

Fundamental limits on the rate of bacterial cell division

³ Nathan M. Belliveau^{†, 1}, Griffin Chure^{†, 2, 3}, Christina L. Hueschen⁴, Hernan G.
⁴ Garcia⁵, Jané Kondev⁶, Daniel S. Fisher⁷, Julie Theriot^{1, 8}, Rob Phillips^{2, 9, *}

*For correspondence:

[†]These authors contributed equally to this work

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

¹⁴

¹⁵ **Abstract** This will be written next

¹⁶

¹⁷ Translation and ribosomal synthesis

¹⁸ Lastly, we turn our attention to the process of synthesizing new proteins, translation. These pro-
¹⁹ cesses stand as good candidates for defining the growth limit as the synthesis of new proteins
²⁰ relies on the generation of ribosomes, themselves proteinaceous molecules. As we will see in the
²¹ coming sections of this work, this poses a "chicken-or-the-egg" problem where the synthesis of
²² ribosomes requires ribosomes in the first place.

²³ We will begin our exploration of protein translation in the same spirit as we have in previous
²⁴ sections – we will draw order-of-magnitude estimates based on our intuition and relying on litera-
²⁵ ture studies and will compare these estimates to the observed data. In doing so, we will estimate
²⁶ both the absolute number of ribosomes necessary for replication of the proteome as well as the
²⁷ synthesis of amino-acyl tRNAs. In the closing sections, we will explore the details of ribosome bio-
²⁸ genesis in granular detail, ultimately presenting a quantitative model tying ribosome abundance
²⁹ to the concentration of amino acids as well as the state of chromosome replication.

³⁰ tRNA synthetases

³¹ We begin by first estimating the number of tRNA ligases in *E. coli* needed to convert free amino-
³² acids to polypeptide chains. At a modest growth rate of ≈ 5000 s, *E. coli* has roughly 3×10^6 proteins
³³ per cell (BNID: 115702; ?). Assuming that the typical protein is on the order of ≈ 300 amino acids
³⁴ in length (BNID: 100017; ?), we can estimate that a total of $\approx 10^9$ amino acids are stitched together
³⁵ by peptide bonds.

³⁶ How many tRNAs are needed to facilitate this remarkable number of amino acid delivery events
³⁷ to the translating ribosomes? It is important to note that tRNAs are recycled after they've passed
³⁸ through the ribosome and can be recharged with a new amino acid, ready for another round of
³⁹ peptide bond formation. While some *in vitro* data exists on the turnover of tRNA in *E. coli* for
⁴⁰ different amino acids, we can make a reasonable estimate by comparing the number of amino
⁴¹ acids to be polymerized to cell division time. Using our stopwatch of 5000 s and 10^9 amino acids,

42 we arrive at a requirement of $\approx 2 \times 10^5$ tRNA molecules. This estimate is in line with experimental
 43 measurements of $\approx 3 \times 10^5$ per cell (BNID: 108611, ?), suggesting we are on the right track.

44 There are many processes which go into synthesizing a tRNA and ligating it with the appropriate
 45 amino acids. As we covered in the previous section, there appear to be more than enough RNA
 46 polymerases per cell to synthesize the needed pool of tRNAs. Without considering the many ways
 47 in which amino acids can be scavenged or synthesized *de novo*, we can explore ligation the as a
 48 potential rate limiting step. The enzymes which link the correct amino acid to the tRNA, known
 49 as tRNA synthetases or tRNA ligases, are incredible in their proofreading of substrates with the
 50 incorrect amino acid being ligated once out of every 10^4 to 10^5 times (BNID: 103469, ?). This is due
 51 in part to the consumption of energy as well as a multi-step pathway to ligation. While the rate
 52 at which tRNA is ligated is highly dependent on the identity of the amino acid, it is reasonable to
 53 state that the typical tRNA synthetase has charging rate of ≈ 20 AA per tRNA synthetase per second
 54 (BNID: 105279, ?).

55 Combining these estimates together, as shown schematically in ??(A), yields an estimate of \approx
 56 10^4 tRNA synthetases per cell with a division time of 5000 s. This point estimate is in very close
 57 agreement with the observed number of synthetases (the sum of all 20 tRNA synthetases in *E.*
 58 *coli*). This estimation strategy seems to adequately describe the observed growth rate dependence
 59 of the tRNA synthetase copy number (shown as the grey line in ??(B)), suggesting that the copy
 60 number scales with the cell volume.

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

67 Protein synthesis

68 As we are now assured that tRNA charging is

69 If we consider an elongation rate of ≈ 15 peptide bonds per second (BNID: 114271, ??), the for-
 70 mation of $\approx 10^9$ peptide bonds would require about 1.5×10^4 ribosomes. This is indeed consistent
 71 with the experimental data shown in ??(B).

72 Ribosomal synthesis

73 So far our estimates have led to protein copy numbers that are consistent with the proteomic
 74 data, or even in excess of what might be needed for each task under limiting growth conditions.
 75 Even in our example of *E. coli* grown under different carbohydrate sources (??(B)), it becomes clear
 76 cells can utilize alternative carbon sources by inducing the expression of additional membrane
 77 transporters and enzymes. Optimal resource allocation and the role of ribosomal proteins have
 78 been an area of intense quantitative study over the last decade by Hwa and others (??). From the
 79 perspective of limiting growth, our earlier estimate of rRNA highlighted the necessity for multiple
 80 copies of rRNA genes in order to make enough rRNA. For *E. coli*'s fastest growth rates at 2 hr^{-1} , the
 81 additional demand for rRNA is further supported by parallelized DNA replication and increased
 82 rRNA gene dosage. This suggests the possibility that synthesis of ribosomes might be rate limiting.
 83 While the transcriptional demand for the ribosomal proteins is substantially lower than rRNA genes,
 84 since proteins can be translated from relatively fewer mRNA, other ribosomal proteins like the
 85 translation elongation factor EF-Tu also present a substantial burden. For EF-Tu in particular, it is
 86 the most highly expressed protein in *E. coli* and is expressed from multiple gene copies, *tufA* and
 87 *tufB*.

88 To gain some intuition into how translation may set the speed limit for bacterial growth, we
 89 again consider the total number of peptide bonds that must be synthesized, N_{AA} . Noting that cell

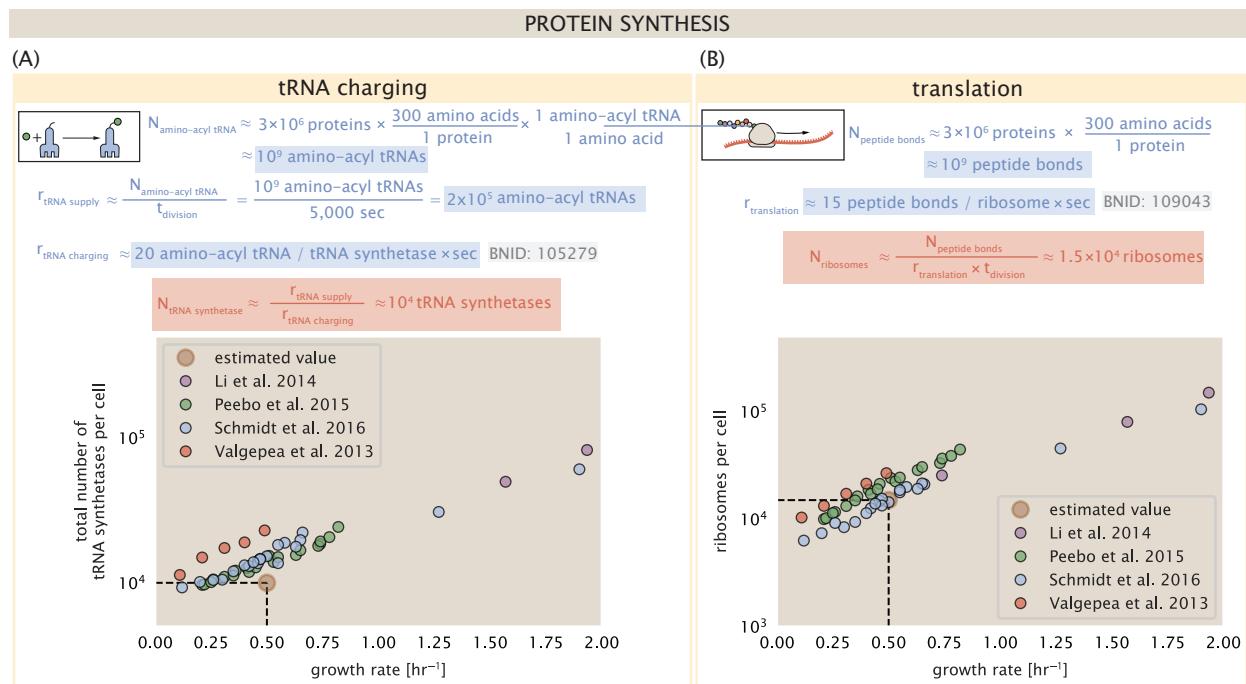


Figure 1. Estimation of the required tRNA synthetases and ribosomes. (A) Estimation for the number of tRNA synthetases that will supply the required amino acid demand. The sum of all tRNA synthetases copy numbers are plotted as a function of growth rate ([ArgS], [CysS], [GlnS], [Glx], [IleS], [LeuS], [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 10^9 peptide bonds with an elongation rate of 15 peptide bonds per second. The average abundance of ribosomes is plotted as a function of growth rate. Our estimated values are shown for a growth rate of 0.5 hr^{-1} .

90 mass grows exponentially (?), we can compute the number of amino acids to be polymerized as

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

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

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

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

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

98 where L_R is the total length in amino acids that make up a ribosome, and Φ_R is the ribosomal mass
 99 fraction. This is plotted as a function of ribosomal fraction Φ_R in ??(A), where we take $L_R \approx 7500$ aa,
 100 corresponding to the length in amino acids for all ribosomal subunits of the 50S and 30S complex
 101 (BNID: 101175, (?)). This formulation assumes that the cell can transcribe the required amount of
 102 rRNA, which appears reasonable for *E. coli*, allowing us to consider the inherent limit on growth set
 103 by the ribosome.

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

114 For reasonable values of Φ_R , between about 0.1 - 0.3 (?), the maximum growth rate is in line with
 115 experimentally reported growth rates around 0.5 - 2 hr $^{-1}$. Importantly, in order for a cell to increase
 116 their growth limit they *must* increase their relative ribosomal abundance. This can be achieved by
 117 either synthesizing more ribosomes or reducing the fraction of non-ribosomal proteins. Reduction
 118 of non-ribosomal proteins is not a straightforward task since (as we have found throughout our
 119 estimates) doubling a cell requires many other enzymes and transporters. Increasing the absolute
 120 ribosomal abundance in *E. coli* will be limited by the number of rRNA operons.

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

123 [expand on.]

124 Discussion

125 [Fill in.]

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

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

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

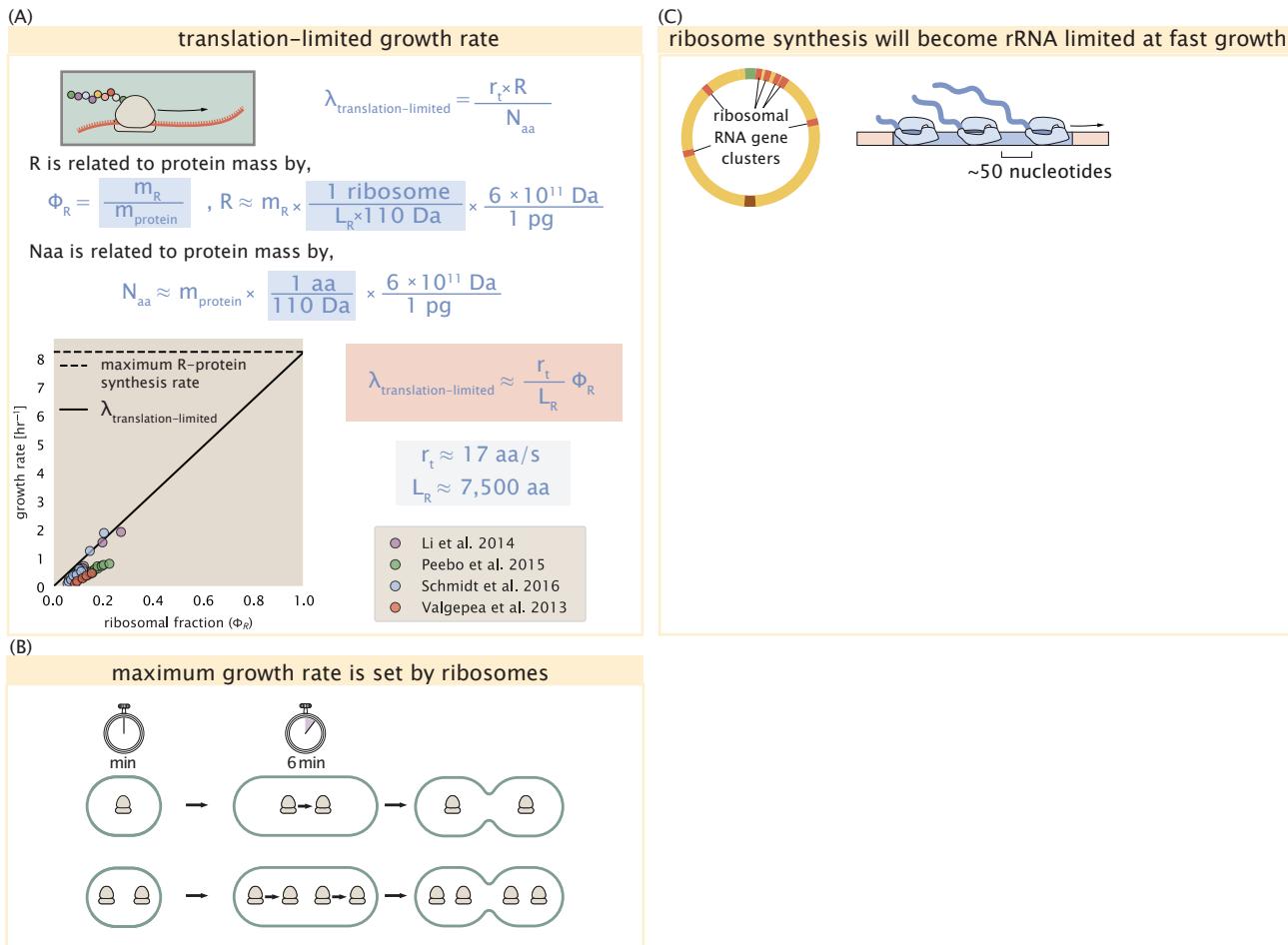


Figure 2. Translation-limited growth rate. (A) Here we consider the translation-limited growth as a function of ribosomal fraction. By mass balance, the time required to double the entire proteome (N_{AA} / r_t) sets the translation-limited growth rate, $\lambda_{\text{translation-limited}}$. Here N_{AA} 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)

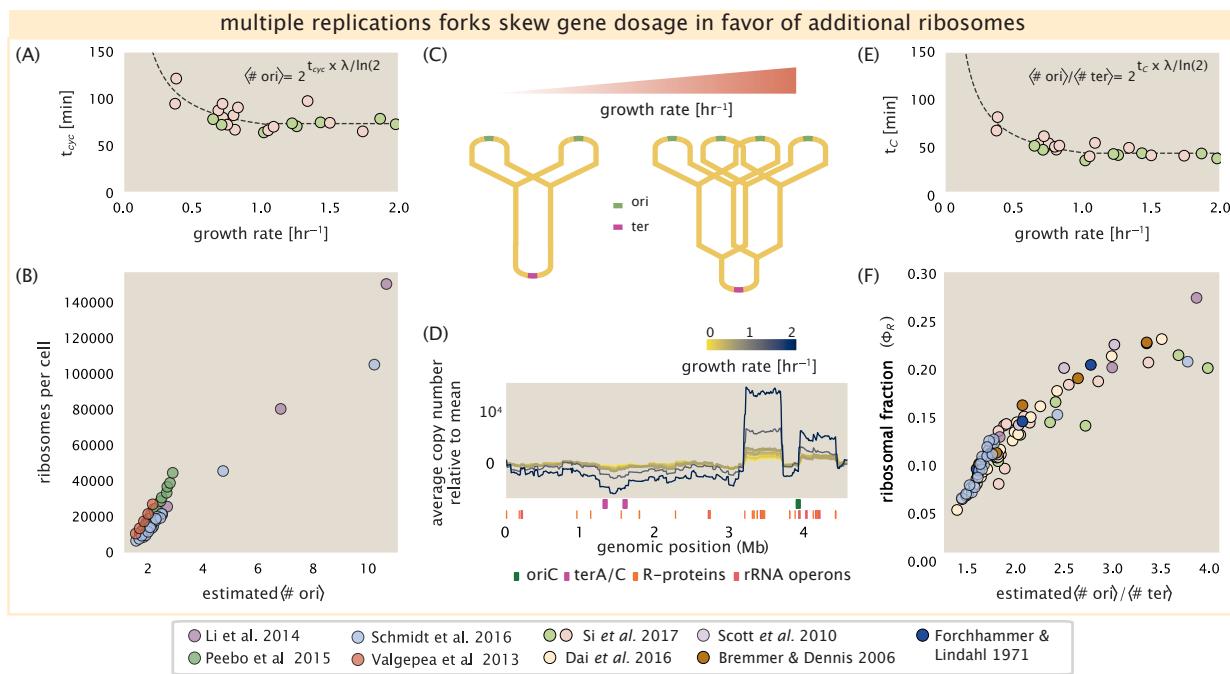


Figure 3. Multiple replication forks skew gene dosage and ribosomal content. (A) Schematic shows the expected increase in replication forks (or number of ori regions) as *E. coli* cells grow faster. (B) A running boxcar average of protein copy number is calculated for each each growth condition considered 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 $\langle \# \text{ori} \rangle$.

131 Parallel DNA replication biases gene dosage in support of ribosome synthesis.

132 *E. coli* cells grow by a so-called "adder" mechanism, whereby cells add a constant volume with each
 133 cell division (?). In conjunction with this, additional rounds of DNA replication are triggered when
 134 cells reach a critical volume per origin of replication (??(A)). This leads to the classically-described
 135 exponential increase in cell size with growth rate ????. In the context of maximizing growth rate, it
 136 is notable that the majority of ribosomal proteins and rRNA operons are found closer to the DNA
 137 origin.

138 While an increase in transcription has been observed for genes closer to the origin in rapidly
 139 growing *E. coli* (?), we were unaware of such characterization at the proteomic level. In order to
 140 see whether there is a relative increase in protein expression for genes closer to the origin at
 141 faster growth, we calculated a running boxcar average (500 kbp window) of protein copy number
 142 as a function of each gene's transcriptional start site (??(B)). While absolute protein copy numbers
 143 can vary substantially across the chromosome, we indeed observe a bias in expression under fast
 144 growth conditions (dark blue), showing the result. The dramatic change in protein copy number
 145 near the origin is primarily due to the increase in ribosomal protein expression. This trend is in
 146 contrast to slower growth conditions (yellow) where the average copy number is more uniform
 147 across the length of the chromosome.

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

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

156 In order to test each of these expectations we considered the experimental data from ?, which
 157 inferred these parameters for cells under nutrient-limited growth. The ratio $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$ de-
 158 pends on how quickly chromosomes are replicated relative the cell's doubling time τ and is given
 159 by $2^{\tau_C/\tau}$. Here τ_C is the time taken to replicate *E. coli*'s chromosome, referred to as the C period of
 160 cell division. In ??(C) we plot the measured τ_C versus τ (computed as $\tau = \log(2)/\lambda$), with data points
 161 in red corresponding to *E. coli* strain MG1655, and blue to strain NCM3722. ? also measured the
 162 total RNA to protein ratio which reflects ribosomal abundance and we show that data along with
 163 other recent measurements from ???. Indeed, we find that the ribosomal fraction increases with
 164 $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$ (??(C)). We note a systematic difference in the relative abundances from ? and ?
 165 that was inconsistent with a number of other measurements of total RNA-to-protein ratios ($\approx \Phi_R$
 166 $\times 2.1$?) and only show the data from ? and ? for relative ribosome abundances (see supplemental
 167 section XX for a more complete discussion). For the data shown, the ribosomal fraction doesn't
 168 increase as much at higher $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$. Since several rRNA operons are actually located ap-
 169 proximately half-way between the origin and terminus, the trend may in part be a consequence of
 170 a diminishing increase in rRNA gene dosage at higher $\langle \# \text{ori} \rangle / \langle \# \text{ter} \rangle$ ratios.

171 We can similarly estimate $\langle \# \text{ori} \rangle$, which depends on how often replication forks are initiated
 172 per cell cycle. This is given by the number of overlapping cell cycles, $2^{\tau_{\text{cyc}}/\tau}$, where τ_{cyc} refers to
 173 the total time of chromosome replication and cell division. ??(E) shows the associated data from ?,
 174 which we use to estimate $\langle \# \text{ori} \rangle$ for each growth condition of the proteomic data. In agreement
 175 with our expectations, we find that ribosome copy number increases with the estimated $\langle \# \text{ori} \rangle$
 176 (??(F)).

177 While it is difficult to distinguish between causality and correlation, the data is consistent with
 178 the need for cells to increase their effective rRNA gene dosage in order to grow according to the
 179 constraint set by Equation 2. These results may also shed some light on the notable increase in
 180 ribosomal content that is observed when sublethal doses of antibiotics (??). Specifically, if rRNA
 181 synthesis is rate limiting, and nutrient conditions largely dictate the extent of overlapping DNA
 182 replication cycles, than addition of antibiotic will lengthen the doubling time and allow an increased
 183 rRNA synthesis relative to the rate of cell division. In Supplemental Section XX, we consider this
 184 further using additional data from ?.

185 Regulation of translating ribosomes helps maintain maximal growth according to nutri-
 186 ent availability.

187 While the above observations show how *E. coli* can vary its ribosomal content to increase growth
 188 rate, it also presents a challenge in the limit of poorer nutrient conditions. Recall from Equation ??
 189 that ribosomal content should decrease to zero as growth decreases to zero. While bacteria tend
 190 to decrease their ribosomal abundance in poorer nutrient conditions, they do so only to some
 191 fixed, non-zero amount (??). Here we find a minimal ribosomal fraction of ≈ 0.06 in the slowest
 192 growth conditions. From the perspective of a bacterium dealing with uncertain nutrient conditions,
 193 there is likely a benefit for the cell to maintain some relative fraction of ribosomes to support rapid
 194 growth as nutrient conditions improve.

195 The challenge however, lies in the cell's ability to maintain growth when ribosomes are in excess
 196 of the rate that nutrients can be harvested and amino acids synthesized for consumption ??A. In
 197 the limit of poor growth conditions, ribosomes would consume their amino acid supply and be
 198 unable to maintain steady-state growth. In reality, *E. coli* is still able to maintain a relatively high
 199 elongation rate even in stationary phase ($\approx 8 \text{ AA/s}$, (??)). A explanation for this is that the cell further
 200 regulates its biological activity in conditions of stress and nutrient-limitation; in particular through

201 the small-molecule alarmones (p)ppGpp (?). In (p)ppGpp null strains, cells are unable to grow in
 202 nutrient-poor media. Indeed, these small molecules play a role in controlling biosynthesis rates
 203 throughout the central dogma [NB citations]. Here we explore this further in the context of growth
 204 by maximizing protein synthesis.

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

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

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

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

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

219 Global regulatory control across central dogma may provide an explanation for the robust scaling laws in *E. coli*.

220 A number of recent papers further highlight the possibility that (p)ppGpp may even provide a causal explanation for the scaling laws in *E. coli*. In the context of ribosomal activity, increased levels of (p)ppGpp are associated with lower ribosomal content, and at slow growth appear to help reduce the fraction of actively translating ribosomes (?). Titration of the cellular (p)ppGpp concentrations (up or down) can invoke similar proteomic changes reminiscent of those observed under nutrient limitation (?). In light of the limiting dependence of ribosome copy number on chromosomal gene dosage, it was recently shown that growth in a (p)ppGpp null strain abolishes both the scaling in cell size and the $\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$ ratio. Instead, cells exhibited a high $\langle \# \text{ ori} \rangle / \langle \# \text{ ter} \rangle$ closer to 4 and

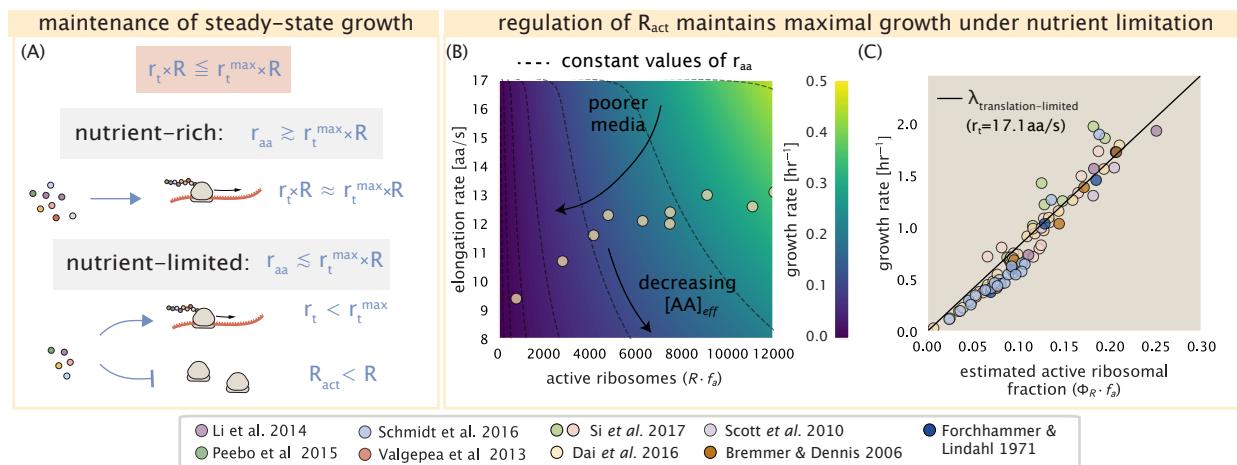


Figure 4. *E. coli* must regulate ribosomal activity in limiting nutrient conditions. (A) Schematic showing translation-specific requirements for maintenance of steady-state growth. In a nutrient rich environment, amino acid supply r_{aa} is sufficiently in excess of the demand by ribosomes translating 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 $R \cdot f_a$. 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.

248 cell size more consistent with a fast growth state where (p)ppGpp levels are low (?).]

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