNTP building blocks into the replicated chromo-
 357 some strand via the DNA polymerase. Replication is initiated at a single region of the chromosome
 358 termed the *oriC* locus at which a pair of DNA polymerases bind and begin their high-fidelity repli-
 359 cation of the genome in opposite directions. Assuming equivalence between the two replication
 360 forks, this means that the two DNA polymerase complexes (termed replisomes) meet at the mid-
 361 way point of the circular chromosome termed the *ter* locus. The kinetics of the five types of DNA
 362 polymerases (I – V) have been intensely studied, revealing that DNA polymerase III performs the
 363 high fidelity processive replication of the genome with the other "accessory" polymerases playing
 364 auxiliary roles (Fijalkowska *et al.*, 2012). *In vitro* measurements have shown that DNA Polymerase
 365 III copies DNA at a rate of ≈ 600 nucleotides per second (BNID: 104120, Milo *et al.* (2010)). There-
 366 fore, to replicate a single chromosome, two replisomes moving at their maximal rate would copy
 367 the entire genome in ≈ 4000 s. Thus, with a division time of 5000 s (our "typical" growth rate for
 368 the purposes of this work), there is sufficient time for a pair of DNA polymerase III complexes to
 369 replicate the entire genome. However, this estimate implies that 4000 s would be the upper-limit
 370 time scale for bacterial division which is at odds with the familiar 20 minute (1200 s) doubling time
 371 of *E. coli* in rich medium.

372 It is well known that *E. coli* can parallelize its DNA replication such that multiple chromosomes
 373 are being replicated at once, with as many as 10 - 12 replication forks at a given time (Bremer
 374 and Dennis, 2008; Si *et al.*, 2017). Thus, even in rapidly growing cultures, we expect only a few
 375 polymerases (≈ 10) are needed to replicate the chromosome per cell doubling. However, as shown
 376 in **Figure 6(B)**, DNA polymerase III is nearly an order of magnitude more abundant. This

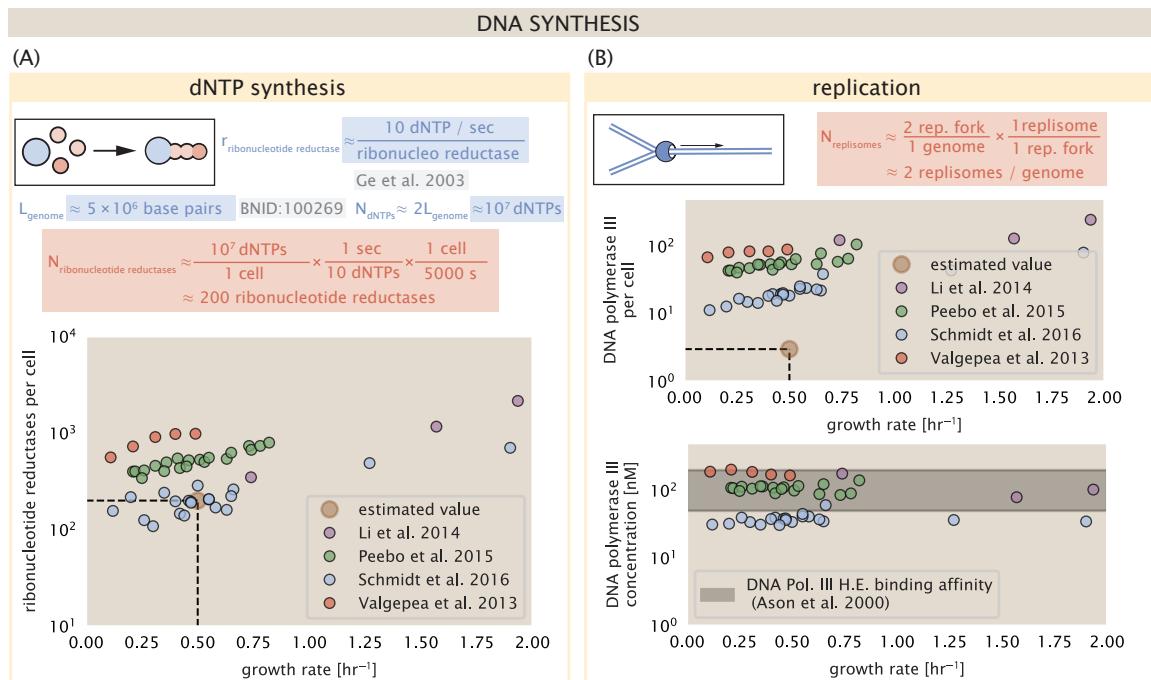


Figure 6. Complex abundance estimates for dNTP synthesis and DNA replication. (A) Estimate of the number of ribonucleotide reductase enzymes needed to facilitate the synthesis of $\approx 10^7$ dNTPs over the course of a 6000 second generation time. Points in the plot correspond to the total number of ribonucleotide reductase I ($[NrdA]_2[NrdB]_2$) and ribonucleotide reductase II ($[NrdE]_2[NrdF]_2$) complexes. (B) An estimate for the minimum number of DNA polymerase holoenzyme complexes needed to facilitate replication of a single genome. Points in the top plot correspond to the total number of DNA polymerase III holoenzyme complexes ($[DnaE]_3[DnaQ]_3[HolE]_3[DnaX]_5[HolB][HolA][DnaN]_4[HolC]_4[HolD]_4$) per cell. Bottom plot shows the effective concentration of DNA polymerase III holoenzyme given cell volumes calculated from the growth rate as in *Si et al. (2019)* (See Appendix Section 4).

discrepancy can be understood by considering its binding constant to DNA. DNA polymerase III is highly processive, facilitated by a strong affinity of the complex to the DNA. *In vitro* biochemical characterization has quantified the K_D of DNA polymerase III holoenzyme to single-stranded and double-stranded DNA to be 50 and 200 nM, respectively (Ason et al., 2000). The bottom plot in **Figure 6** (B) shows that the concentration of the DNA polymerase III across all data sets and growth conditions is within this range. Thus, while the copy number of the DNA polymerase III is in excess of the strict number required to replicate the genome, its copy number appears to vary such that its concentration is approximately equal to the dissociation constant to the DNA. While the processes regulating the initiation of DNA replication are complex and involve more than just the holoenzyme, these data indicate that the kinetics of replication rather than the explicit copy number of the DNA polymerase III holoenzyme is the more relevant feature of DNA replication to consider. In light of this, the data in **Figure 6(B)** suggests that for bacteria like *E. coli*, DNA replication does not represent a rate-limiting step in cell division. However, it is worth noting that for bacterium like *C. crescentus* whose chromosomal replication is initiated only once per cell cycle (Jensen et al., 2001), the time to double their chromosome likely represents an upper limit to their growth rate.

RNA Synthesis

With the machinery governing the replication of the genome accounted for, we now turn our attention to the next stage of the central dogma – the transcription of DNA to form RNA. We primarily consider three major groupings of RNA, namely the RNA associated with ribosomes (rRNA), the RNA encoding the amino-acid sequence of proteins (mRNA), and the RNA which links codon sequence to amino-acid identity during translation (tRNA). Despite the varied function of these RNA species, they share a commonality in that they are transcribed from DNA via the action of RNA polymerase. In the coming paragraphs, we will consider the synthesis of RNA as a rate limiting step in bacterial division by estimating how many RNA polymerases must be present to synthesize all necessary rRNA, mRNA, and tRNA.

rRNA

We begin with an estimation of the number of RNA polymerases needed to synthesize the rRNA that serve as catalytic and structural elements of the ribosome. Each ribosome contains three rRNA molecules of lengths 120, 1542, and 2904 nucleotides (BNID: 108093, Milo et al. (2010)), meaning each ribosome contains ≈ 4500 nucleotides. As the *E. coli* RNA polymerase transcribes DNA to RNA at a rate of ≈ 40 nucleotides per second (BNID: 101904, Milo et al. (2010)), it takes a single RNA polymerase ≈ 100 s to synthesize the RNA needed to form a single functional ribosome. Therefore, in a 5000 s division time, a single RNA polymerase transcribing rRNA at a time would result in only ≈ 50 functional ribosomal rRNA units – far below the observed number of $\approx 10^4$ ribosomes per cell.

Of course, there can be more than one RNA polymerase transcribing the rRNA genes at any given time. To elucidate the *maximum* number of rRNA units that can be synthesized given a single copy of each rRNA gene, we will consider a hypothesis in which the rRNA operon is completely tiled with RNA polymerase. *In vivo* measurements of the kinetics of rRNA transcription have revealed that RNA polymerases are loaded onto the promoter of an rRNA gene at a rate of ≈ 1 per second (BNID: 111997; 102362, Milo et al. (2010)). If RNA polymerases are being constantly loaded on to the rRNA genes at this rate, then we can assume that ≈ 1 functional rRNA unit is synthesized per second. With a 5000 second division time, this hypothesis leads to a maximal value of 5000 functional rRNA units, still undershooting the observed number of 10^4 ribosomes per cell.

E. coli has evolved a clever mechanism to surpass this kinetic limit for the rate of rRNA production. Rather than having only one copy of each rRNA gene, *E. coli* has seven copies of the operon (BNID: 100352, Milo et al. (2010)) four of which are localized directly adjacent to the origin of replication (Birnbaum and Kaplan, 1971). As fast growth also implies an increased gene dosage due to paralellized chromosomal replication, the total number of rRNA genes can be on the order of $\approx 10 - 70$ copies at moderate to fast growth rates (Stevenson and Schmidt, 2004). Using our standard

426 time scale of a 5000 second division time, we can make the lower-bound estimate that the typical
 427 cell will have 7 copies of the rRNA operon. Synthesizing one functional rRNA unit per second per
 428 rRNA operon, a total of 4×10^4 rRNA units can be synthesized, comfortably above the observed
 429 number of ribosomes per cell.

430 How many RNA polymerases are then needed to constantly transcribe 7 copies of the rRNA
 431 genes? We approach this estimate by considering the maximum number of RNA polymerases tiled
 432 along the rRNA genes with a loading rate of 1 per second and a transcription rate of 40 nucleotides
 433 per second. Considering that a RNA polymerase has a physical footprint of approximately 40 nu-
 434 cleotides (BNID: 107873, *Milo et al. (2010)*), we can expect ≈ 1 RNA polymerase per 80 nucleotides.
 435 With a total length of ≈ 4500 nucleotides per operon and 7 operons per cell, the maximum number
 436 of RNA polymerases that can be transcribing rRNA at any given time is ≈ 400 . As we will see in the
 437 coming sections, the synthesis of rRNA is the dominant requirement of the RNA polymerase pool.

438 mRNA

439 To form a functional protein, all protein coding genes must first be transcribed from DNA to form
 440 an mRNA molecule. While each protein requires an mRNA blueprint, many copies of the protein
 441 can be synthesized from a single mRNA. Factors such as strength of the ribosomal binding site,
 442 mRNA stability, and rare codon usage frequency dictate the number of proteins that can be made
 443 from a single mRNA, with yields ranging from 10^1 to 10^4 (BNID: 104186; 100196; 106254, *Milo et al.*
 444 (*2010*)). Computing the geometric mean of this range yields ≈ 1000 proteins synthesized per mRNA,
 445 a value that agrees with experimental measurements of the number of proteins per cell ($\approx 3 \times 10^6$,
 446 BNID: 100088, *Milo et al. (2010)*) and total number of mRNA per cell ($\approx 3 \times 10^3$, BNID:100064, *Milo*
 447 *et al. (2010)*).

448 This estimation captures the *steady-state* mRNA copy number, meaning that at any given time,
 449 there will exist approximately 3000 unique mRNA molecules. To determine the *total* number of
 450 mRNA that need to be synthesized over the cell's lifetime, we must consider degradation of the
 451 mRNA. In most bacteria, mRNAs are rather unstable with life times on the order of several minutes
 452 (BNID: 104324; 106253; 111927; 111998, *Milo et al. (2010)*). For convenience, we assume that the
 453 typical mRNA in our cell of interest has a typical lifetime of ≈ 300 seconds. Using this value, we
 454 can determine the total mRNA production rate to maintain a steady-state copy number of 3000
 455 mRNA per cell. While we direct the reader to the appendix for a more detailed discussion of mRNA
 456 transcriptional dynamics, we state here that the total mRNA production rate must be on the order
 457 of ≈ 15 mRNA per second. In *E. coli*, the average protein is ≈ 300 amino acids in length (BNID:
 458 108986; *Milo et al. (2010)*), meaning that the corresponding mRNA is ≈ 900 nucleotides which we
 459 will further approximate as ≈ 1000 nucleotides to account for the non-protein coding regions on
 460 the 5' and 3' ends. This means that the cell must have enough RNA polymerase molecules about
 461 to sustain a transcription rate of $\approx 1.5 \times 10^4$ nucleotides per second. Knowing that a single RNA
 462 polymerase polymerizes RNA at a clip of 40 nucleotides per second, we arrive at a comfortable
 463 estimate of ≈ 250 RNA polymerase complexes needed to satisfy the mRNA demands of the cell. It
 464 is worth noting that this number is approximately half of that required to synthesize enough rRNA,
 465 as we saw in the previous section. We find this to be a striking result as these 250 RNA polymerase
 466 molecules are responsible for the transcription of the ≈ 4000 protein coding genes that are not
 467 ribosome associated.

468 tRNA

469 The final class of RNA molecules worthy of quantitative consideration is the the pool of tRNAs
 470 used during translation to map codon sequence to amino acid identity. Unlike mRNA or rRNA,
 471 each individual tRNA is remarkably short, ranging from 70 to 95 nucleotides each (BNID: 109645;
 472 102340, *Milo et al. (2010)*). What they lack in length, they make up for in abundance. There are
 473 approximately ≈ 3000 tRNA molecules present for each of the 20 amino acids (BNID: 105280, *Milo*
 474 *et al. (2010)*), although the precise copy number is dependent on the identity of the ligated amino

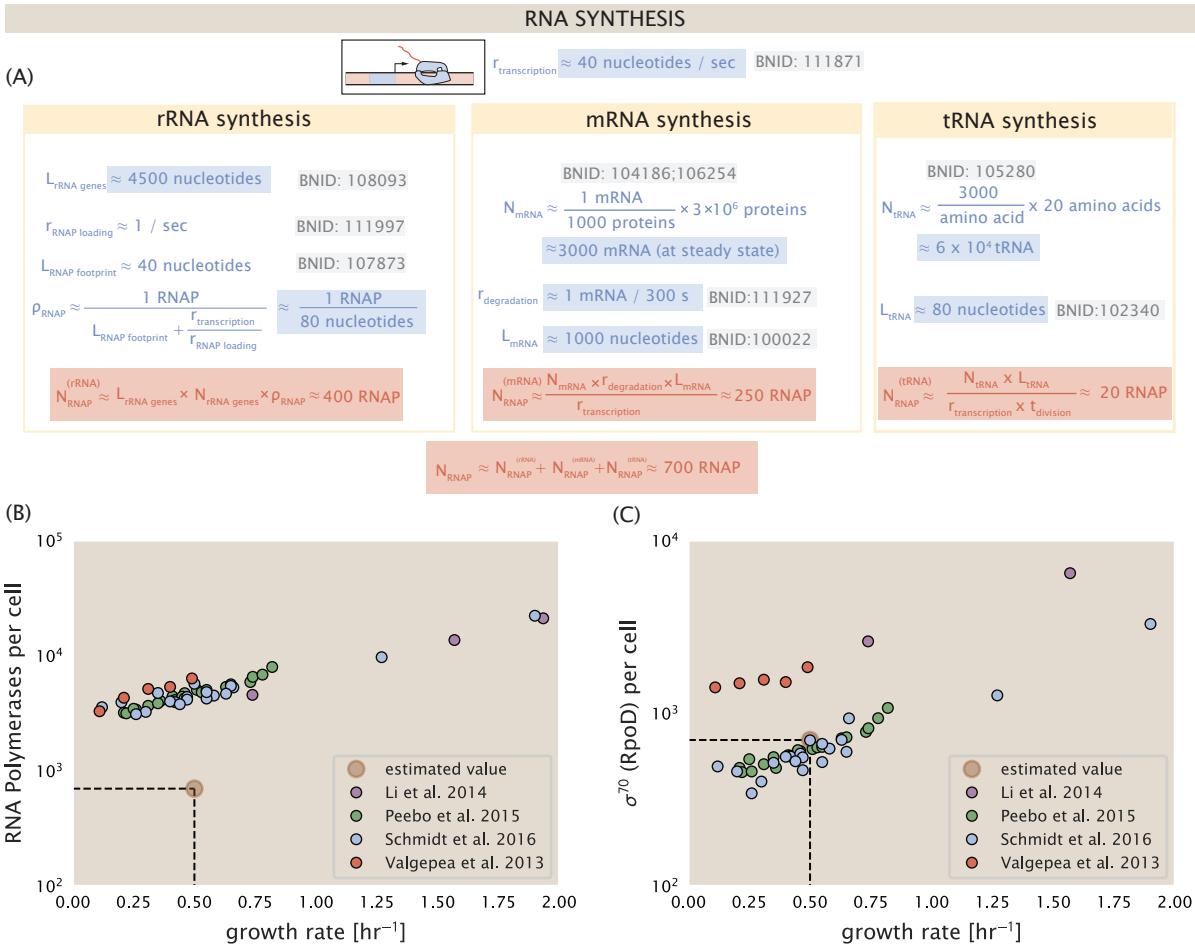


Figure 7. Estimation of the RNA polymerase demand and comparison with experimental data. (A)

Estimations for the number of RNA polymerase needed to synthesize sufficient quantities of rRNA, mRNA, and tRNA from left to right, respectively. Bionumber Identifiers (BNIDs) are provided for key quantities used in the estimates. (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 $[RpoA]_2[RpoC][RpoB]$. (C) The abundance of σ^{70} as a function of growth rate. Estimated value for the number of RNAP is shown in (B) and (C) as a translucent brown point at a growth rate of 0.5 hr^{-1} .

acid. Using these values, we make the estimate that $\approx 5 \times 10^6$ nucleotides are sequestered in tRNA per cell. Unlike mRNA, tRNA is remarkably stable with typical lifetimes *in vivo* on the order of ≈ 48 hours (Abelson et al., 1974; Svenningsen et al., 2017) – well beyond the timescale of division. Once again using our rule-of-thumb for the rate of transcription to be 40 nucleotides per second and assuming a division time of ≈ 5000 seconds, we arrive at an estimate of ≈ 20 RNA polymerases to synthesize enough tRNA. This requirement pales in comparison to the number of polymerases needed to generate the rRNA and mRNA pools and can be neglected as a significant transcriptional burden.

483 RNA Polymerase and σ -factor Abundance

484 These estimates, summarized in Figure 7 (A), reveal that synthesis of rRNA and mRNA are the dominant RNA species synthesized by RNA polymerase, suggesting the need for ≈ 700 RNA polymerases per cell. As is revealed in Figure 7 (B), this estimate is about an order of magnitude below the observed number of RNA polymerase complexes per cell ($\approx 5000 - 7000$). The disagreement between the estimated number of RNA polymerases and these observations are at least consistent with

489 recent literature revealing that $\approx 80\%$ of RNA polymerases in *E. coli* are not transcriptionally active
 490 (**Patrick et al., 2015**). Our estimate ignores the possibility that some fraction is only nonspecifically
 491 bound to DNA, as well as the obstacles that RNA polymerase and DNA polymerase present for each
 492 other as they move along the DNA (**Finkelstein and Greene, 2013**).

493 In addition, it is also vital to consider the role of σ -factors which help RNA polymerase identify
 494 and bind to transcriptional start sites (**Browning and Busby, 2016**). Here we consider σ^{70} (RpoD)
 495 which is the dominant "general-purpose" σ -factor in *E. coli*. While initially thought of as being solely
 496 involved in transcriptional initiation, the past two decades of single-molecule work has revealed
 497 a more multipurpose role for σ^{70} including facilitating transcriptional elongation (**Kapanidis et al.,
 498 2005; Goldman et al., 2015; Perdue and Roberts, 2011; Mooney and Landick, 2003; Mooney et al.,
 499 2005**). **Figure 7** (B) is suggestive of such a role as the number of σ^{70} proteins per cell is in close
 500 agreement with our estimate of the number of transcriptional complexes needed.

501 While these estimates and comparison with experimental data reveal an interesting dynamic
 502 at play between the transcriptional demand and copy numbers of the corresponding machinery,
 503 these findings illustrate that transcription cannot be the rate limiting step in bacterial division. **Fig-
 504 ure 7** (A) reveals that the availability of RNA polymerase is not a limiting factor for cell division as the
 505 cell always has an apparent ~ 10 -fold excess than needed. Furthermore, if more transcriptional
 506 activity was needed to satisfy the cellular requirements, more σ^{70} -factors could be expressed to
 507 utilize a larger fraction of the RNA polymerase pool.

508 Translation and ribosomal synthesis

509 Lastly, we turn our attention to the process of synthesizing new proteins, translation. These pro-
 510 cesses stand as good candidates for defining the growth limit as the synthesis of new proteins
 511 relies on the generation of ribosomes, themselves proteinaceous molecules. As we will see in the
 512 coming sections of this work, this poses a "chicken-or-the-egg" problem where the synthesis of
 513 ribosomes requires ribosomes in the first place.

514 We will begin our exploration of protein translation in the same spirit as we have in previous
 515 sections – we will draw order-of-magnitude estimates based on our intuition and relying on litera-
 516 ture studies and will compare these estimates to the observed data. In doing so, we will estimate
 517 both the absolute number of ribosomes necessary for replication of the proteome as well as the
 518 synthesis of amino-acyl tRNAs. In the closing sections, we will explore the details of ribosome bio-
 519 genesis in granular detail, ultimately presenting a quantitative model tying ribosome abundance
 520 to the concentration of amino acids as well as the state of chromosome replication.

521 tRNA synthetases

522 We begin by first estimating the number of tRNA ligases in *E. coli* needed to convert free amino-
 523 acids to polypeptide chains. At a modest growth rate of ≈ 5000 s, *E. coli* has roughly 3×10^6 proteins
 524 per cell (BNID: 115702; **Milo et al. (2010)**). Assuming that the typical protein is on the order of \approx
 525 300 amino acids in length (BNID: 100017; **Milo et al. (2010)**), we can estimate that a total of $\approx 10^9$
 526 amino acids are stitched together by peptide bonds.

527 How many tRNAs are needed to facilitate this remarkable number of amino acid delivery events
 528 to the translating ribosomes? It is important to note that tRNAs are recycled after they've passed
 529 through the ribosome and can be recharged with a new amino acid, ready for another round of
 530 peptide bond formation. While some *in vitro* data exists on the turnover of tRNA in *E. coli* for
 531 different amino acids, we can make a reasonable estimate by comparing the number of amino
 532 acids to be polymerized to cell division time. Using our stopwatch of 5000 s and 10^9 amino acids,
 533 we arrive at a requirement of $\approx 2 \times 10^5$ tRNA molecules. This estimate is in line with experimental
 534 measurements of $\approx 3 \times 10^5$ per cell (BNID: 108611, **Milo et al. (2010)**), suggesting we are on the
 535 right track.

536 There are many processes which go into synthesizing a tRNA and ligating it with the appropriate
 537 amino acids. As we covered in the previous section, there appear to be more than enough RNA

538 polymerases per cell to synthesize the needed pool of tRNAs. Without considering the many ways
 539 in which amino acids can be scavenged or synthesized *de novo*, we can explore ligation the as a
 540 potential rate limiting step. The enzymes which link the correct amino acid to the tRNA, known as
 541 tRNA synthetases or tRNA ligases, are incredible in their proofreading of substrates with the incor-
 542 rect amino acid being ligated once out of every 10^4 to 10^5 times (BNID: 103469, *Milo et al. (2010)*).
 543 This is due in part to the consumption of energy as well as a multi-step pathway to ligation. While
 544 the rate at which tRNA is ligated is highly dependent on the identity of the amino acid, it is reason-
 545 able to state that the typical tRNA synthetase has charging rate of ≈ 20 AA per tRNA synthetase per
 546 second (BNID: 105279, *Milo et al. (2010)*).

547 Combining these estimates together, as shown schematically in *Figure 8(A)*, yields an estimate
 548 of $\approx 10^4$ tRNA synthetases per cell with a division time of 5000 s. This point estimate is in very close
 549 agreement with the observed number of synthetases (the sum of all 20 tRNA synthetases in *E. coli*).
 550 This estimation strategy seems to adequately describe the observed growth rate dependence of
 551 the tRNA synthetase copy number (shown as the grey line in *Figure 8(B)*), suggesting that the copy
 552 number scales with the cell volume.

553 In total, the estimated and observed $\approx 10^4$ tRNA synthetases occupy only a meager fraction of
 554 the total cell proteome, around 0.5% by abundance. It is reasonable to assume that if tRNA charg-
 555 ing was a rate limiting process, cells would be able to increase their growth rate by devoting more
 556 cellular resources to making more tRNA synthases. As the synthesis of tRNAs and the correspond-
 557 ing charging can be highly parallelized, we can argue that tRNA charging is not a rate limiting step
 558 in cell division, at least for the growth conditions explored in this work.

559 Protein synthesis

560 With the number of tRNA synthetases accounted for, we now consider the abundance of the pro-
 561 tein synthesis machines themselves, ribosomes. Ribosomes are enormous protein/rRNA com-
 562 plexes that facilitate the peptide bond formation between amino acids in the correct sequence
 563 as defined by the coding mRNA. Before we examine the synthesis of the ribosome proteins and
 564 the limits that may place on the observed bacterial growth rates, let's consider replication of the
 565 cellular proteome.

566 As described in the previous section, *E. coli* consists of $\approx 3 \times 10^6$ proteins at a growth rate of \approx
 567 5000 s. If we again assume that each protein is composed of ≈ 300 amino acids and each amino
 568 acid is linked together by one peptide bond, we arrive at an estimate that the cellular proteome
 569 consists of $\approx 10^{10}$ peptide bonds. While the rate at which ribosomes translates is well known to
 570 have a growth rate dependence *Dai et al. (2018)* and is a topic which we discuss in detail in the
 571 coming sections. However, for the purposes of our order-of-magnitude estimate, we can make
 572 the approximation that translation occurs at a rate of ≈ 15 amino acids per second per ribosome
 573 (BNID: 100233, *Milo et al. (2010)*). Under this approximation and assuming a division time of 5000
 574 s, we can arrive at an estimate of $\approx 10^4$ ribosomes are needed to replicate the cellular proteome,
 575 shown in *Figure 8(B)*. This point estimate, while glossing over important details such as chromo-
 576 some copy number and growth-rate dependent translation rates, proves to be notably accurate
 577 when compared to the experimental observations (*Figure 8(B)*).

578 Translation as a growth-rate limiting step

579 Thus far in our work, the general back-of-the-envelope estimates have been reasonably successful
 580 in explaining what sets the scale of absolute protein copy number. In many cases, these estimates
 581 can be adapted to consider a continuum of growth rates in lieu of a single 5000 s point estimate,
 582 the details of which are described in the Supplemental Information. A recurring theme we have
 583 relied on is the ability of the cell to parallelize different processes to transport or synthesize the
 584 required amount of the corresponding biomolecule. For example, we saw in our example of *E. coli*
 585 grown on different carbon sources that expression of particular transporters can be induced, often
 586 producing more than needed acquire enough carbon to build new cell mass (??(B)). In examining

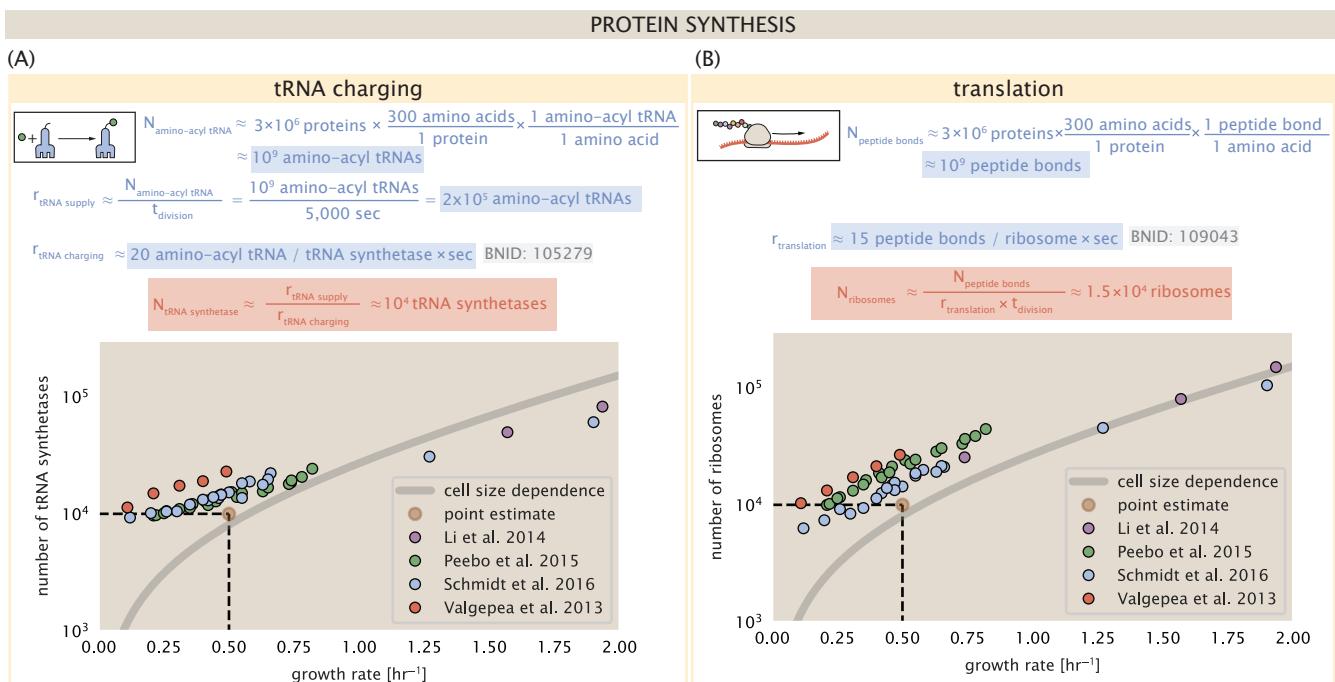


Figure 8. Estimation of the required tRNA synthetases and ribosomes. (A) Estimation for the number of tRNA synthetases that will supply the required amino acid demand. The sum of all tRNA synthetases copy numbers are plotted as a function of growth rate ([ArgS], [CysS], [GlnS], [Glx], [IleS], [LeuS], [ValS], [AlaS]₂, [AsnS]₂, [AspS]₂, [TyrS]₂, [TrpS]₂, [ThrS]₂, [SerS]₂, [ProS]₂, [PheS]₂[PheT]₂, [MetG]₂, [LysS]₂, [GlyS]₂[GlyQ]₂). (B) Estimation of the number of ribosomes required to synthesize 10⁹ 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 hr⁻¹. Grey lines correspond to the estimated complex abundance calculated at different growth rates. See Supplemental Information XX for a more detail description of this calculation.

replication of the DNA, we described how cells can replicate multiple copies of the chromosome at any given time, permitting growth rates faster than the limit at which the chromosome can be faithfully replicated. As a final example, we showed how increasing the gene dosage of the rRNA operons is necessary to produce enough rRNA to form functional ribosomes. However, when it comes to ribosome biogenesis, namely the translation of ribosomal proteins, such parallelization is not possible, suggesting that translation may be a key factor determining the cellular growth rate.

Optimal resource allocation and the role of ribosomal proteins have been an area of intense quantitative study over the last decade by Hwa and others (Scott et al., 2010; Hui et al., 2015). From the perspective of limiting growth, our earlier estimate of rRNA highlighted the necessity for multiple copies of rRNA genes in order to make enough rRNA. For *E. coli*'s fastest growth rates at 2 hr⁻¹, the additional demand for rRNA is further supported by parallelized DNA replication and increased rRNA gene dosage. This suggests the possibility that synthesis of ribosomes might be rate limiting. While the transcriptional demand for the ribosomal proteins is substantially lower than rRNA genes, since proteins can be translated from relatively fewer mRNA, other ribosomal proteins like the translation elongation factor EF-Tu also present a substantial burden. For EF-Tu in particular, it is the most highly expressed protein in *E. coli* and is expressed from multiple gene copies, *tufA* and *tufB*.

To gain some intuition into how translation may set the speed limit for bacterial growth, we again consider the total number of peptide bonds that must be synthesized, N_{AA} . Noting that cell mass grows exponentially (Godin et al., 2010), we can compute the number of amino acids to be

608 polymerized as

$$N_{AA} = \frac{r_t R}{\lambda}, \quad (1)$$

609 where λ is the cell growth rate in s^{-1} , r_t is the maximum translation rate in amino acids per second,
 610 and R is the average ribosome copy number per cell. Knowing the number of peptide bonds to be
 611 formed permits us to compute the translation-limited growth rate as

$$\lambda_{\text{translation-limited}} = \frac{r_t R}{N_{AA}}. \quad (2)$$

612 Alternatively, since N_{AA} is related to the total protein mass through the molecular weight of
 613 each protein, we can also consider the growth rate in terms of the fraction of the total proteome
 614 mass that is dedicated to ribosomal protein mass. By making the approximation that an average
 615 amino acid has a molecular weight of 110 Da (see **Figure 9(A)**), we can rewrite the growth rate as,

$$\lambda_{\text{translation-limited}} \approx \frac{r_t}{L_R} \Phi_R, \quad (3)$$

616 where L_R is the total length in amino acids that make up a ribosome, and Φ_R is the ribosomal mass
 617 fraction. This is plotted as a function of ribosomal fraction Φ_R in **Figure 9(A)**, where we take $L_R \approx$
 618 7500 aa, corresponding to the length in amino acids for all ribosomal subunits of the 50S and 30S
 619 complex (BNID: 101175, (Milo et al., 2010)). This formulation assumes that the cell can transcribe
 620 the required amount of rRNA, which appears reasonable for *E. coli*, allowing us to consider the
 621 inherent limit on growth set by the ribosome.

622 The growth rate defined by Equation 3 reflects mass-balance under steady-state growth and
 623 has long provided a rationalization to the apparent linear increase in *E. coli*'s ribosomal content
 624 as a function of growth rate (Maaloe, 1979; Scott et al., 2010). For our purposes, there are several
 625 important consequences of this trend. Firstly, we note there is a maximum growth rate of $\lambda \approx$
 626 6hr^{-1} , or doubling time of about 7 minutes (dashed line). This growth rate can be viewed as an
 627 inherent maximum growth rate due to the need for the cell to double the cell's entire ribosomal
 628 mass. Interestingly, this limit is independent of the absolute number of ribosomes and is simply
 629 given by time to translate an entire ribosome, L_R/r_t . As shown in **Figure 9(B)**, we can reconcile this
 630 with the observation that in order to double the average number of ribosomes, each ribosome
 631 must produce a second ribosome. Unlike DNA replication or rRNA transcription, this is a process
 632 that cannot be parallelized.

633 For reasonable values of Φ_R , between about 0.1 - 0.3 (Scott et al., 2010), the maximum growth
 634 rate is in line with experimentally reported growth rates around 0.5 - 2 hr^{-1} . Importantly, in order
 635 for a cell to increase their growth limit they *must* increase their relative ribosomal abundance. This
 636 can be achieved by either synthesizing more ribosomes or reducing the fraction of non-ribosomal
 637 proteins. Reduction of non-ribosomal proteins is not a straightforward task since (as we have
 638 found throughout our estimates) doubling a cell requires many other enzymes and transporters.
 639 Increasing the absolute ribosomal abundance in *E. coli* will be limited by the number of rRNA oper-
 640 ons.

641 Here we again return to rRNA synthesis, but here consider the maximum rRNA that can be
 642 produced at different growth rates.

643 [expand on.]

644 Discussion

645 [Fill in.]

646 Maximizing growth rate requires coordination of biosynthesis at all growth rates.

647 However, the mechanism behind growth rate control has remained elusive and has only been
 648 described at a phenomenological level.

649 Here we attempt to place our observations across the proteomic data sets in the context of *E.*
 650 *coli* maximizing its steady-state growth rate across a wide array of conditions.

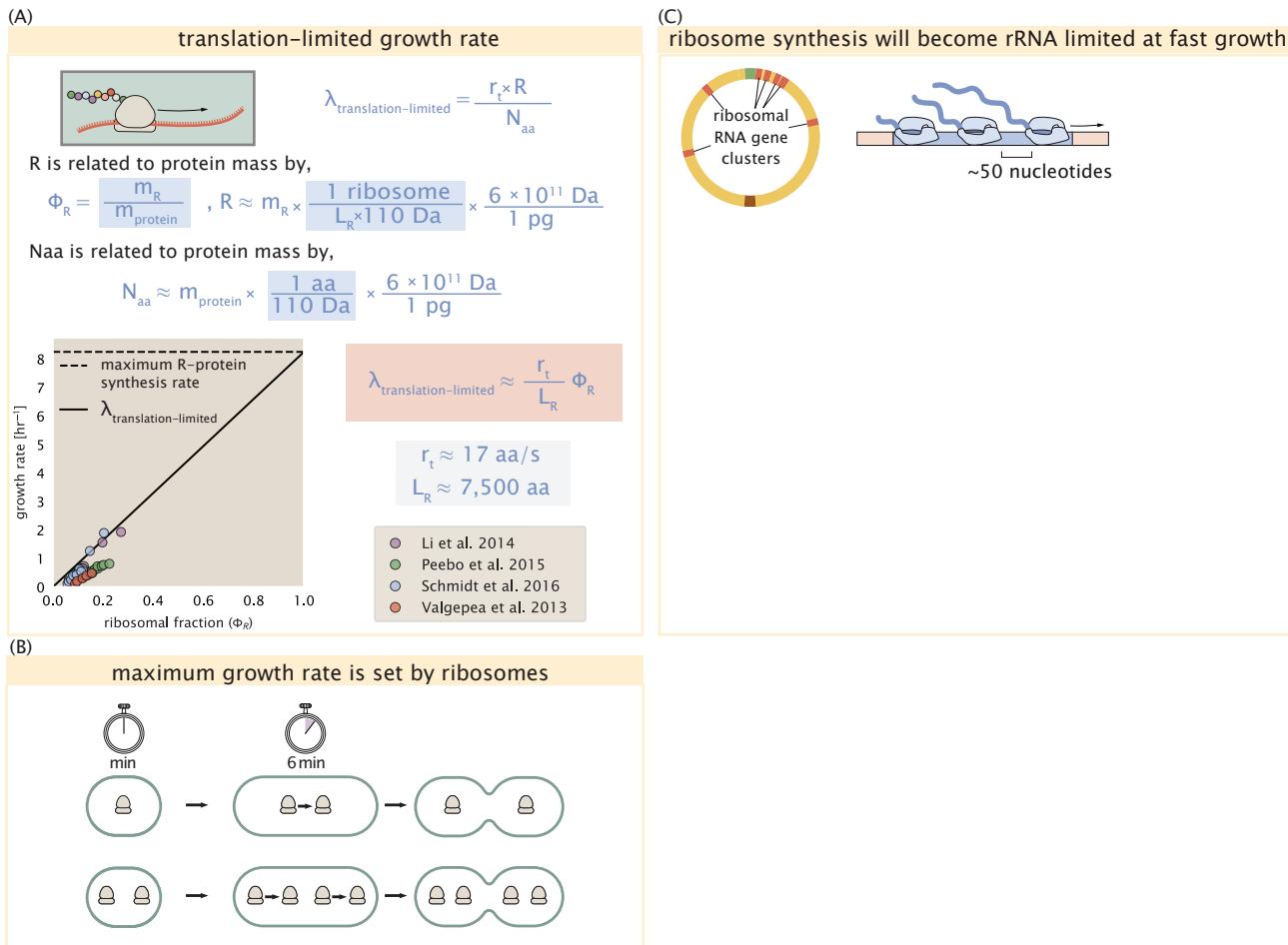


Figure 9. Translation-limited growth rate. (A) Here we consider the translation-limited growth as a function of ribosomal fraction. By mass balance, the time required to double the entire proteome (N_{AA} / r_t) sets the translation-limited growth rate, $\lambda_{\text{translation-limited}}$. Here N_{aa} is effectively the number of peptide bonds that must be translated, r_t is the translation elongation rate, and R is the number of ribosomes. This can also be re-written in terms of the ribosomal mass fraction $\Phi_R = m_R / m_{\text{protein}}$, where m_R is the total ribosomal mass and m_{protein} is the mass of all proteins in the cell. L_R refers to the summed length of the ribosome in amino acids. $\lambda_{\text{translation-limited}}$ is plotted as a function of Φ_R (solid line). (B) The dashed line in part (A) identifies a maximum growth rate that is set by the ribosome. Specifically, this growth rate corresponds to the time required to translate an entire ribosome, L_R / r_t . This is a result that is independent of the number of ribosomes in the cell as shown schematically here. (C)

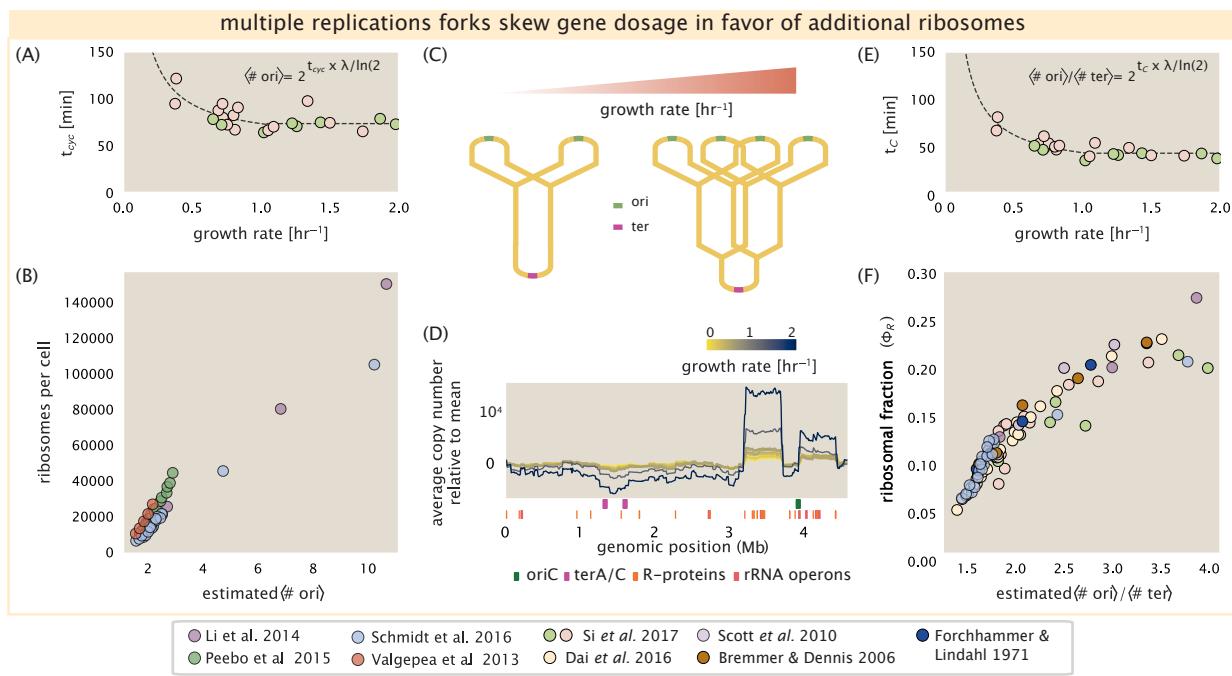


Figure 10. Multiple replication forks skew gene dosage and ribosomal content. (A) Schematic shows the expected increase in replication forks (or number of ori regions) as *E. coli* cells grow faster. (B) A running boxcar average of protein copy number is calculated for each each growth condition considered by Schmidt *et al.*. A 0.5 Mb averaging window was used. Protein copy numbers are reported relative to their condition-specific means in order to center all data sets. (C) and (E) show experimental data from Si *et al.* (2017) Solid lines show fits to the data, which were used to estimate $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$ and $\langle \# \text{ori} \rangle$ [NB: to note fit equations]. Red data points correspond to measurements in strain MG1655, while light green points are for strain NCM3722. (D) Plot compares our estimate of $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$ to the experimental measurements of ribosomal abundance. Ribosomal fraction was approximated from the RNA/protein ratios of Dai *et al.* (2016) (yellow) and Si *et al.* (2017) (light red and light green) by the conversion RNA/protein ratio $\approx \Phi_R \cdot 2.1$. (F) Plot of the ribosome copy number estimated from the proteomic data against the estimated $\langle \# \text{ori} \rangle$.

651 Parallel DNA replication biases gene dosage in support of ribosome synthesis.
 652 *E. coli* cells grow by a so-called "adder" mechanism, whereby cells add a constant volume with
 653 each cell division (Taheri-Araghi *et al.*, 2015). In conjunction with this, additional rounds of DNA
 654 replication are triggered when cells reach a critical volume per origin of replication (Figure 10(A)).
 655 This leads to the classically-described exponential increase in cell size with growth rate Schaechter
 656 *et al.* (1958); Si *et al.* (2017, 2019). In the context of maximizing growth rate, it is notable that the
 657 majority of ribosomal proteins and rRNA operons are found closer to the DNA origin.

658 While an increase in transcription has been observed for genes closer to the origin in rapidly
 659 growing *E. coli* (Scholz *et al.*, 2019), we were unaware of such characterization at the proteomic
 660 level. In order to see whether there is a relative increase in protein expression for genes closer to
 661 the origin at faster growth, we calculated a running boxcar average (500 kbp window) of protein
 662 copy number as a function of each gene's transcriptional start site (Figure 10(B)). While absolute
 663 protein copy numbers can vary substantially across the chromosome, we indeed observe a bias in
 664 expression under fast growth conditions (dark blue), showing the result. The dramatic change in
 665 protein copy number near the origin is primarily due to the increase in ribosomal protein expres-
 666 sion. This trend is in contrast to slower growth conditions (yellow) where the average copy number
 667 is more uniform across the length of the chromosome.

668 If ribosomal genes (rRNA and ribosomal proteins) are being synthesized at their maximal rate
 669 according to their rRNA gene dosage and maximal transcription rate, we can make two related
 670 hypotheses about how their ribosome abundance should vary with chromosomal content. First,

671 the ribosomal protein fraction should increase in proportion to the average ratio of DNA origins to
 672 DNA termini ($\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$ ratio). This is a consequence of the skew in DNA dosage as cells grow
 673 faster. The second hypothesis is that the absolute number of ribosomes should increase with the
 674 number of DNA origins ($\langle \# \text{ ori} \rangle$), since this will reflect the total gene dosage at a particular growth
 675 condition.

676 In order to test each of these expectations we considered the experimental data from *Si et al.*
 677 (2017), which inferred these parameters for cells under nutrient-limited growth. The ratio $\langle \# \text{ ori} \rangle /$
 678 $\langle \# \text{ ter} \rangle$ depends on how quickly chromosomes are replicated relative the cell's doubling time τ and
 679 is given by $2^{\tau_C/\tau}$. Here τ_C is the time taken to replicate *E. coli*'s chromosome, referred to as the C
 680 period of cell division. In **Figure 10(C)** we plot the measured τ_C versus τ (computed as $\tau = \log(2)/\lambda$),
 681 with data points in red corresponding to *E. coli* strain MG1655, and blue to strain NCM3722. *Si*
 682 *et al.* (2017) also measured the total RNA to protein ratio which reflects ribosomal abundance and
 683 we show that data along with other recent measurements from *Dai et al.* (2016, 2018). Indeed, we
 684 find that the ribosomal fraction increases with $\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$ (**Figure 10(C)**). We note a systematic
 685 difference in the relative abundances from *Peebo et al.* (2015) and *Valgepea et al.* (2013) that was
 686 inconsistent with a number of other measurements of total RNA-to-protein ratios ($\approx \Phi_R \times 2.1$ *Dai*
 687 *et al.* (2016)) and only show the data from *Schmidt et al.* (2016) and *Li et al.* (2014) for relative
 688 ribosome abundances (see supplemental section XX for a more complete discussion). For the data
 689 shown, the ribosomal fraction doesn't increase as much at higher $\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$. Since several
 690 rRNA operons are actually located approximately half-way between the origin and terminus, the
 691 trend may in part be a consequence of a diminishing increase in rRNA gene dosage at higher $\langle \#$
 692 $\text{ori} \rangle / \langle \# \text{ ter} \rangle$ ratios.

693 We can similarly estimate $\langle \# \text{ ori} \rangle$, which depends on how often replication forks are initiated
 694 per cell cycle. This is given by the number of overlapping cell cycles, $2^{\tau_{\text{cyc}}/\tau}$, where τ_{cyc} , refers to
 695 the total time of chromosome replication and cell division. **Figure 10(E)** shows the associated data
 696 from *Si et al.* (2019), which we use to estimate $\langle \# \text{ ori} \rangle$ for each growth condition of the proteomic
 697 data. In agreement with our expectations, we find that ribosome copy number increases with the
 698 estimated $\langle \# \text{ ori} \rangle$ (**Figure 10(F)**).

699 While it is difficult to distinguish between causality and correlation, the data is consistent with
 700 the need for cells to increase their effective rRNA gene dosage in order to grow according to the
 701 constraint set by Equation 2. These results may also shed some light on the notable increase
 702 in ribosomal content that is observed when sublethal doses of antibiotics (*Scott et al.*, 2010; *Dai*
 703 *et al.*, 2016). Specifically, if rRNA synthesis is rate limiting, and nutrient conditions largely dictate the
 704 extent of overlapping DNA replication cycles, than addition of antibiotic will lengthen the doubling
 705 time and allow an increased rRNA synthesis relative to the rate of cell division. In Supplemental
 706 Section XX, we consider this further using additional data from *Si et al.* (2017).

707 Regulation of translating ribosomes helps maintain maximal growth according to nutrient
 708 availability.

709 While the above observations show how *E. coli* can vary its ribosomal content to increase growth
 710 rate, it also presents a challenge in the limit of poorer nutrient conditions. Recall from Equation 3
 711 that ribosomal content should decrease to zero as growth decreases to zero. While bacteria tend to
 712 decrease their ribosomal abundance in poorer nutrient conditions, they do so only to some fixed,
 713 non-zero amount (*Scott et al.*, 2010; *Liebermeister et al.*, 2014). Here we find a minimal ribosomal
 714 fraction of ≈ 0.06 in the slowest growth conditions. From the perspective of a bacterium dealing
 715 with uncertain nutrient conditions, there is likely a benefit for the cell to maintain some relative
 716 fraction of ribosomes to support rapid growth as nutrient conditions improve.

717 The challenge however, lies in the cell's ability to maintain growth when ribosomes are in ex-
 718 cess of the rate that nutrients can be harvested and amino acids synthesized for consumption
 719 **Figure 11A**. In the limit of poor growth conditions, ribosomes would consume their amino acid
 720 supply and be unable to maintain steady-state growth. In reality, *E. coli* is still able to maintain a

721 relatively high elongation rate even in stationary phase (≈ 8 AA/s, (Dai et al., 2016, 2018)). A explanation
 722 for this is that the cell further regulates its biological activity in conditions of stress and
 723 nutrient-limitation; in particular through the small-molecule alarmones (p)ppGpp (Harris and The-
 724 riot, 2018). In (p)ppGpp null strains, cells are unable to grow in nutrient-poor media. Indeed, these
 725 small molecules play a role in controlling biosynthesis rates throughout the central dogma [NB
 726 citations]. Here we explore this further in the context of growth by maximizing protein synthesis.

727 We consider slow growth conditions (λ less than 0.5 hr^{-1}) by assuming that the decrease in
 728 elongation rate is due to a limiting supply of amino acids and a need for the cell to maintain ex-
 729 cess nutrients for cellular homeostasis under steady-state growth. There is some experimental
 730 support showing that in poorer nutrient growth conditions, cells have lower amino acids concen-
 731 trations (Bennett et al., 2009). We proceed by coarse graining the cell's amino acid supply as an
 732 single, effective rate-limiting species (see Supplemental Section XX for a more complete discussion).
 733 Under such a scenario, the elongation rate can be described as simply depending on the maximum
 734 elongation rate ($\approx 17.1 \text{ aa/s}$, (Dai et al., 2016, 2018)), an effective K_d , and the limiting amino acid
 735 concentration $[AA]_{eff}$. Specifically, the elongation rate is given by,

$$r_t = r_t^{max} \cdot \frac{1}{1 + K_d/[AA]_{eff}}. \quad (4)$$

736 For cells growing in minimal media + glucose, the amino acid concentration is of order 100 mM
 737 (BNID: 110093, (Milo et al., 2010; Bennett et al., 2009)). With a growth rate of about 0.6 hr^{-1} and
 738 elongation rate of 12.5 aa per second (Dai et al., 2016), we can estimate an effective K_d of about 40
 739 mM. Ultimately the steady state amino acid concentration will depend on the difference between
 740 the supply of amino acids r_{aa} and consumption by ribosomes $r_t \cdot R \cdot f_a$, where f_a accounts for the
 741 possible reduction of actively translating ribosomes.

742 In Figure 11B we consider how the maximal growth rate and elongation rates vary as a func-
 743 tion of the number of actively translating ribosomes in this slow growth regime (see Supplemen-
 744 tal Section XX for a complete description of this model). If we consider r_{AA} to be reflective of a
 745 specific growth condition, by considering lines of constant r_{AA} , we find that cells grow fastest by
 746 maximizing their fraction of actively translating ribosomes. When we consider the experimental
 747 measurements from Dai et al. (2018), we see that although cells indeed reduce $R \times f_a$, they do so
 748 in a way that keeps $[AA]_{eff}$ relatively constant. Given our estimate for the K_d of 40 mM, we would
 749 only expect a decrease from 100 mM to about 35 mM in the slowest growth conditions. While
 750 experimental data is limited, amino acid concentrations only decrease to about 60 mM for cells
 751 grown in minimal media + acetate ($\lambda = 0.3 \text{ hr}^{-1}$ in our proteomic data; value obtained from Bennett
 752 et al. (2009)), qualitatively consistent with our expectations.

753 Given the quantitative data from Dai et al. (2018), which determined f_a across the entire range
 754 of growth rates across our data, we next estimated the active fraction of ribosomal protein. As
 755 shown in Figure 11(C), we find that cells grow at a rate near the expected translation maximum
 756 expected from Equation 1, using the maximum elongation rate of $r_t = 17.1 \text{ aa per second}$. This is in
 757 contrast to the reality that ribosomes are translating at almost half this rate in the poorest growth
 758 conditions. This highlights that there are alternative ways to grow according to the translated-
 759 limited growth rate that is expected based with ribosomes translating at their maximal elongation
 760 rate. Specifically, it is by adjusting $r_t \times R \times f_a$ to match maximal growth rate set by Equation 2, through
 761 the parameters $r_{tmax} \times R'$, that cells are able to maximize their growth rate under steady-state.

762 Global regulatory control across central dogma may provide an explanation for the ro-
 763 bust scaling laws in *E. coli*.

764 A number of recent papers further highlight the possibility that (p)ppGpp may even provide a causal
 765 explanation for the scaling laws in *E. coli*. In the context of ribosomal activity, increased levels of
 766 (p)ppGpp are associated with lower ribosomal content, and at slow growth appear to help reduce the
 767 fraction of actively translating ribosomes (Dai et al., 2016, 2018). Titration of the cellular (p)ppGpp

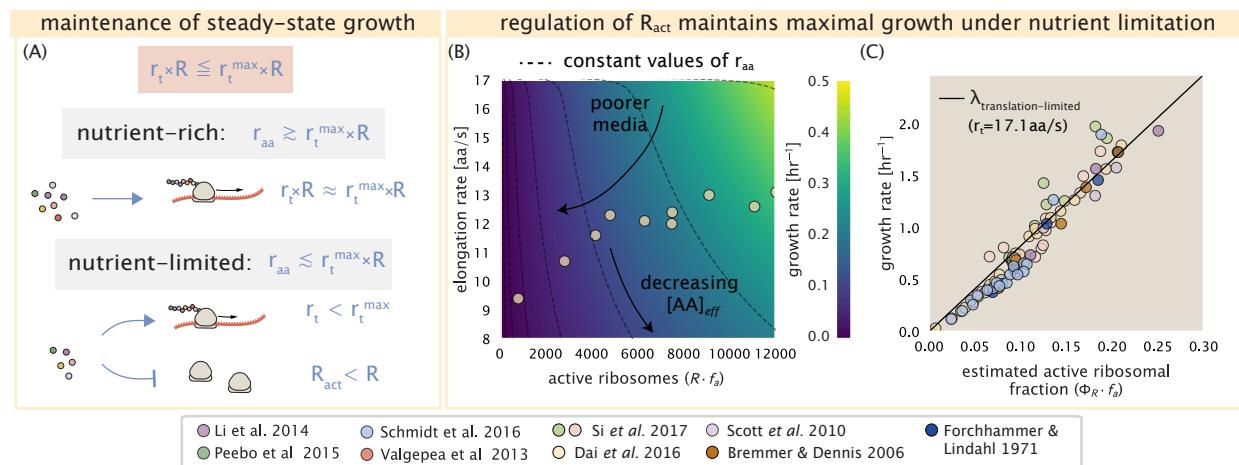


Figure 11. *E. coli* must regulate ribosomal activity in limiting nutrient conditions. (A) Schematic showing translation-specific requirements for maintenance of steady-state growth. In a nutrient rich environment, amino acid supply r_{aa} is sufficiently in excess of the demand by ribosomes translating at their maximal rate. In poorer nutrient conditions, reduced amino acid supply r_{aa} will decrease the rate of elongation. In a regime where r_{aa} is less than $r_t \cdot R$, the number of actively translating ribosomes will need to be reduced in order to maintain steady-state growth. (B) Translation elongation rate is plotted as a function of the number of actively translating ribosomes $R \cdot f_g$. Dashed lines correspond to a range of amino acid synthesis rates r_{aa} , from 10^3 to 10^6 . Growth rates are calculated according to Equation 1, assuming a constant ribosomal fraction of 8 percent. See appendix XX for additional details. (C) Experimental data from Dai *et al.* are used to estimate the fraction of actively translating ribosomes. The solid line represents the translation-limited growth rate for ribosomes elongating at 17.1 AA/s.

768 concentrations (up or down) can invoke similar proteomic changes reminiscent of those observed
 769 under nutrient limitation (Zhu and Dai, 2019). In light of the limiting dependence of ribosome copy
 770 number on chromosomal gene dosage, it was recently shown that growth in a (p)ppGpp null strain
 771 abolishes both the scaling in cell size and the $\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$ ratio. Instead, cells exhibited a high
 772 $\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$ closer to 4 and cell size more consistent with a fast growth state where (p)ppGpp
 773 levels are low (Fernández-Coll *et al.*, 2020).]

774 [NB, expand on to consider how activity of RNAP and other aspects(?) may follow a similar
 775 behaviour and are under related control mechanisms.]

776 References

- 777 Abelson, H., Johnson, L., Penman, S., and Green, H. (1974). Changes in RNA in relation to growth of the fibroblast:
 778 II. The lifetime of mRNA, rRNA, and tRNA in resting and growing cells. *Cell*, 1(4):161–165.
- 779 Aidelberg, G., Towbin, B. D., Rothschild, D., Dekel, E., Bren, A., and Alon, U. (2014). Hierarchy of non-glucose
 780 sugars in *Escherichia coli*. *BMC Systems Biology*, 8(1):133.
- 781 Antonenko, Y. N., Pohl, P., and Denisov, G. A. (1997). Permeation of ammonia across bilayer lipid membranes
 782 studied by ammonium ion selective microelectrodes. *Biophysical Journal*, 72(5):2187–2195.
- 783 Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S.,
 784 Eppig, J. T., Harris, M. A., Hill, D. P., Isbel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E.,
 785 Ringwald, M., Rubin, G. M., and Sherlock, G. (2000). Gene Ontology: tool for the unification of biology. *Nature Genetics*,
 786 25(1):25–29.
- 787 Ason, B., Bertram, J. G., Hingorani, M. M., Beechem, J. M., O'Donnell, M., Goodman, M. F., and Bloom, L. B.
 788 (2000). A Model for *Escherichia coli* DNA Polymerase III Holoenzyme Assembly at Primer/Template Ends:
 789 DNA Triggers A Change In Binding Specificity of the γ Complex Clamp Loader. *Journal of Biological Chemistry*,
 790 275(4):3006–3015.
- 791 Assentoft, M., Kaptan, S., Schneider, H.-P., Deitmer, J. W., de Groot, B. L., and MacAulay, N. (2016). Aquaporin 4
 792 as a NH₃ Channel. *Journal of Biological Chemistry*, 291(36):19184–19195.
- 793 Bauer, S. and Ziv, E. (1976). Dense growth of aerobic bacteria in a bench-scale fermentor. *Biotechnology and
 794 Bioengineering*, 18(1):81–94. _eprint: <https://onlinelibrary.wiley.com/doi/10.1002/bit.260180107>.
- 795 Belliveau, N. M., Barnes, S. L., Ireland, W. T., Jones, D. L., Sweredoski, M. J., Moradian, A., Hess, S., Kinney, J. B.,
 796 and Phillips, R. (2018). Systematic approach for dissecting the molecular mechanisms of transcriptional
 797 regulation in bacteria. *Proceedings of the National Academy of Sciences*, 115(21):E4796–E4805.
- 798 Bennett, B. D., Kimball, E. H., Gao, M., Osterhout, R., Van Dien, S. J., and Rabinowitz, J. D. (2009). Absolute
 799 metabolite concentrations and implied enzyme active site occupancy in *Escherichia coli*. *Nature Chemical
 800 Biology*, 5(8):593–599.
- 801 Birnbaum, L. S. and Kaplan, S. (1971). Localization of a Portion of the Ribosomal RNA Genes in *Escherichia coli*.
 802 *Proceedings of the National Academy of Sciences*, 68(5):925–929.
- 803 Booth, I. R., Mitchell, W. J., and Hamilton, W. A. (1979). Quantitative analysis of proton-linked transport systems.
 804 The lactose permease of *Escherichia coli*. *Biochemical Journal*, 182(3):687–696.
- 805 Bremer, H. and Dennis, P. P. (2008). Modulation of Chemical Composition and Other Parameters of the Cell at
 806 Different Exponential Growth Rates. *EcoSal Plus*, 3(1).
- 807 Browning, D. F. and Busby, S. J. W. (2016). Local and global regulation of transcription initiation in bacteria.
 808 *Nature Reviews Microbiology*, 14(10):638–650.
- 809 Cox, G. B., Newton, N. A., Gibson, F., Snoswell, A. M., and Hamilton, J. A. (1970). The function of ubiquinone in
 810 *Escherichia coli*. *Biochemical Journal*, 117(3):551–562.
- 811 Dai, X., Zhu, M., Warren, M., Balakrishnan, R., Okano, H., Williamson, J. R., Fredrick, K., and Hwa, T. (2018).
 812 Slowdown of Translational Elongation in *Escherichia coli* under Hyperosmotic Stress. - PubMed - NCBI. *mBio*,
 813 9(1):281.
- 814 Dai, X., Zhu, M., Warren, M., Balakrishnan, R., Patsalo, V., Okano, H., Williamson, J. R., Fredrick, K., Wang, Y.-P.,
 815 and Hwa, T. (2016). Reduction of translating ribosomes enables *Escherichia coli* to maintain elongation rates
 816 during slow growth. *Nature Microbiology*, 2(2):16231.
- 817 Escalante, A., Salinas Cervantes, A., Gosset, G., and Bolívar, F. (2012). Current knowledge of the *Escherichia coli*
 818 phosphoenolpyruvate-carbohydrate phosphotransferase system: Peculiarities of regulation and impact on
 819 growth and product formation. *Applied Microbiology and Biotechnology*, 94(6):1483–1494.
- 820 Feist, A. M., Henry, C. S., Reed, J. L., Krummenacker, M., Joyce, A. R., Karp, P. D., Broadbelt, L. J., Hatzimanikatis,
 821 V., and Palsson, B. Ø. (2007). A genome-scale metabolic reconstruction for *Escherichia coli* K-12 MG1655 that
 822 accounts for 1260 ORFs and thermodynamic information. *Molecular Systems Biology*, 3(1):121.

- 823 Fernández-Coll, L., Maciąg-Dorszynska, M., Tailor, K., Vadia, S., Levin, P. A., Szalewska-Palasz, A., Cashel, M.,
824 and Dunny, G. M. (2020). The Absence of (p)ppGpp Renders Initiation of *Escherichia coli* Chromosomal DNA
825 Synthesis Independent of Growth Rates. *mBio*, 11(2):45.
- 826 Fijalkowska, I. J., Schaaper, R. M., and Jonczyk, P. (2012). DNA replication fidelity in *Escherichia coli*: A multi-DNA
827 polymerase affair. *FEMS Microbiology Reviews*, 36(6):1105–1121.
- 828 Finkelstein, I. J. and Greene, E. C. (2013). Molecular Traffic Jams on DNA. *Annual Review of Biophysics*, 42(1):241–
829 263.
- 830 Gama-Castro, S., Salgado, H., Santos-Zavaleta, A., Ledezma-Tejeida, D., Muñiz-Rascado, L., García-Sotelo, J. S.,
831 Alquicira-Hernández, K., Martínez-Flores, I., Pannier, L., Castro-Mondragón, J. A., Medina-Rivera, A., Solano-
832 Lira, H., Bonavides-Martínez, C., Pérez-Rueda, E., Alquicira-Hernández, S., Porrón-Sotelo, L., López-Fuentes,
833 A., Hernández-Koutoucheva, A., Moral-Chávez, V. D., Rinaldi, F., and Collado-Vides, J. (2016). RegulonDB
834 version 9.0: High-level integration of gene regulation, coexpression, motif clustering and beyond. *Nucleic
835 Acids Research*, 44(D1):D133–D143.
- 836 Ge, J., Yu, G., Ator, M. A., and Stubbe, J. (2003). Pre-Steady-State and Steady-State Kinetic Analysis of *E. coli* Class
837 I Ribonucleotide Reductase. *Biochemistry*, 42(34):10071–10083.
- 838 Godin, M., Delgado, F. F., Son, S., Grover, W. H., Bryan, A. K., Tzur, A., Jorgensen, P., Payer, K., Grossman, A. D.,
839 Kirschner, M. W., and Manalis, S. R. (2010). Using buoyant mass to measure the growth of single cells. *Nature
840 Methods*, 7(5):387–390.
- 841 Goldman, S. R., Nair, N. U., Wells, C. D., Nickels, B. E., and Hochschild, A. (2015). The primary σ factor in *Es-
842 cherichia coli* can access the transcription elongation complex from solution *in vivo*. *eLife*, 4:e10514.
- 843 Harris, L. K. and Theriot, J. A. (2018). Surface Area to Volume Ratio: A Natural Variable for Bacterial Morphogen-
844 esis. *Trends in microbiology*, 26(10):815–832.
- 845 Harris, R. M., Webb, D. C., Howitt, S. M., and Cox, G. B. (2001). Characterization of PitA and PitB from *Escherichia
846 coli*. *Journal of Bacteriology*, 183(17):5008–5014.
- 847 Heldal, M., Norland, S., and Tumyr, O. (1985). X-ray microanalytic method for measurement of dry matter and
848 elemental content of individual bacteria. *Applied and Environmental Microbiology*, 50(5):1251–1257.
- 849 Henkel, S. G., Beek, A. T., Steinsiek, S., Stagge, S., Bettenbrock, K., de Mattos, M. J. T., Sauter, T., Sawodny, O.,
850 and Ederer, M. (2014). Basic Regulatory Principles of *Escherichia coli*'s Electron Transport Chain for Varying
851 Oxygen Conditions. *PLoS ONE*, 9(9):e107640.
- 852 Hui, S., Silverman, J. M., Chen, S. S., Erickson, D. W., Basan, M., Wang, J., Hwa, T., and Williamson, J. R. (2015).
853 Quantitative proteomic analysis reveals a simple strategy of global resource allocation in bacteria. *Molecular
854 Systems Biology*, 11(2):e784–e784.
- 855 Ingledew, W. J. and Poole, R. K. (1984). The respiratory chains of *Escherichia coli*. *Microbiological Reviews*,
856 48(3):222–271.
- 857 Ireland, W. T., Beeler, S. M., Flores-Bautista, E., Belliveau, N. M., Sweredoski, M. J., Moradian, A., Kinney, J. B.,
858 and Phillips, R. (2020). Deciphering the regulatory genome of *Escherichia coli*, one hundred promoters at a
859 time. *bioRxiv*.
- 860 Jacob, F. and Monod, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular
861 Biology*, 3(3):318–356.
- 862 Jensen, R. B., Wang, S. C., and Shapiro, L. (2001). A moving DNA replication factory in *Caulobacter crescentus*.
863 *The EMBO journal*, 20(17):4952–4963.
- 864 Jun, S., Si, F., Pugatch, R., and Scott, M. (2018). Fundamental principles in bacterial physiology - history, recent
865 progress, and the future with focus on cell size control: A review. *Reports on Progress in Physics*, 81(5):056601.
- 866 Kapanidis, A. N., Margeat, E., Laurence, T. A., Doose, S., Ho, S. O., Mukhopadhyay, J., Kortkhonjia, E., Mekler, V.,
867 Ebright, R. H., and Weiss, S. (2005). Retention of Transcription Initiation Factor Σ 70 in Transcription Elonga-
868 tion: Single-Molecule Analysis. *Molecular Cell*, 20(3):347–356.
- 869 Khademi, S., O'Connell, J., Remis, J., Robles-Colmenares, Y., Miercke, L.J.W., and Stroud, R. M. (2004). Mechanism
870 of Ammonia Transport by Amt/MEP/Rh: Structure of AmtB at 1.35 Å. *Science*, 305(5690):1587–1594.

- 871 Khademian, M. and Imlay, J. A. (2017). *Escherichia coli* cytochrome c peroxidase is a respiratory oxidase that
872 enables the use of hydrogen peroxide as a terminal electron acceptor. *Proceedings of the National Academy
873 of Sciences*, 114(33):E6922–E6931.
- 874 Li, G.-W., Burkhardt, D., Gross, C., and Weissman, J. S. (2014). Quantifying absolute protein synthesis rates
875 reveals principles underlying allocation of cellular resources. *Cell*, 157(3):624–635.
- 876 Liebermeister, W., Noor, E., Flamholz, A., Davidi, D., Bernhardt, J., and Milo, R. (2014). Visual account of protein
877 investment in cellular functions. *Proceedings of the National Academy of Sciences*, 111(23):8488–8493.
- 878 Liu, M., Durfee, T., Cabrera, J. E., Zhao, K., Jin, D. J., and Blattner, F. R. (2005). Global Transcriptional Programs
879 Reveal a Carbon Source Foraging Strategy by *Escherichia coli*. *Journal of Biological Chemistry*, 280(16):15921–
880 15927.
- 881 Maaløe, O. (1979). *Regulation of the Protein-Synthesizing Machinery—Ribosomes, tRNA, Factors, and So On*. Gene
882 Expression. Springer.
- 883 Milo, R., Jorgensen, P., Moran, U., Weber, G., and Springer, M. (2010). BioNumbers—the database of key num-
884 bers in molecular and cell biology. *Nucleic Acids Research*, 38(suppl_1):D750–D753.
- 885 Monod, J. (1947). The phenomenon of enzymatic adaptation and its bearings on problems of genetics and
886 cellular differentiation. *Growth Symposium*, 9:223–289.
- 887 Mooney, R. A., Darst, S. A., and Landick, R. (2005). Sigma and RNA Polymerase: An On-Again, Off-Again Rela-
888 tionship? *Molecular Cell*, 20(3):335–345.
- 889 Mooney, R. A. and Landick, R. (2003). Tethering Σ 70 to RNA polymerase reveals high *in vivo* activity of σ factors
890 and Σ 70-dependent pausing at promoter-distal locations. *Genes & Development*, 17(22):2839–2851.
- 891 Neidhardt, F. C., Ingraham, J., and Schaechter, M. (1991). *Physiology of the Bacterial Cell - A Molecular Approach*,
892 volume 1. Elsevier.
- 893 Ojkic, N., Serbanescu, D., and Banerjee, S. (2019). Surface-to-volume scaling and aspect ratio preservation in
894 rod-shaped bacteria. *eLife*, 8:642.
- 895 Patrick, M., Dennis, P. P., Ehrenberg, M., and Bremer, H. (2015). Free RNA polymerase in *Escherichia coli*.
896 *Biochimie*, 119:80–91.
- 897 Peebo, K., Valgepea, K., Maser, A., Nahku, R., Adamberg, K., and Vilu, R. (2015). Proteome reallocation in *Es-*
898 *cherichia coli* with increasing specific growth rate. *Molecular BioSystems*, 11(4):1184–1193.
- 899 Perdue, S. A. and Roberts, J. W. (2011). σ^{70} -dependent Transcription Pausing in *Escherichia coli*. *Journal of
900 Molecular Biology*, 412(5):782–792.
- 901 Phillips, R. (2018). Membranes by the Numbers. In *Physics of Biological Membranes*, pages 73–105. Springer,
902 Cham, Cham.
- 903 Ramos, S. and Kaback, H. R. (1977). The relation between the electrochemical proton gradient and active trans-
904 port in *Escherichia coli* membrane vesicles. *Biochemistry*, 16(5):854–859.
- 905 Rosenberg, H., Gerdes, R. G., and Chegwidden, K. (1977). Two systems for the uptake of phosphate in *Escherichia
906 coli*. *Journal of Bacteriology*, 131(2):505–511.
- 907 Rudd, S. G., Valerie, N. C. K., and Helleday, T. (2016). Pathways controlling dNTP pools to maintain genome
908 stability. *DNA Repair*, 44:193–204.
- 909 Sánchez-Romero, M. A., Molina, F., and Jiménez-Sánchez, A. (2011). Organization of ribonucleoside diphosphate
910 reductase during multifork chromosome replication in *Escherichia coli*. *Microbiology*, 157(8):2220–2225.
- 911 Schaechter, M., Maaløe, O., and Kjeldgaard, N. O. (1958). Dependency on Medium and Temperature of Cell Size
912 and Chemical Composition during Balanced Growth of *Salmonella typhimurium*. *Microbiology*, 19(3):592–606.
- 913 Schmidt, A., Kochanowski, K., Vedelaar, S., Ahrné, E., Volkmer, B., Callipo, L., Knoops, K., Bauer, M., Aebersold,
914 R., and Heinemann, M. (2016). The quantitative and condition-dependent *Escherichia coli* proteome. *Nature
915 Biotechnology*, 34(1):104–110.

- 916 Scholz, S. A., Diao, R., Wolfe, M. B., Fivenson, E. M., Lin, X. N., and Freddolino, P. L. (2019). High-Resolution
 917 Mapping of the *Escherichia coli* Chromosome Reveals Positions of High and Low Transcription. *Cell Systems*,
 918 8(3):212–225.e9.
- 919 Scott, M., Gunderson, C. W., Mateescu, E. M., Zhang, Z., and Hwa, T. (2010). Interdependence of cell growth and
 920 gene expression: origins and consequences. *Science*, 330(6007):1099–1102.
- 921 Sekowska, A., Kung, H.-F., and Danchin, A. (2000). Sulfur Metabolism in *Escherichia coli* and Related Bacteria:
 922 Facts and Fiction. *Journal of Molecular Microbiology and Biotechnology*, 2(2):34.
- 923 Si, F., Le Treut, G., Sauls, J. T., Vadia, S., Levin, P. A., and Jun, S. (2019). Mechanistic Origin of Cell-Size Control
 924 and Homeostasis in Bacteria. *Current Biology*, 29(11):1760–1770.e7.
- 925 Si, F., Li, D., Cox, S. E., Sauls, J. T., Azizi, O., Sou, C., Schwartz, A. B., Erickstad, M. J., Jun, Y., Li, X., and Jun, S. (2017).
 926 Invariance of Initiation Mass and Predictability of Cell Size in *Escherichia coli*. *Current Biology*, 27(9):1278–1287.
- 927 Sirko, A., Zatyka, M., Sadowy, E., and Hulanicka, D. (1995). Sulfate and thiosulfate transport in *Escherichia coli* K-
 928 12: Evidence for a functional overlapping of sulfate- and thiosulfate-binding proteins. *Journal of Bacteriology*,
 929 177(14):4134–4136.
- 930 Stasi, R., Neves, H. I., and Spira, B. (2019). Phosphate uptake by the phosphonate transport system PhnCDE.
 931 *BMC Microbiology*, 19.
- 932 Stevenson, B. S. and Schmidt, T. M. (2004). Life History Implications of rRNA Gene Copy Number in *Escherichia*
 933 *coli*. *Applied and Environmental Microbiology*, 70(11):6670–6677.
- 934 Stouthamer, A. H. and Bettenhaussen, C. W. (1977). A continuous culture study of an ATPase-negative mutant
 935 of *Escherichia coli*. *Archives of Microbiology*, 113(3):185–189.
- 936 Svenningsen, S. L., Kongstad, M., Stenum, T. S. n., Muñoz-Gómez, A. J., and Sørensen, M. A. (2017). Transfer RNA
 937 is highly unstable during early amino acid starvation in *Escherichia coli*. *Nucleic Acids Research*, 45(2):793–804.
- 938 Szenk, M., Dill, K. A., and de Graff, A. M. R. (2017). Why Do Fast-Growing Bacteria Enter Overflow Metabolism?
 939 Testing the Membrane Real Estate Hypothesis. *Cell Systems*, 5(2):95–104.
- 940 Taheri-Araghi, S., Bradde, S., Sauls, J. T., Hill, N. S., Levin, P. A., Paulsson, J., Vergassola, M., and Jun, S. (2015).
 941 Cell-size control and homeostasis in bacteria. - PubMed - NCBI. *Current Biology*, 25(3):385–391.
- 942 Tatusov, R. L., Galperin, M. Y., Natale, D. A., and Koonin, E. V. (2000). The COG database: a tool for genome-scale
 943 analysis of protein functions and evolution. *Nucleic Acids Research*, 28(1):33–36.
- 944 Taymaz-Nikerel, H., Borujeni, A. E., Verheijen, P. J. T., Heijnen, J. J., and van Gulik, W. M. (2010).
 945 Genome-derived minimal metabolic models for *Escherichia coli* mg1655 with estimated in vivo
 946 respiratory ATP stoichiometry. *Biotechnology and Bioengineering*, 107(2):369–381. _eprint:
 947 <https://onlinelibrary.wiley.com/doi/pdf/10.1002/bit.22802>.
- 948 The Gene Ontology Consortium (2018). The Gene Ontology Resource: 20 years and still GOing strong. *Nucleic
 949 Acids Research*, 47(D1):D330–D338.
- 950 Valgepea, K., Adamberg, K., Seiman, A., and Vilu, R. (2013). *Escherichia coli* achieves faster growth by increasing
 951 catalytic and translation rates of proteins. *Molecular BioSystems*, 9(9):2344.
- 952 van Heeswijk, W. C., Westerhoff, H. V., and Boogerd, F. C. (2013). Nitrogen Assimilation in *Escherichia coli*: Putting
 953 Molecular Data into a Systems Perspective. *Microbiology and Molecular Biology Reviews*, 77(4):628–695.
- 954 Weber, J. and Senior, A. E. (2003). ATP synthesis driven by proton transport in F1FO-ATP synthase. *FEBS Letters*,
 955 545(1):61–70.
- 956 Willsky, G. R., Bennett, R. L., and Malamy, M. H. (1973). Inorganic Phosphate Transport in *Escherichia coli*: Involvement
 957 of Two Genes Which Play a Role in Alkaline Phosphatase Regulation. *Journal of Bacteriology*, 113(2):529–
 958 539.
- 959 Zhang, L., Jiang, W., Nan, J., Almqvist, J., and Huang, Y. (2014a). The *Escherichia coli* CysZ is a pH dependent sulfate
 960 transporter that can be inhibited by sulfite. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1838(7):1809–
 961 1816.
- 962 Zhang, Z., Aboulwafa, M., and Saier, M. H. (2014b). Regulation of crp gene expression by the catabolite repres-
 963 sor/activator, cra, in *Escherichia coli*. *Journal of Molecular Microbiology and Biotechnology*, 24(3):135–141.
- 964 Zhu, M. and Dai, X. (2019). Growth suppression by altered (p)ppGpp levels results from non-optimal resource
 965 allocation in *Escherichia coli*. *Nucleic Acids Research*, 47(9):4684–4693.