

# Fundamental limits on the rate of bacterial cell division

<sup>3</sup> **Nathan M. Belliveau<sup>†, 1</sup>, Griffin Chure<sup>†, 2, 3</sup>, Christina L. Hueschen<sup>4</sup>, Hernan G.**  
<sup>4</sup> **Garcia<sup>5</sup>, Jané Kondev<sup>6</sup>, Daniel S. Fisher<sup>7</sup>, Julie Theriot<sup>1, 8</sup>, Rob Phillips<sup>2, 9, \*</sup>**

\*For correspondence:

<sup>†</sup>These authors contributed equally to this work

<sup>5</sup> <sup>1</sup>Department of Biology, University of Washington, Seattle, WA, USA; <sup>2</sup>Division of  
<sup>6</sup> Biology and Biological Engineering, California Institute of Technology, Pasadena, CA,  
<sup>7</sup> USA; <sup>3</sup>Department of Applied Physics, California Institute of Technology, Pasadena, CA,  
<sup>8</sup> USA; <sup>4</sup>Department of Chemical Engineering, Stanford University, Stanford, CA, USA;  
<sup>9</sup> <sup>5</sup>Department of Molecular Cell Biology and Department of Physics, University of  
<sup>10</sup> California Berkeley, Berkeley, CA, USA; <sup>6</sup>Department of Physics, Brandeis University,  
<sup>11</sup> Waltham, MA, USA; <sup>7</sup>Department of Applied Physics, Stanford University, Stanford, CA,  
<sup>12</sup> USA; <sup>8</sup>Allen Institute for Cell Science, Seattle, WA, USA; <sup>9</sup>Department of Physics,  
<sup>13</sup> California Institute of Technology, Pasadena, CA, USA; \*Contributed equally

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<sup>15</sup> **Abstract** This will be written next

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## <sup>17</sup> Uptake of Nutrients

<sup>18</sup> In order to build new cellular mass, the molecular and elemental building blocks must be scav-  
<sup>19</sup> enged from the environment in different forms. Carbon, for example, is acquired via the transport  
<sup>20</sup> of carbohydrates and sugar alcohols with some carbon sources receiving preferential treatment  
<sup>21</sup> in their consumption (?). Phosphorus, sulfur, and nitrogen, on the other hand, are harvested pri-  
<sup>22</sup> marily in the forms of inorganic salts, namely phosphate, sulfate, and ammonia (??????). All of  
<sup>23</sup> these compounds have different permeabilities across the cell membrane and most require some  
<sup>24</sup> energetic investment either via ATP hydrolysis or through the proton electrochemical gradient to  
<sup>25</sup> bring the material across the hydrophobic cell membrane. Given the diversity of biological trans-  
<sup>26</sup> port mechanisms and the vast number of inputs needed to build a cell, we begin by considering  
<sup>27</sup> transport of some of the most important cellular ingredients: carbon, nitrogen, oxygen, hydrogen,  
<sup>28</sup> phosphorus, and sulfur.

<sup>29</sup> The elemental composition of *E. coli* has received much quantitative attention over the past  
<sup>30</sup> half century (????), providing us with a starting point for estimating the copy numbers of various  
<sup>31</sup> transporters. While there is some variability in the exact elemental percentages (with different un-  
<sup>32</sup> certainties), we can estimate that the dry mass of a typical *E. coli* cell is  $\approx$  45% carbon (BNID: 100649,  
<sup>33</sup> ?),  $\approx$  15% nitrogen (BNID: 106666, ?),  $\approx$  3% phosphorus (BNID: 100653, ?), and 1% sulfur (BNID:  
<sup>34</sup> 100655, ?). In the coming paragraphs, we will engage in a dialogue between back-of-the-envelope  
<sup>35</sup> estimates for the numbers of transporters needed to facilitate these chemical stoichiometries and  
<sup>36</sup> the experimental proteomic measurements of the biological reality. Such an approach provides  
<sup>37</sup> the opportunity to test if our biological knowledge is sufficient to understand the scale at which  
<sup>38</sup> these complexes are produced. Specifically, we will make these estimates considering a modest  
<sup>39</sup> doubling time of 5000 s, a growth rate of  $\approx$  0.5 hr<sup>-1</sup>, the range in which the majority of the experi-  
<sup>40</sup> mental measurements reside.

**41    Nitrogen Transport**

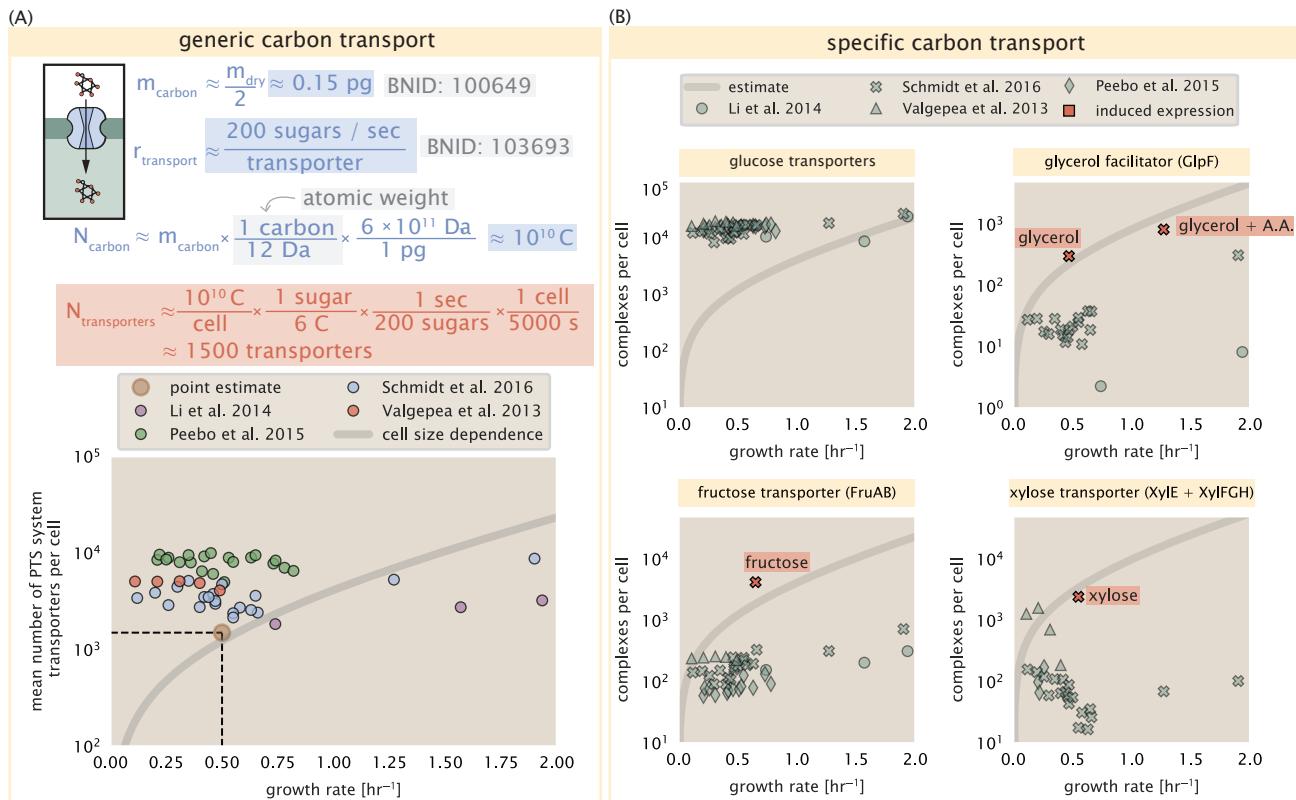
42    Before we begin our back-of-the-envelope estimations, we must address which elemental sources  
 43    must require proteinaceous transport, meaning that the cell cannot acquire appreciable amounts  
 44    simply via diffusion from the membrane. The permeability of the lipid membrane to a large num-  
 45    ber of solutes has been extensively characterized over the past century. Large, polar molecular  
 46    species (such as various sugar molecules, sulfate, and phosphate) have low permeabilities while  
 47    small, non-polar compounds (such as oxygen, carbon dioxide, and ammonia) can readily diffuse  
 48    across the membrane. Ammonia, a primary source of nitrogen in typical laboratory conditions, has  
 49    a permeability on par with water ( $\approx 10^5$  nm/s, BNID:110824 ?). In particularly nitrogen-poor condi-  
 50    tions, *E. coli* expresses a transporter (AmtB) which appears to aid in nitrogen assimilation, though  
 51    the mechanism and kinetic details of transport is still a matter of debate (??). Beyond ammonia,  
 52    another plentiful source of nitrogen come in the form of glutamate, which has its own complex  
 53    metabolism and scavenging pathways. However, nitrogen is plentiful in the growth conditions ex-  
 54    amined in this work, permitting us to neglect nitrogen transport as a potential rate limiting process  
 55    in cell division in typical experimental conditions. We direct the reader to the supplemental infor-  
 56    mation for a more in-depth discussion of permeabilities and a series of calculations revealing that  
 57    active nitrogen transport can be neglected for the purposes of this article.

**58    Carbon Transport**

59    We begin our estimations with the most abundant element in *E. coli* by mass, carbon. Using  $\approx 0.3$   
 60    pg as the typical *E. coli* dry mass (BNID: 103904, ?), we estimate that  $\approx 10^{10}$  carbon atoms must be  
 61    brought into the cell in order to double all of the carbon-containing molecules (??(A, top))). Typi-  
 62    cal laboratory growth conditions, such as those explored in the aforementioned proteomic data  
 63    sets, provide carbon as a single class of sugar such as glucose, galactose, or xylose to name a few.  
 64    *E. coli* has evolved myriad mechanisms by which these sugars can be transported across the cell  
 65    membrane. One such mechanism of transport is via the PTS system which is a highly modular  
 66    system capable of transporting a diverse range of sugars (?). The glucose-specific component of  
 67    this system transports  $\approx 200$  glucose molecules per second per transporter (BNID: 114686, ?). Mak-  
 68    ing the assumption that this is a typical sugar transport rate, coupled with the need to transport  
 69     $10^{10}$  carbon atoms, we arrive at the conclusion that on the order of 1,000 transporters must be ex-  
 70    pressed in order to bring in enough carbon atoms to divide in 5000 s, diagrammed in the top panel  
 71    of ??(A). This estimate, along with the observed average number of the PTS system carbohydrate  
 72    transporters present in the proteomic data sets (????), is shown in ??(A). While we estimate 1500  
 73    transporters are needed with a 5000 s division time, we can abstract this calculation to consider  
 74    any particular growth rate given knowledge of the cell density and volume as a function of growth  
 75    rate and direct the reader to the SI for more information. As revealed in ??(A), experimental mea-  
 76    surements exceed the estimate by several fold, illustrating that transport of carbon in to the cell is  
 77    not rate limiting for cell division.

78    The estimate presented in ??(A) neglects any specifics of the regulation of carbon transport  
 79    system and presents a data-averaged view of how many carbohydrate transporters are present  
 80    on average. Using the diverse array of growth conditions explored in the proteomic data sets, we  
 81    can explore how individual carbon transport systems depend on the population growth rate. In  
 82    ??(B), we show the total number of carbohydrate transporters specific to different carbon sources.  
 83    A striking observation, shown in the top-left plot of ??(B), is the constancy in the expression of  
 84    the glucose-specific transport systems. Additionally, we note that the total number of glucose-  
 85    specific transporters is tightly distributed  $\approx 10^4$  per cell, the approximate number of transporters  
 86    needed to sustain rapid growth of several divisions per hour, as indicated by the grey shaded line.  
 87    This illustrates that *E. coli* maintains a substantial number of complexes present for transporting  
 88    glucose which is known to be the preferential carbon source (??).

89    It is now understood that a large number of metabolic operons are regulated with dual-input  
 90    logic gates that are only expressed when glucose concentrations are low (mediated by cyclic-AMP



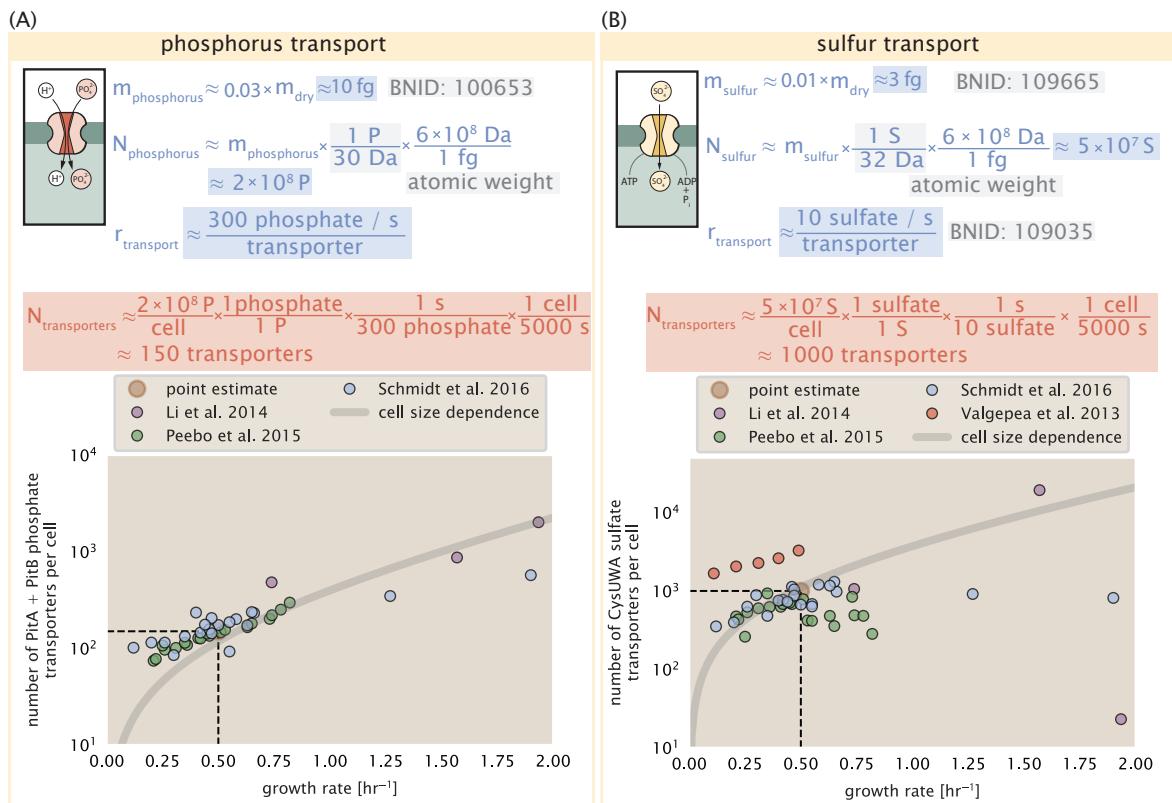
**Figure 1. The abundance of carbon transport systems across growth rates.** (A) A simple estimate for the minimum number of generic carbohydrate transport systems (top) assumes  $\approx 10^{10}$  C are needed to complete division, each transported sugar contains  $\approx 6$  C, and each transporter conducts sugar molecules at a rate of  $\approx 200$  per second. Bottom plot shows the estimated number of transporters needed at a growth rate of  $\approx 0.5$  per hr (light-brown point and dashed lines). Colored points correspond to the mean number of 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.

receptor protein CRP) and the concentration of other carbon sources are elevated (??). A famed example of such dual-input regulatory logic is in the regulation of the *lac* operon which is only natively activated in the absence of glucose and the presence of allolactose, an intermediate in lactose metabolism (?), though we now know of many other such examples (??). This illustrates that once glucose is depleted from the environment, cells have a means to dramatically increase the abundance of the specific transporter needed to digest the next sugar that is present. Several examples of induced expression of specific carbon-source transporters are shown in ??(B). Points colored in red (labeled by red text-boxes) correspond to growth conditions in which the specific carbon source (glycerol, xylose, or fructose) is present. These plots show that, in the absence of the particular carbon source, expression of the transporters is maintained on the order of  $\sim 10^2$  per cell. However, when induced, the transporters become highly-expressed and fall close to the predicted number of transporters needed to facilitate growth on that substrate alone, shown as a transparent grey line. Together, this generic estimation and the specific examples of induced expression suggest that transport of carbon across the cell membrane, while critical for growth, is not the rate-limiting step of cell division.

## 106 Phosphorus and Sulfur Transport

107 We now turn our attention towards other essential elements, namely phosphorus and sulfur. Phosphorus is critical to the cellular energy economy in the form of high-energy phosphodiester bonds 108 making up DNA, RNA, and the NTP energy pool as well as playing a critical role in the post-translational 109 modification of proteins and defining the polar-heads of lipids. In total, phosphorus makes up 110  $\approx 3\%$  of the cellular dry mass which in typical experimental conditions is in the form of inorganic 111 phosphate. The cell membrane has remarkably low permeability to this highly-charged and critical 112 molecule, therefore requiring the expression of active transport systems. In *E. coli*, the proton elec- 113 trochemical gradient across the inner membrane is leveraged to transport inorganic phosphate 114 into the cell (?). Proton-solute symporters are widespread in *E. coli* (??) and can have rapid trans- 115 port rates of 50 to 100 molecules per second for sugars and other solutes (BNID: 103159; 111777, ?). 116 As a more extreme example, the proton transporters in the F<sub>1</sub>-F<sub>0</sub> ATP synthase, which leverage the 117 proton electrochemical gradient for rotational motion, can shuttle protons across the membrane 118 at a rate of  $\approx 1000$  per second (BNID: 104890; 103390, (?)). In *E. coli* the PitA phosphate transport 119 system has been shown to be very tightly coupled with the proton electrochemical gradient with a 120 1:1 proton:phosphate stoichiometric ratio (??). Taking the geometric mean of the aforementioned 121 estimates gives a plausible rate of phosphate transport on the order of 300 per second. Illustrated 122 in ??(A), we can estimate that  $\approx 150$  phosphate transporters are necessary to maintain an  $\approx 3\%$  123 dry mass with a 5000 s division time. This estimate is again satisfied when we examine the ob- 124 served copy numbers of PitA in proteomic data sets (plot in ??(A)). While our estimate is very much 125 in line with the observed numbers, we emphasize that this is likely a slight overestimate of the 126 number of transporters needed as there are other phosphorous scavenging systems, such as the 127 ATP-dependent phosphate transporter Pst system which we have neglected.

128 Satisfied that there are a sufficient number of phosphate transporters present in the cell, we 129 now turn to sulfur transport as another potentially rate limiting process. Similar to phosphate, sul- 130 fide is highly-charged and not particularly membrane permeable, requiring active transport. While 131 there exists a H<sup>+</sup>/sulfate symporter in *E. coli*, it is in relatively low abundance and is not well char- 132 acterized (?). Sulfate is predominantly acquired via the ATP-dependent ABC transporter CysUWA 133 system which also plays an important role in selenium transport (??). While specific kinetic details 134 of this transport system are not readily available, generic ATP transport systems in prokaryotes 135 transport on the order of 1 to 10 molecules per second (BNID: 109035, ?). Combining this generic 136 transport rate, measurement of sulfur comprising 1% of dry mass, and a 5000 second division 137 time yields an estimate of  $\approx 1000$  CysUWA complexes per cell (??(B)). Once again, this estimate is 138 in notable agreement with proteomic data sets, suggesting that there are sufficient transporters 139 present to acquire the necessary sulfur. In a similar spirit of our estimate of phosphorus transport,



**Figure 2. 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]<sub>2</sub>[CysU][CysW][Sbp/CysP]. Grey line in (A) and (B) represents the estimated number of transporters per cell at a continuum of growth rates.

141 we emphasize that this is likely an overestimate of the number of necessary transporters as we  
 142 have neglected other sulfur scavenging systems that are in lower abundance.

### 143 **Limits on Transporter Expression**

144 So which, if any, of these processes may be rate limiting for growth? As suggested by ?? (B), induced  
 145 expression can lead to an order-of-magnitude (or more) increase in the amount of transporters  
 146 needed to facilitate transport. Thus, if acquisition of nutrients was the limiting state in cell division,  
 147 could expression simply be increased to accommodate faster growth? A way to approach this  
 148 question is to compute the amount of space in the bacterial membrane that could be occupied  
 149 by nutrient transporters. Considering a rule-of-thumb for the surface area of *E. coli* of about 6  
 150  $\mu\text{m}^2$  (BNID: 101792, ?), we expect an areal density for 1000 transporters to be approximately 200  
 151 transporters/ $\mu\text{m}^2$ . For a typical transporter occupying about 50 nm<sup>2</sup>/dimer, this amounts to about  
 152 only 1 percent of the total inner membrane (?). In addition, bacterial cell membranes typically have  
 153 densities of 10<sup>5</sup> proteins/ $\mu\text{m}^2$  (?), implying that the cell could accommodate more transporters of  
 154 a variety of species if it were rate limiting. As we will see in the next section, however, occupancy  
 155 of the membrane can impose other limits on the rate of energy production.

### 156 **Protein synthesis**

157 Lastly, we turn our attention to the process of translation. So far our estimates have led to protein  
 158 copy numbers that are consistent with the proteomic data, or even in excess of what might be

needed for each task under limiting growth conditions. Even in our example of *E. coli* grown under different carbohydrate sources (??(B)), cells can utilize alternative carbon sources by inducing the expression of additional membrane transporters and enzymes. 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 (??). 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, suggesting 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 many 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 by multiple genes on the chromosome, tufA and tufB.

We can begin to gain some intuition into how translation might limit growth by noting that the total number of peptide bonds generated as the cell doubles,  $N_{aa}$ , will be given by,  $\tau \cdot r_t \cdot R/\ln(2)$ . Here,  $\tau$  refers to the doubling time of the cell under steady-state growth and the factor  $\ln(2)$  is due to exponential growth.  $r_t$  is the maximum translation elongation rate, and  $R$  is the average number of ribosomes per cell. With the growth rate related to the cell doubling time by  $\lambda = \ln(2)/\tau$ , we can write the translation-limited growth rate as,

$$\lambda_{\text{translation-limited}} = \frac{r_t \cdot R}{N_{aa}}. \quad (1)$$

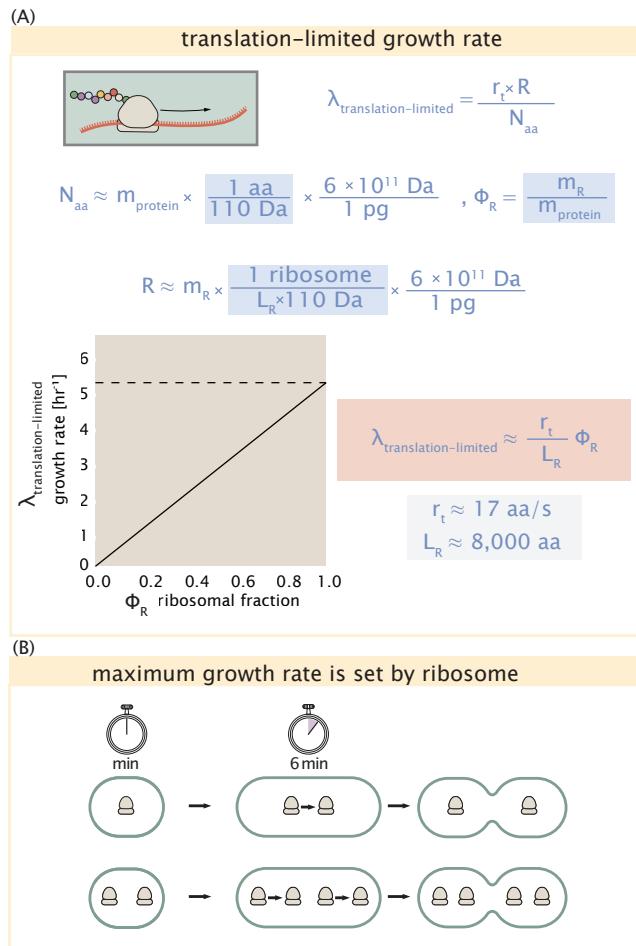
Alternatively, since  $N_{aa}$  is related to the total protein mass through the molecular weight of each protein, we can also consider the growth rate in terms of ribosomal mass fraction. By making the approximation that an average amino acid has a molecular weight of 110 Da (see ??(A)), we can rewrite the growth rate as,

$$\lambda_{\text{translation-limited}} \approx \frac{r_t}{L_R} \Phi_R, \quad (2)$$

where  $L_R$  is the total length in amino acids that make up a ribosome, and  $\Phi_R$  is the ribosomal mass fraction. This is plotted as a function of ribosomal fraction  $\Phi_R$  in ??(A), where we take  $L_R \approx 7459$  aa, corresponding to the length in amino acids for all ribosomal subunits of the 50S and 30S complex (BNID: 101175, (?)). This formulation assumes that the cell can transcribe the required amount of rRNA, which appears reasonable for *E. coli* under the allowing us to consider the inherent limit on growth set by the ribosome.

The growth rate defined by Equation ?? reflects mass-balance under steady-state growth and has long provided a rationalization to the apparent linear increase in *E. coli*'s ribosomal content as a function of growth rate (??). For our purposes, there are several important consequences of this trend. Perhaps the first thing to notice is that there is a maximum growth rate at about  $\lambda \approx 6\text{hr}^{-1}$ , or doubling time of about 7 minutes (dashed line). This growth rate can be viewed as an inherent maximum growth rate due to the need for the cell to double the cell's entire ribosomal mass. Interestingly, this limit is independent of the absolute number of ribosomes, but rather is simply given by time to translate an entire ribosome,  $L_R/r_t$ . As shown in ??(B), we can reconcile this with the observation that in order to double the average number of ribosomes, each ribosome must produce a second ribosome. This is a process that cannot be parallelized.

For reasonable values of  $\Phi_R$ , between about 0.1 - 0.3 (?), the maximum growth rate is in line with experimentally reported growth rates around  $0.5 - 2\text{ hr}^{-1}$ . Here we are implicitly assuming that translation proceeds randomly, without preference between ribosomal or non-ribosomal mRNA, which appears reasonable. Importantly, in order for a cell to scale this growth limit set by  $\Phi_R$ , cells must increase their ribosomal abundance. This can be achieved by either synthesizing more ribosomes or reducing the fraction of non-ribosomal proteins. Reduction of non-ribosomal proteins is not straight forward since, as we have found throughout our estimates, doubling a cell requires many other enzymes and transporters. Increasing the absolute ribosomal abundance for the case of *E. coli* is limited by the number of rRNA operons.



**Figure 3. 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 ( $N_{\text{aa}} / r_t$ ) sets the translation-limited growth rate,  $\lambda_{\text{translation-limited}}$ . Here  $N_{\text{aa}}$  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  $m_{\text{protein}}$  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  $\Phi_R$  (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.

**205    Multiple replication forks bias ribosome abundance.**

206    *E. coli* cells grow by an adder mechanism, whereby cells add a constant volume with each cell di-  
 207    vision (?). In conjunction with this, additional rounds of DNA replication are triggered when cells  
 208    reach a critical volume per origin of replication (??(A)). This leads to the classically-described ex-  
 209    ponential increase in cell size with growth rate ???. The mechanism behind growth rate control  
 210    however, has remained elusive. In the context of maximizing growth rate, it is notable that the ma-  
 211    jority of ribosomal proteins and rRNA operons are found closer to the DNA origin. Given the need  
 212    to increase to total gene dosage of rRNA operons at faster growth rates, and the intimate relation-  
 213    ship between ribosomal content and growth rate we considered above, this raises the possibility  
 214    that the observed size scaling and increase in chromosomal content might simply be a means for  
 215    the cell to tune biosynthesis according to its physiological state.

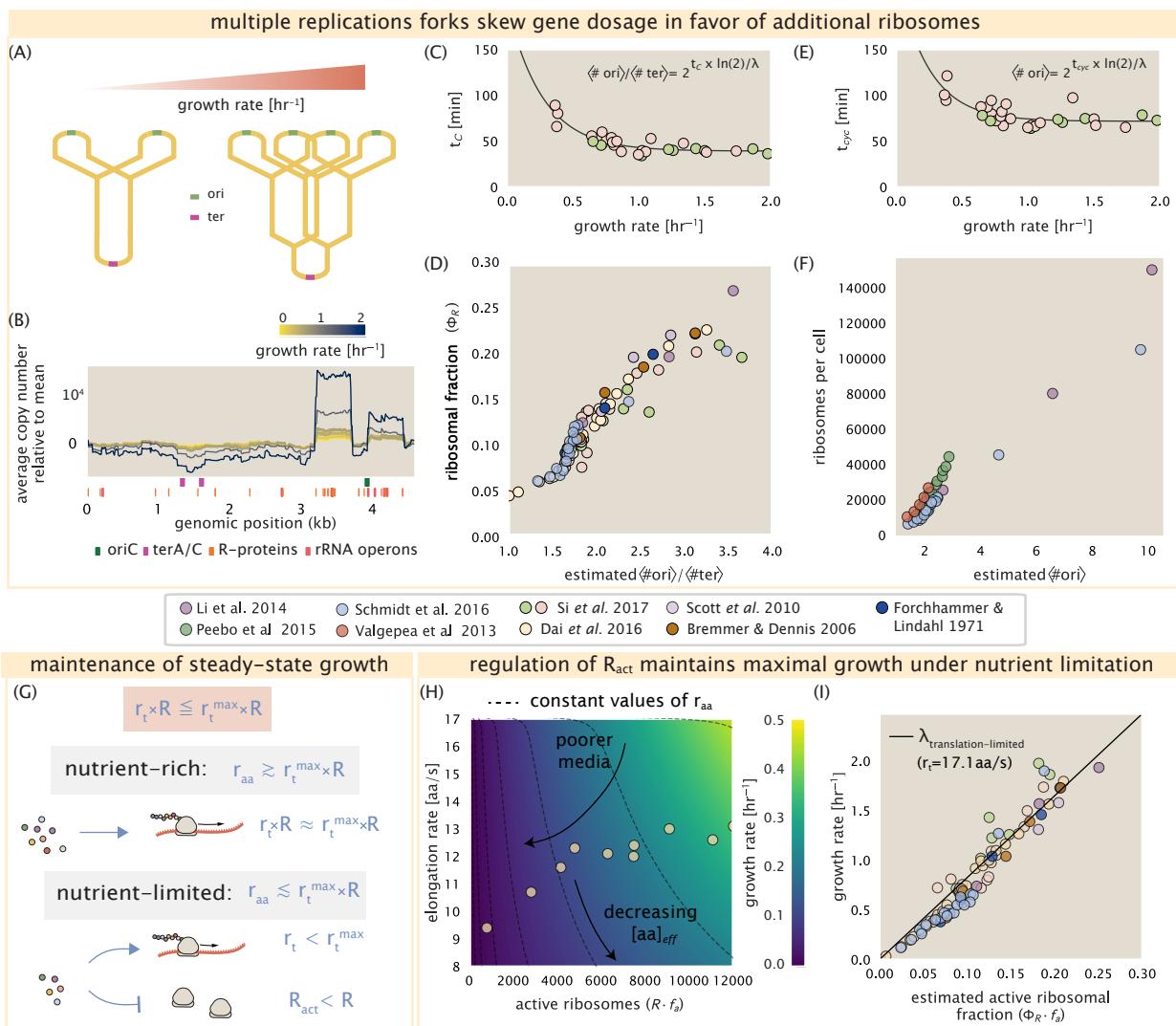
216    While an increase in transcription has been observed for genes near the origin in rapidly grow-  
 217    ing *E. coli* (?), we were unaware of such characterization at the proteomic level. In order to test  
 218    whether there is a relative increase in protein expression for genes closer to the origin, we calcu-  
 219    lated a running boxcar average of protein copy number as a function of each gene's transcriptional  
 220    start site. While absolute protein copy numbers can vary substantially across the chromosome, we  
 221    indeed observe a bias in expression under fast growth conditions (??(B), showing the result using  
 222    a 0.5 kb averaging window). The dramatic change in protein copy number near the origin mainly  
 223    reflects the increase in ribosomal protein expression. This trend is in contrast to slower growth con-  
 224    ditions where the average copy number is more uniform across the length of the chromosome.

225    If ribosomal genes (rRNA and ribosomal proteins) are being synthesized according to their avail-  
 226    able gene dosage we can make two related hypotheses about how their abundance should vary  
 227    with chromosomal content. The first is that the ribosomal protein fraction should increase in pro-  
 228    portion to the average ratio of DNA origins to DNA termini ( $\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$  ratio). This is a conse-  
 229    quence of the skew in DNA dosage as cells grow faster. The second is that the absolute number of  
 230    ribosomes should increase linearly with the number of DNA origins ( $\langle \# \text{ ori} \rangle$ ), since this will reflect  
 231    the total gene dosage at a particular growth condition.

232    In order to test each of these expectations we considered the experimental data from Si *et*  
 233    *al.* (2017), which inferred these parameters for cells under nutrient-limited growth.  $\langle \# \text{ ori} \rangle / \langle \#$   
 234     $\text{ter} \rangle$  ratio depends on how quickly chromosomes are replicated relative the cell's doubling time  $\tau$   
 235    and is given by  $2^{\tau_C/\tau}$ . Here  $\tau_C$  is the time taken to replicate *E. coli*'s' chromosome, referred to as  
 236    the C period of cell division. In ??(C) we plot  $\tau_C$  versus  $\tau$  that were measured, with data points in  
 237    red corresponding to *E. coli* strain MG1655, and blue to strain NCM3722. In their work they also  
 238    measured the total RNA to protein ratio which reflects ribosomal abundance and we show that data  
 239    along with other recent measurements from Dai *et al.*. Indeed we find that the ribosomal fraction  
 240    increases with  $\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$  (??(C)). We note a systematic difference in the relative abundances  
 241    from Peebo *et al.* and Valgepea *et al.* that was inconsistent with a number of other measurements  
 242    of total RNA-to-protein ratios ( $\approx \Phi_R \times 2.1 ?$ ) and only show the data from Schmidt *et al.* and Li *et al.*  
 243    for relative ribosome abundances (see supplemental section XX for a more complete discussion).

244    We can similarly estimate  $\langle \# \text{ ori} \rangle$ , which depends on how often replication forks are initiated  
 245    per cell cycle. This is given by the number of overlapping cell cycles,  $2^{\tau_{\text{cyc}}/\tau}$ , where  $\tau_{\text{cyc}}$  refers to  
 246    the total time of chromosome replication and cell division. ??(E) shows the associated data from  
 247    Si *et al.*, which we use to estimate  $\langle \# \text{ ori} \rangle$  for each growth condition of the proteomic data. In  
 248    agreement with our expectations, we find a strong correlation between the ribosome copy number  
 249    and estimated  $\langle \# \text{ ori} \rangle$  (??(F)).

250    These results may also shed some light on the notable increase in ribosomal content that is  
 251    observed when sublethal doses of antibiotics. Specifically, if rRNA synthesis is rate limiting, and  
 252    nutrient conditions largely dictate the extent of overlapping DNA replication cycles, than addition  
 253    of antibiotic will lengthen the doubling time and allow an increase in the abundance of rRNA that  
 254    can be synthesized over a division cycle. In Supplemental Section XX we consider this further using



**Figure 4. 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 growth condition considered by Schmidt *et al.*. A 0.5 kb 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) plots the ribosome copy number estimated from the proteomic data against our estimate of  $\langle \# \text{ori} \rangle$ . (G) 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 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. (H) [in progress], (I) 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.

255 additional data from Si *et al.* (2017).

**256 Regulation of translating ribosomes helps maintain maximal growth according to  
257 nutrient availability.**

258 While the above analysis provides an explanation for how *E. coli* appears to vary its ribosomal  
259 content to maximize growth, it also presents a challenge in the limit of poorer nutrient conditions.  
260 Recall from Equation ?? that ribosomal content should decrease to zero as growth decreases to  
261 zero. While bacteria tend to decrease their ribosomal abundance in poorer nutrient conditions,  
262 they do so only to some fixed, non-zero amount (??). Here we find a minimal ribosomal fraction  
263 of about 6 percent in the slowest growth conditions. From the perspective of a bacterium dealing  
264 with uncertain nutrient conditions, there is likely a benefit for the cell to maintain some relative  
265 fraction of ribosomes to support rapid growth as nutrient conditions improve.

266 The challenge however, lies in the cell's ability to maintain steady-state growth when ribosomes  
267 are in excess of the rate that nutrients can be harvested and amino acids synthesized for consump-  
268 tion ??G. One explanation for this is that the elongation rate decreases in poorer growth conditions.  
269 Cells, however, are still able to maintain a relatively high elongation rate even in stationary phase  
270 ( $\approx 8 \text{ aa/s}$ , ??)). A second explanation is that there are mechanisms to regulate biological activity  
271 in conditions of stress and nutrient-limitation; in particular through the small-molecule alarmones  
272 (p)ppGpp (?). Here we explore these two observations to better understand their consequence on  
273 growth rate.

274 We consider slow growth conditions ( $\lambda$  less than  $0.5 \text{ hr}^{-1}$ ) by assuming that the decrease in  
275 elongation rate is due to a limiting supply of amino acids and a need for the cell to maintain excess  
276 nutrients for cellular homeostasis under steady-state growth. There is some experimental support  
277 showing that in poorer nutrient growth conditions, cells have lower amino acids concentrations (?).  
278 We proceed by coarse grainig the cell's amino acid supply as an single, effective rate-limiting species  
279 (see Appendix XX for a more complete discussion). Under such a scenario, the elongation rate can  
280 described as simply depending on the maximum elongation rate ( $\approx 17.1 \text{ aa/s}$ , ??)), an effective  $K_d$ ,  
281 and the limiting amino acid concentration  $[aa]_{eff}$ . Specifically, the elongation rate is given by,

$$r_t = r_t^{max} \cdot \frac{1}{1 + K_d/[aa]_{eff}}. \quad (3)$$

282 For cells growing in minimal media + glucose, the amino acid concentration is of order 100 mM  
283 (BNID: 110093, ??)). With a growth rate of about  $0.6 \text{ hr}^{-1}$  and elongation rate of  $12.5 \text{ aa per second}$   
284 (?), we can estimate an effective  $K_d$  of about 40 mM. Ultimately the steady state amino acid concen-  
285 tration will depend on the difference between the supply of amino acids  $r_{aa}$  and consumption by  
286 ribosomes  $r_t \cdot R \cdot f_a$ , where  $f_a$  accounts for the possible reduction of actively translating ribosomes.

287 In ??E we consider how the maximal growth rate and elongation rates vary as a function of the  
288 number of actively translating ribosomes in this slow growth regime (see Supplemental Section XX  
289 for a complete description of the model). If we consider  $r_a a$  to be reflective of a specific growth  
290 condition, by considering lines of constant  $r_a a$ , we find that cells grow fastest by maximizing their  
291 fraction of actively translating ribosomes. When we consider the experimental measurements  
292 from Dai *et al.*, we see that although cells indeed reduce  $R \cdot f_a$ , they do so in a way that keeps  $[aa]_{eff}$   
293 relatively constant. Given our estimate for the  $K_d$  of 40 mM, we would only expect a decrease  
294 from 100 mM to about 35 mM in the slowest growth conditions. While experimental data is lim-  
295 ited, amino acid concentrations only decrease to about 60 mM for cells grown in minimal media +  
296 acetate ( $\lambda 0.3 \text{ hr}^{-1}$  in our proteomic data; value obtained from ?), qualitatively consistent with our  
297 expectations.

298 Given the quantitative data from Dai *et al.*, which determined  $f_a$  across the entire range of  
299 growth rates across our data, we next estimated the active fraction of ribosomal protein. As shown  
300 in ??(G), we find that cells grow at a rate near the expected translation maximum expected from  
301 Equation 1, using the maximum elongation rate of  $r_t = 17.1 \text{ aa per second}$ . This is in contrast to the

302 reality that cells ribosomes are translating at almost half this rate in the poorest growth conditions.  
303 This suggests that there are alternative ways to grow according to the translated-limited growth  
304 rate. Even though *E. coli* cells do not scale their ribosomal content to zero, they appear to achieve  
305 the same end by regulating the fraction of their ribosomes in poorer nutrient conditions.

306 [NB, important to include in discussion section: A number of recent papers highlight the pos-  
307 sibility that (p)ppGpp may even provide a causal explanation for the nutrient-limit scaling law. In  
308 the context of ribosomal activity, increased levels of (p)ppGpp is associated with lower ribosomal  
309 content, and at slow growth appear to reduce the fraction of actively translating ribosomes (??).  
310 Titration of the cellular (p)ppGpp concentrations (up or down) can invoke similar proteomic changes  
311 reminiscent of those observed under nutrient limitation (?). In light of the limiting dependence of  
312 ribosome copy number on chromosomal content, it was recently shown that growth (p)ppGpp null  
313 strain abolished both the cell size scaling and changes to the  $\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$  ratio. Rather, cells  
314 exhibited a  $\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$  closer to 4 and cell size more consistent with a fast growth state where  
315 (p)ppGpp levels are low (?).]