

Appendix for: Fundamental limits on the rate of bacterial growth

Nathan M. Belliveau^{†, 1}, Griffin Chure^{†, 2}, Christina L. Hueschen³, Hernan G. Garcia⁴, Jane Kondev⁵, Daniel S. Fisher⁶, Julie A. Theriot^{1, 7, *}, Rob Phillips^{8, 9, *}

¹Department of Biology, University of Washington, Seattle, WA, USA; ²Department of Applied Physics, California Institute of Technology, Pasadena, CA, USA; ³Department of Chemical Engineering, Stanford University, Stanford, CA, USA; ⁴Department of Molecular Cell Biology and Department of Physics, University of California Berkeley, Berkeley, CA, USA; ⁵Department of Physics, Brandeis University, Waltham, MA, USA; ⁶Department of Applied Physics, Stanford University, Stanford, CA, USA; ⁷Allen Institute for Cell Science, Seattle, WA, USA; ⁸Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA; ⁹Department of Physics, California Institute of Technology, Pasadena, CA, USA; *Co-corresponding authors. Address correspondence to phillips@pboc.caltech.edu and jtheriot@uw.edu; [†]These authors contributed equally to this work

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Additional Estimates of Fundamental Biological Processes

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

Nutrient Transport

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

Nitrogen

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

Phosphorus

Phosphorus is critical to the cellular energy economy in the form of high-energy phosphodiester bonds making up DNA, RNA, and the NTP energy pool as well as playing a critical role in the post-translational modification of proteins and defining the polar-heads of lipids. In total, phosphorus makes up $\approx 3\%$ of the cellular dry mass which in typical experimental conditions is in the form of inorganic phosphate. The cell membrane has remarkably low permeability to this highly-charged and critical molecule, therefore requiring the expression of active transport systems. In *E. coli*, the proton electrochemical gradient across the inner membrane is leveraged to transport inorganic phosphate into the cell (*Rosenberg et al., 1977*). Proton-solute symporters are widespread in *E. coli* (*Ramos and Kaback, 1977; Booth et al., 1979*) and can have rapid transport rates of 50 to 100 molecules per second for sugars and other solutes (BNID: 103159; 111777). As a more extreme example, the proton transporters in the F_1 - F_0 ATP synthase, which use the proton electrochemical gradient for rotational motion, can shuttle protons across the membrane at a rate of ≈ 1000 per second (BNID: 104890; 103390). In *E. coli* the PitA phosphate transport system has been shown to be very tightly coupled with the proton electrochemical gradient with a 1:1 proton:phosphate stoichiometric ratio (*Harris et al., 2001; Feist et al., 2007*). Taking the geometric mean of the aforementioned estimates gives a plausible rate of phosphate transport on the order of 300 per second. Illustrated in **Figure 2-Figure Supplement 1(A)**, we can estimate that ≈ 200 phosphate transporters are necessary to maintain an $\approx 3\%$ dry mass with a 5000 s division time. This estimate is consistent with observation when we examine the observed copy numbers of PitA in proteomic data sets (plot in **Figure 2-Figure Supplement 1(A)**). While our estimate is very much in line with the observed numbers, we emphasize that this is likely a slight overestimate of the number of transporters needed as there are other phosphorous scavenging systems, such as the ATP-dependent phosphate transporter Pst system which we have neglected.

Sulfur

Similar to phosphate, sulfate is highly-charged and not particularly membrane permeable, requiring active transport. While there exists a H⁺/sulfate symporter in *E. coli*, it is in relatively low abundance and is not well characterized (*Zhang et al., 2014*). Sulfate is predominantly acquired via the ATP-dependent ABC transporter CysUWA system which also plays an important role in selenium transport (*Sekowska et al., 2000; Sirko et al., 1995*). While specific kinetic details of this transport system are not readily available, generic ATP transport systems in prokaryotes transport on the order of 1 to 10 molecules per second (BNID: 109035). Combining this generic transport rate, measurement of sulfur comprising 1% of dry mass, and a 5000 second division time yields an estimate of ≈ 1000 CysUWA complexes per cell (**Figure 2-Figure Supplement 1(B)**). Once again, this estimate is in notable agreement with proteomic data sets, suggesting that there are sufficient transporters present to acquire the necessary sulfur. In a similar spirit of

our estimate of phosphorus transport, we emphasize that this is likely an overestimate of the number of necessary transporters as we have neglected other sulfur scavenging systems that are in lower abundance.

Additional Process of the Central Dogma

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

dNTP synthesis

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

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

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

mRNA and tRNA Synthesis

In **Figure 7** of the main text, we presented estimates for the number of RNA polymerases needed to synthesize enough mRNA and tRNA molecules. Here, we present a more detailed rationalization of these estimates.

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

This estimation captures the *steady-state* mRNA copy number, meaning that at any given time, there will exist approximately 3000 unique mRNA molecules. To determine the *total* number of mRNA that need to be synthesized over the cell's lifetime, we must consider degradation of the mRNA. In most bacteria, mRNAs are rather unstable with life times on the order of several minutes (BNID: 104324; 106253; 111927; 111998). For convenience, we assume that the typical mRNA in our cell of interest has a typical lifetime of ≈ 300 seconds. Using this value, we can determine the total mRNA production rate to maintain a steady-state copy number of 3000 mRNA per cell. While we direct the reader to the appendix for a more detailed discussion of mRNA transcriptional dynamics, we state here that the total mRNA production rate must be on the order of ≈ 15 mRNA per second. In *E. coli*, the average protein is ≈ 300 amino acids in length (BNID: 108986), meaning that the corresponding mRNA is ≈ 900 nucleotides which we will further approximate as ≈ 1000 nucleotides to account for the non-protein coding regions on the 5' and 3' ends. This means that the cell must have enough RNA polymerase molecules about to sustain a transcription rate of $\approx 1.5 \times 10^4$ nucleotides per second. Knowing that a

single RNA polymerase polymerizes RNA at a clip of 40 nucleotides per second, we arrive at a comfortable estimate of ≈ 250 RNA polymerase complexes needed to satisfy the mRNA demands of the cell. It is worth noting that this number is approximately half of that required to synthesize enough rRNA, as we saw in the previous section. We find this to be a striking result as these 250 RNA polymerase molecules are responsible for the transcription of the ≈ 4000 protein coding genes that are not ribosome associated.

We now turn our attention to the synthesis of tRNA. Unlike mRNA or rRNA, each individual tRNA is remarkably short, ranging from 70 to 95 nucleotides each (BNID: 109645; 102340). What they lack in length, they make up for in abundance, with reported values ranging from $\approx 5 \times 10^4$ (BNID: 105280) to $\approx 5 \times 10^5$ (BNID: 108611). To test tRNA synthesis as a possible growth-rate limiting stage, we will err towards a higher abundance of $\approx 5 \times 10^5$ per cell. Combining the abundance and tRNA length measurements, we make the estimate that $\approx 5 \times 10^7$ 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 ≈ 200 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.

tRNA Charging

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

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

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

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

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

Experimental Details Behind Proteomic Data

Here we provide a brief summary of the experiments behind each proteomic data set considered. The purpose of this section is to identify how the authors arrived at absolute protein abundances. In the following section (see section on Summary of Proteomic Data) we will then provide a summary of the protein abundance measurements. Table A1 provides an overview of the publications we considered. These are predominately mass spectrometry-based, with the exception of the work from Li *et al.* (2014) which used

Table A1. Overview of proteomic data sets.

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

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

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

ribosomal profiling, and the fluorescence-based counting done in Taniguchi *et al.* (2010). After having compiled and comparing these measurements, we noted substantial deviations in the measurements from Taniguchi *et al.* (2010) and Soufi *et al.* (2015) (shown in the following section), and decided to only use the data from Taniguchi *et al.* (2010); Li *et al.* (2014); Valgepea *et al.* (2013); Peebo *et al.* (2015) in the main text. For completeness, we include these additional datasets in our discussion of the experimental data.

Fluorescence based measurements

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

Ribosomal profiling measurements

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

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

Mass spectrometry measurements

Perhaps not surprisingly, the data is predominantly mass spectrometry based. This is largely due to tremendous improvements in the sensitivity of mass spectrometers, as well as improvements in sample preparation and data analysis pipelines. It is now a relatively routine task to extract protein from a cell and quantify the majority of proteins present by shotgun proteomics. In general, this involves lysing cells, enzymatically digesting the proteins into short peptide fragments, and then introducing them into the mass

spectrometer (e.g. with liquid chromatography and electrospray ionization), which itself can have multiple rounds of detection and further fragmentation of the peptides.

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

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

Summary of Proteomic Data

In the work of the main text we only used the data from *Valgepea et al. (2013)*; *Li et al. (2014)*; *Peebo et al. (2015)*; *Schmidt et al. (2016)*. As shown in *Figure A1(A)*, the reported total protein abundances in the work of *Taniguchi et al. (2010)* and *Soufi et al. (2015)* differed quite substantially from the other work. For the work of *Taniguchi et al. (2010)* this is in part due to a lower coverage in total proteomic mass quantified, though we also noticed that most proteins appear undercounted when compared to the other data.

Figure A1(B) summarizes the total protein mass for each data set used in our final compiled data set. Our inclination initially was to leave reported copy numbers untouched, but a notable discrepancy between the scaling of the total protein per cell between *Schmidt et al. (2016)* and the other data sets forced us to dig deeper into those measurements (compare *Schmidt et al. (2016)* and *Li et al. (2014)* data in *Figure A1(A)*). The particular trend in *Schmidt et al. (2016)* appears to be due to assumptions made about cell size and we provide a more extensive discussion and analysis of their data in Additional Considerations of Schmidt *et al.* Data Set. As a compromise, and in an effort to treat all data equally, we instead applied a correction factor to all protein abundance values based on a data-driven estimate of total protein per cell. Here we used cell size measurements from *Si et al. (2017, 2019)*, and an estimate of total protein content through expected dry mass. Total protein per cell was then determined using available data on total DNA, RNA, and protein from *Basan et al. (2015)*; *Dai et al. (2016)*, which account for the majority of dry mass in the cell. We describe these details further in sections on Estimation of Cell Size and Surface Area and Estimation of Total Protein Content per Cell that follows.

Lastly, in *Figure A2* we show the total proteomic coverage and overlap of proteins quantified across each data set. In part (A) we plot the total number of unique proteins, while in part (B) we plot a Venn diagram to also show the intersections across each data set. Overall, the overlap in quantified proteins is quite high, with 1157 proteins quantified across all data sets. The sequencing based approach of *Li et al. (2014)* has substantially higher coverage compared to the mass spectrometry data sets (3394 genes versus the 2041 genes quantified in the work of *Schmidt et al. (2016)*). However, in terms of total protein mass, the data from *Li et al. (2014)*; *Schmidt et al. (2016)*; *Peebo et al. (2015)* each quantify roughly equivalent total protein mass. An exception to this is in the data from *Valgepea et al. (2013)*, where we find that the total protein quantified in *Valgepea et al. (2013)* is 90-95 % of the total protein mass (when using the data from *Schmidt et al. (2016)* as a reference).

Estimation of Cell Size and Surface Area

Since most of the proteomic data sets lack cell size measurements, we chose instead to use a common estimate of size for any analysis requiring cell size or surface area. Since each of the data sets used either K-12 MG1655 or its derivative, BW25113 (from the

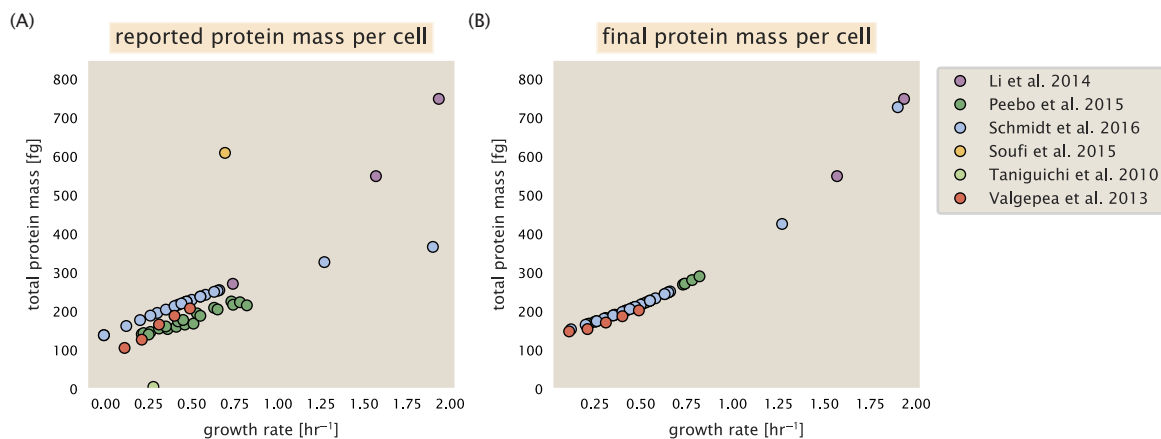


Figure A1. Summary of the growth-rate dependent total protein abundance for each data set. (A) Total protein abundance per cell as originally reported in the data sets of *Taniguchi et al. (2010)*; *Valgepea et al. (2013)*; *Li et al. (2014)*; *Soufi et al. (2015)*; *Peebo et al. (2015)*; *Schmidt et al. (2016)*. Note that the data from *Peebo et al. (2015)* only reported protein abundances per unit volume and total protein per cell was found by multiplying these by the growth-rate dependent cell size as determined by *Si et al. (2017)*. (B) Adjusted total protein abundances across the proteomic data sets are summarized. Protein abundances were adjusted so that all data shared a common set of growth-rate dependent total protein per cell and cellular protein concentration following the cell size expectations of *Si et al. (2017)* (see section on Estimation of Cell Size and Surface Area for further details).

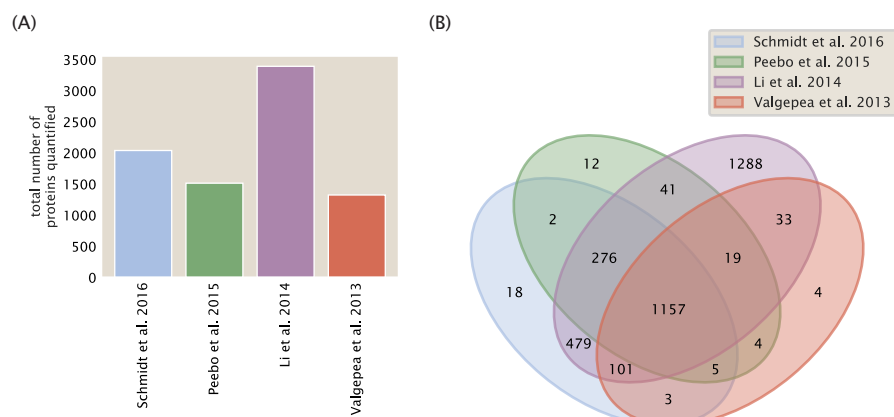


Figure A2. Comparison of proteomic coverage across different data sets. (A) Total number of unique proteins quantified in the data sets of *Valgepea et al. (2013)*; *Li et al. (2014)*; *Schmidt et al. (2016)*; *Peebo et al. (2015)*. (B) Venn diagram showing the number of unique proteins and their intersections across each of the four data sets in (A). The intersection of all four data sets identifies 1157 proteins with measured protein copy number values. The intersection of each of the shaded colors identifies the number of unique proteins quantified only in those overlapping data sets.

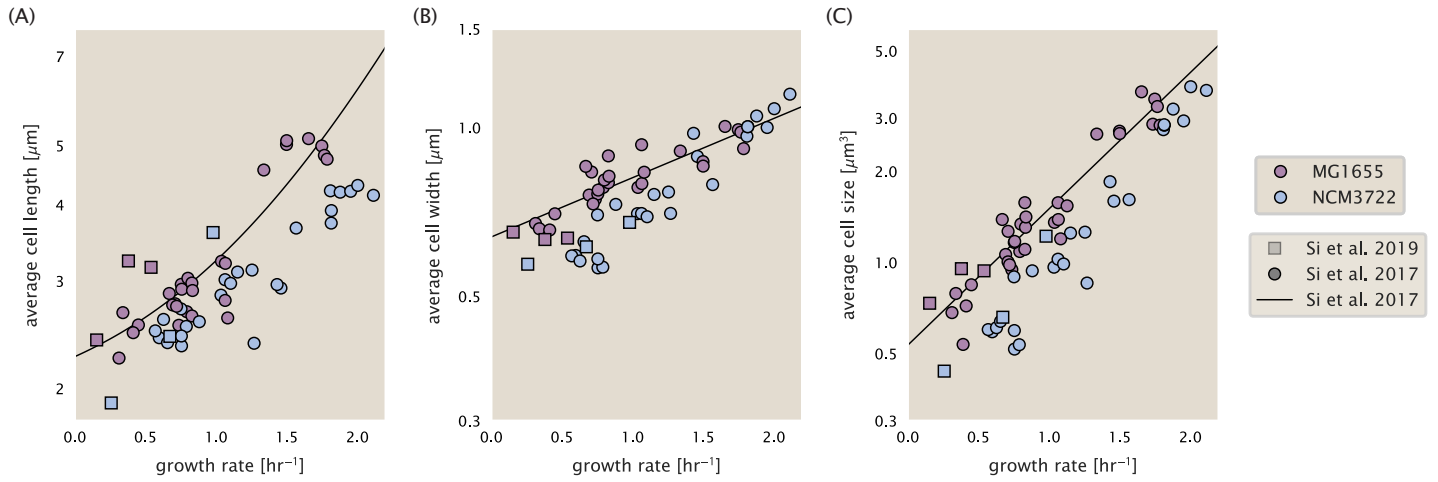


Figure A3. Summary of size measurements from Si et al. 2017, 2019. Cell lengths and widths were measured from cell contours obtained from phase contrast images, and refer to the long and short axis respectively. (A) Cell lengths and (B) cell widths show the mean measurements reported (they report 140-300 images and 5,000-30,000 for each set of samples; which likely means about 1,000-5,000 measurements per mean value reported here since they considered about 6 conditions at a time). Fits were made to the MG1655 strain data; length: $0.5 e^{1.09 \cdot \lambda} + 1.76 \mu\text{m}$, width: $0.64 e^{0.24 \cdot \lambda} \mu\text{m}$. (C) Cell size was calculated as cylinders with two hemispherical ends (Equation 1). The MG1655 strain data gave a best fit of $0.533 e^{1.037 \cdot \lambda} \mu\text{m}^3$.

lab of Barry L. Wanner; the parent strain of the Keio collection (*Datsenko and Wanner, 2000; Baba et al., 2006*)), below we fit the MG1655 cell size data from the supplemental material of *Si et al. (2017, 2019)* using the `optimize.curve_fit` function from the Scipy python package (*Virtanen et al., 2020*). A quick comment on nomenclature: throughout the text, we usually refer to cell size, in units of μm^3 ; however, on occasion we will mention size as a volume in units of fL.

The average size measurements from each of their experiments are shown in **Figure A3**, with cell length and width shown in (A) and (B), respectively. The length data was well described by the exponential function $0.5 e^{1.09 \cdot \lambda} + 1.76 \mu\text{m}$, while the width data was well described by $0.64 e^{0.24 \cdot \lambda} \mu\text{m}$. In order to estimate cell size we take the cell as a cylinder with two hemispherical ends (*Si et al., 2017; Basan et al., 2015*). Specifically, cell size is estimated from,

$$V = \pi \cdot r^2 \cdot (l - 2r/3), \quad (1)$$

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

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

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

Estimation of Total Protein Content per Cell

In order to estimate total protein per cell for a particular growth rate, we begin by estimating the cell size from the fit shown in **Figure A3(C)** (cell size = $0.533 e^{1.037 \cdot \lambda} \mu\text{m}^3$, as noted in the previous section). We then estimate the total protein content from the total dry mass of the cell. Here we begin by noting that for almost the entire range of growth rates considered here, protein, DNA, and RNA were reported to account for at least 90 % of the dry mass (*Basan et al. (2015)*). The authors also found that the total dry mass concentration was roughly constant across growth conditions. Under such a scenario, we can calculate the total dry mass concentration for protein, DNA, and RNA, which is given by $1.1 \text{ g/ml} \times 30 \% \times 90 \%$ or about $[M_p] = 300 \text{ fg per fL}$. Multiplying this by our prediction of cell size gives the total dry mass per cell.

However, even if dry mass concentration is relatively constant across growth conditions, it is not obvious how protein concentration might vary due to the substantial increase in rRNA at faster growth rates (*Dai et al. (2016)*). The increase in rRNA increases from the linear increase in ribosomal content with faster growth rate (*Scott et al. (2010)*), since it makes up about 2/3 or the ribosomal mass. To proceed we therefore relied on experimental measurements of total DNA content per cell from *Basan et al. (2015)*, and RNA to protein ratios that were measured in *Dai et al.* (and cover the entire range of growth conditions considered here). These are reproduced in **Figure A4(A)** and (B), respectively.

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

$$M_p = \frac{[M_p + M_{RNA}]}{1 + (RP_{ratio})}. \quad (3)$$

(RP_{ratio} refers to the RNA to protein ratio as measured by Dai *et al.*. In **Figure A4(C)** we plot the estimated cellular concentrations for protein, DNA, and RNA from these calculations, and in **Figure A4(D)** we plot their total expected mass per cell. This latter quantity is the growth rate-dependent total protein mass that was used to estimate total protein abundance across all data sets (and summarized in **Figure A1(B)**).

Estimating Volume and Number of Amino Acids from Ribosome Copy Number

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

Given the exponential scaling of V and N_{pep} with growth rate, we performed a linear regression of the log transform of these parameters as a function of the log transform of the ribosome copy number. Using optimization by minimization, we estimated the best-fit values of the intercept and slope for each regression. **Figure A5** shows the result of each regression as a dashed line.

Additional Considerations of Schmidt *et al.* Data Set

While the data set from Schmidt *et al.* (2016) remains a heroic effort that our labs continue to return to as a resource, there were steps taken in their calculation of protein copy number that we argue needed further consideration. In particular, the authors made an assumption of constant cellular protein concentration across all growth conditions and used measurements of cell volume that appear inconsistent with an expected exponential scaling of cell size with growth rate that is well-documented in *E. coli* (Schaechter *et al.* (1958); Taheri-Araghi *et al.* (2015); Si *et al.* (2017)).

We begin by looking at their cell volume measurements, which are shown in blue in **Figure A6**. As a comparison, we also plot cell sizes reported in three other recent papers: measurements from Taheri-Araghi *et al.* and Si *et al.* come from the lab of Suckjoon Jun, while those from Basan *et al.* come from the lab of Terence Hwa. Each set of measurements used microscopy and cell segmentation to determine the length and width, and then calculated cell size by treating the cell as a cylinder with two hemispherical ends, as we considered in the previous section. While there is notable discrepancy between the two research groups, which are both using strain NCM3722, Basan *et al.* found that this came specifically from uncertainty in determining the cell width. This is prone to inaccuracy given the small cell size and optical resolution limits (further described in their supplemental text). Perhaps the more concerning point is that while each of these alternative measurements show an exponential increase in cell size at faster growth rates, the measurements used by Schmidt *et al.* appear to plateau. This resulted in an analogous trend in their final reported total cellular protein per cell as shown in **Figure A7** (purple data points), and is in disagreement with other measurements of total protein at these growth rates (Basan *et al.*, 2015).

Since it is not obvious how measurements of cell size influenced their reported protein abundances, in the following subsections we begin by considering how the authors determined total protein mass per cell. We then consider three different approaches to estimate the growth-rate dependent total protein mass and compare these estimates with those reported by Schmidt *et al.* (2016). Those results are summarized in **Figure A6(B)**, with the original values from both Schmidt *et al.* (2016) and Li *et al.* (2014) shown in **Figure A6(A)** for reference. For most growth conditions, we find reasonable agreement between our estimates and the reported total protein per cell. However, for the fastest growth conditions, with glycerol + supplemented amino acids, and LB media, all estimates are substantially higher than those originally reported. This is the main reason why we chose to readjust protein abundance as shown in **Figure A1(B)** (with the calculation described in section Estimation of Total Protein Content per Cell).

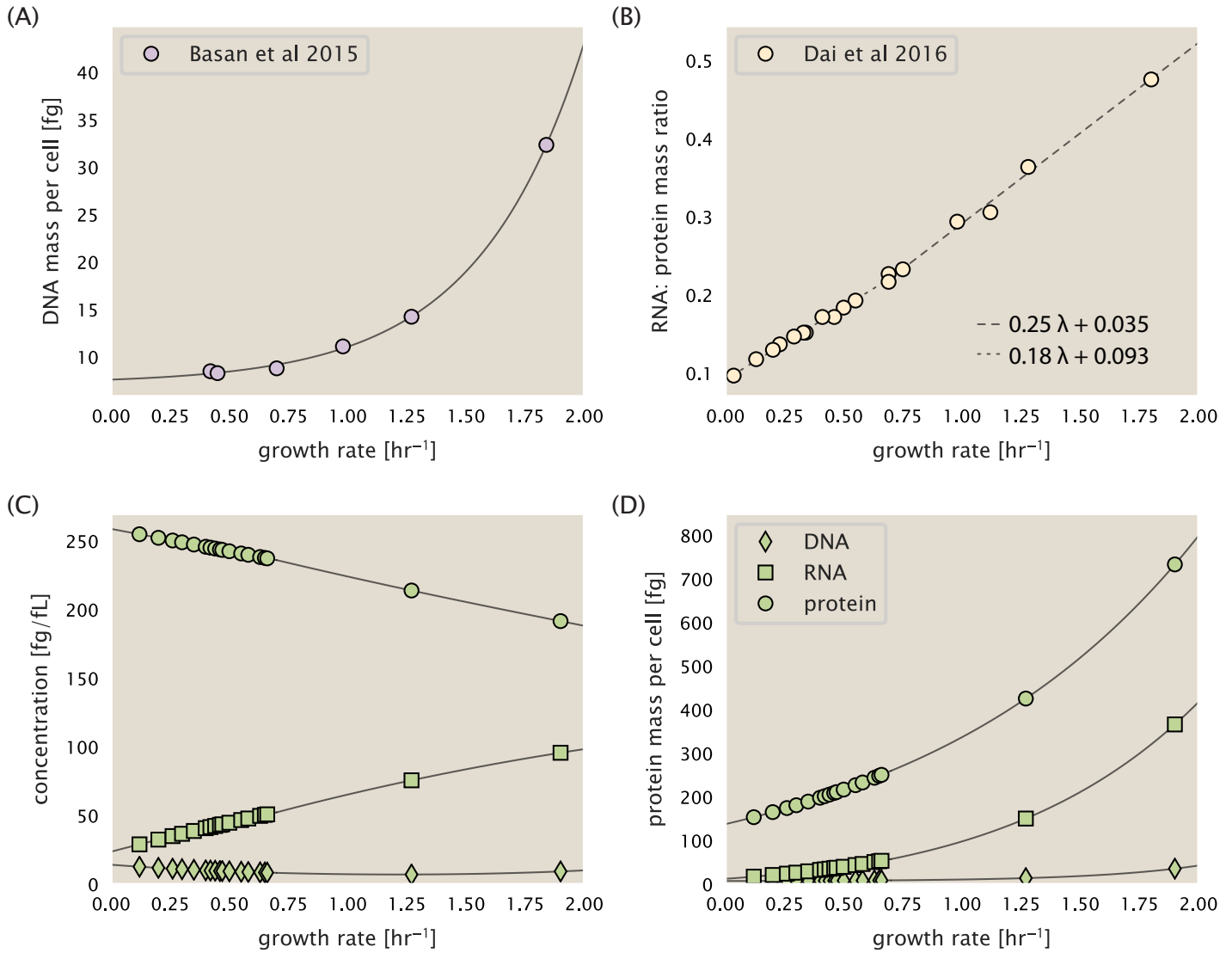


Figure A4. Empirical estimate of cellular protein, DNA, and RNA as a function of growth rate. (A) Measured DNA mass per cell as a function of growth rate, reproduced from Basan *et al.* 2015. The data was fit to an exponential curve (DNA mass in fg per cell is given by $0.42 e^{2.23 \cdot \lambda} + 7.2$ fg per cell, where λ is the growth rate in hr⁻¹). (B) RNA to protein measurements as a function of growth rate. The data was fit to two lines (shown in black) due to the change in slope at slower growth rates *Neidhardt et al. (1991)*; *Dai et al. (2016)*. For growth rates below 0.7 hr⁻¹, the RNA/protein ratio is $0.18 \cdot \lambda + 0.093$, while for growth rates faster than 0.7 hr⁻¹ the RNA/protein ratio is given by $0.25 \cdot \lambda + 0.035$. For (A) and (B) cells are grown under varying levels of nutrient limitation, with cells grown in minimal media with different carbon sources for the slowest growth conditions, and rich-defined media for fast growth rates. (C) Estimation of cellular protein, DNA, and RNA concentration. (D) Total cellular mass estimated for protein, DNA, and RNA using the cell size calculated in Estimation of Cell Size and Surface Area. Symbols (diamond: DNA, square: RNA, circle: protein) show estimated values of mass concentration and mass per cell for the specific growth rates in *Schmidt et al. (2016)*.

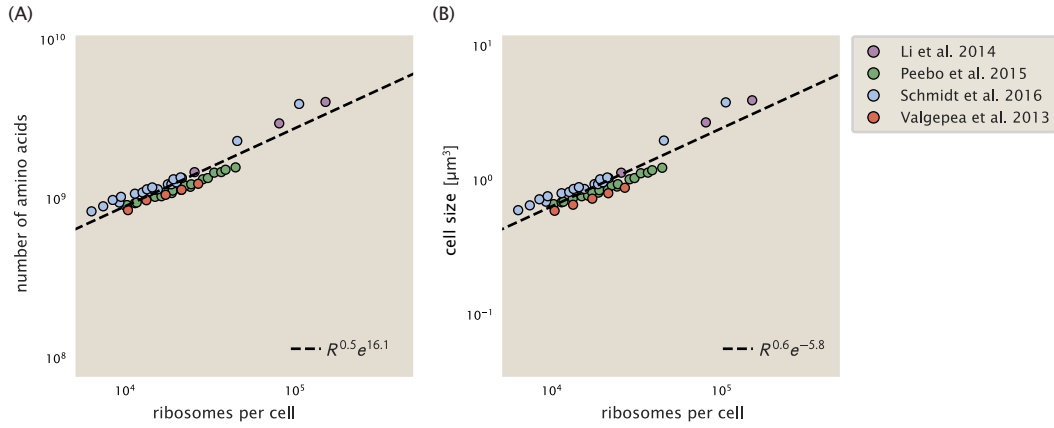


Figure A5. Phenomenological regression of cell volume and number of amino acids per cell as a function of the ribosome copy number. (A) Estimated total number of peptide bonds per cell N_{pep} as a function of number of ribosomes per cell. (B) Estimated cell size as described in Estimation of Cell Size and Surface Area, as a function of number of ribosomes per cell. Colored points correspond to the measured value (or calculated value in the case of the cell size) with colors denoting different data sets. The dashed black line shows the result of the fit, with the functional form of the equation given in the legend with R representing the ribosome copy number.

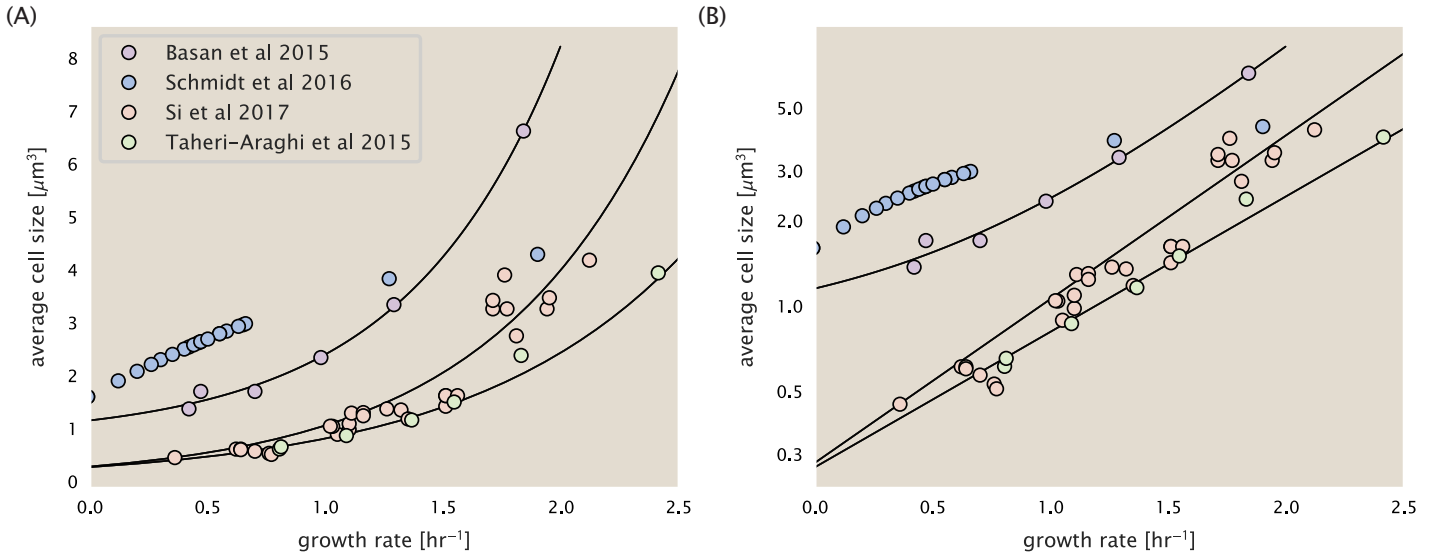


Figure A6. Measurements of cell size as a function of growth rate. (A) Plot of the reported cell sizes from several recent papers. The data in blue come from Volkmer and Heinemann, 2011 (*Volkmer and Heinemann (2011)*) and were used in the work of Schmidt *et al.*. Data from the lab of Terence Hwa are shown in purple (*Basan et al. (2015)*), while the two data sets shown in green and light red come from the lab of Suckjoon Jun (*Taheri-Araghi et al. (2015)*; *Si et al. (2017)*). (B) Same as in (A) but with the data plotted on a logarithmic y-axis to highlight the exponential scaling that is expected for *E. coli*.

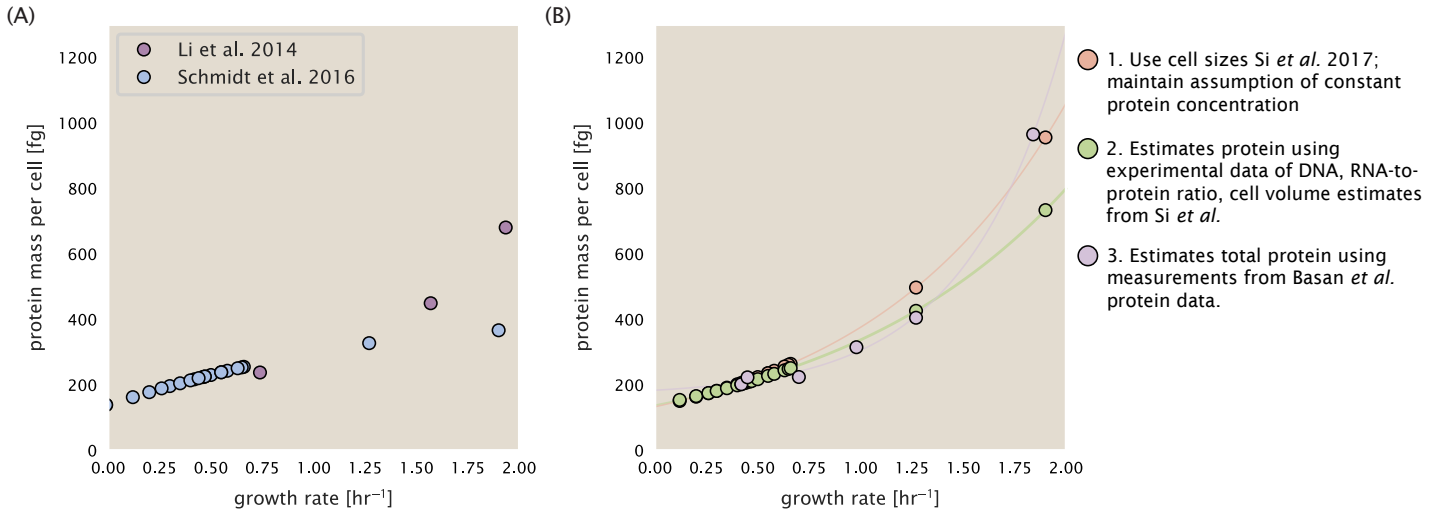


Figure A7. Alternative estimates of total cellular protein for the growth conditions considered in Schmidt *et al.* (A) The original protein mass from Schmidt *et al.* and Li *et al.* are shown in purple and blue, respectively. (B) Three alternative estimates of total protein per cell. 1. *light red*: Rescaling of total protein mass assuming a growth rate independent protein concentration and cell volumes estimated from Si *et al.* 2017. 2. *light green*: Rescaling of total protein mass using estimates of growth rate-dependent protein concentrations and cell volumes estimated from Si *et al.* 2017. Total protein per cell is calculated by assuming a 1.1 g/ml cellular mass density, 30% dry mass, with 90% of the dry mass corresponding to DNA, RNA, and protein (Basan *et al.*, 2015). See Estimation of Total Protein Content per Cell for details on calculation. 3. *light purple*: Rescaling of total protein mass using the experimental measurements from Basan *et al.* 2015.

Effect of cell volume on reported absolute protein abundances

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

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

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

$$M_{p,i} = [M_p]_{orig} \cdot V_i \quad (4)$$

where $M_{p,i}$ represents the total protein mass per cell and V_i is the cell volume for each growth condition i as measured in Volkmer and Heinemann, 2011. Here the thinking is that the values of $M_{p,i}$ reflects the total cellular protein for growth condition i , where any discrepancy from their absolute protein abundance is assumed to be due to protein loss during sample preparation. The protein abundances from their absolute abundance measurements noted above were therefore scaled to their estimates and are shown in Figure Figure A7 (purple data points).

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

$$M'_{P,i} = [M_P]_{Si} \cdot V_{Si,i} \quad (5)$$

where $M'_{P,i}$ is the new protein mass prediction, and $V_{Si,i}$ refers to the new volume prediction for each condition i . These are shown as red data points in Figure [Figure A7\(B\)](#).

Relaxing assumption of constant protein concentration across growth conditions

We next relax the assumption that cellular protein concentration is constant and instead, attempt to estimate it using experimental data. Here we use the estimation of total protein mass per cell detailed in [Estimation of Total Protein Content per Cell](#) for all data points in the [Schmidt *et al.* \(2016\)](#) data set. The green data points in [Figure A7\(B\)](#) show this prediction, and this represents the approach used to estimate total protein per cell for all data sets.

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

One of the challenges in our estimates in the preceding sections is the need to estimate protein concentration and cell volumes. These are inherently difficult to measure accurately due to the small size of *E. coli*. Indeed, for all the additional measurements of cell volume included in Figure [Figure A6](#), no measurements were performed for cells growing at rates below 0.5 hr^{-1} . It therefore remains to be determined whether our extrapolated cell volume estimates are appropriate, with the possibility that the logarithmic scaling of cell size might break down for slower growth.

In our last approach we therefore attempt to estimate total protein using experimental data that required no estimates of concentration or cell volume. Specifically, in the work of Basan *et al.*, the authors measured total protein per cell for a broad range of growth rates (reproduced in Figure [Figure A8](#)). These were determined by first measuring bulk protein from cell lysate, measured by the colorimetric Biuret method ([You *et al.* \(2013\)](#)), and then abundance per cell was calculated from cell counts from either plating cells or a Coulter counter. While it is unclear why Schmidt *et al.* was unable to take a similar approach, the results from Basan *et al.* appear more consistent with our expectation that cell mass will increase exponentially with faster growth rates. In addition, although they do not consider growth rates below about 0.5 hr^{-1} , it is interesting to note that the protein mass per cell appears to plateau to a minimum value at slow growth. In contrast, our estimates using cell volume so far have predicted that total protein mass should continue to decrease slightly for slower growing cells. By fitting this data to an exponential function dependent on growth rate, we could then estimate the total protein per cell for each growth condition considered by [Schmidt *et al.* \(2016\)](#). These are plotted as red data points in [Figure A7\(B\)](#).

Calculation of Complex Abundance

All protein data quantified the abundance of individual proteins per cell. However, this work requires estimates on the abundance of individual protein *complexes*, rather than the copy number of individual proteins. In our analysis of the protein copy number data, it became clear that the reported copy numbers do not always align with those based on reported stoichiometry. As one example of this, the F-O subunit of ATP synthase consists of three protein subunits with a stoichiometry of $[\text{AtpB}][\text{AtpF}]_2[\text{AtpE}]_{10}$ (also referred to as subunits a, b, and c, respectively). In the experimental data of [Schmidt *et al.* \(2016\)](#), the values deviate from this quite substantially, with approximately 1000 AtpB, 9000 AtpF, and 300 AtpE reported per cell (minimal media + glucose growth condition). This highlights the technical challenges that still remain in our ability to quantify cellular composition, particularly for membrane-bound proteins like the ATP synthase complex considered here. In this section, we outline the approach we used to annotate proteins as part of each macromolecular complex and how we used averaging across the individual protein measurements to estimate an absolute complex abundances per cell.

Protein complexes, and proteins individually, often have a variety of names, both longform and shorthand. As individual proteins can have a variety of different synonyms, we sought to ensure that each protein annotated in the data sets used the same synonym. To do use, we relied heavily on the EcoCyc Genomic Database ([Keseler *et al.*, 2017](#)). Each protein in available data sets included an annotation of one of the gene name synonyms as well as an accession ID – either a UniProt or Blattner "b-number". We programmatically matched up individual accession IDs between the proteins in different data sets. In cases where accession IDs matched but the gene names were different, we manually verified that the gene product was the same between the datasets and

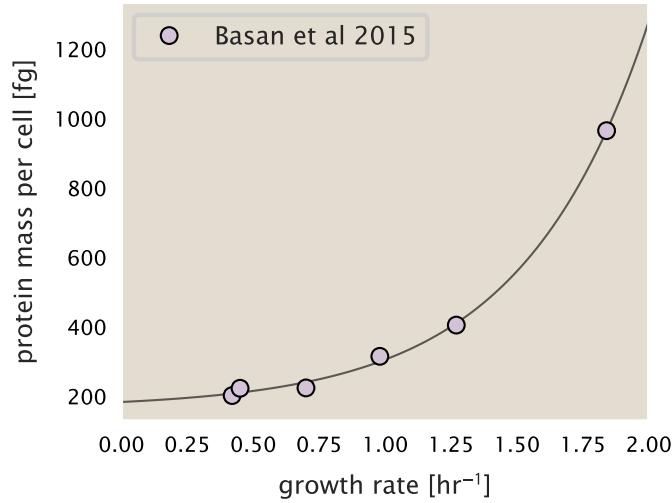


Figure A8. Total cellular protein reported in Basan *et al.* 2015. Measured protein mass as a function of growth rate as reproduced from Basan *et al.* 2015, with cells grown under different levels of nutrient limitation. The data was fit to an exponential curve where protein mass in fg per cell is given by $14.65 e^{2.180 \cdot \lambda} + 172$ fg per cell, where λ is the growth rate in hr^{-1}).

chose a single synonym. All code used in the data cleaning and unification procedures can be found on the associated [GitHub repository] (DOI:XXX) associated with this paper as well as on the associated [paper website](#).

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

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

$$N_{\text{complex}}(\text{subunit } i) = \frac{p_{\text{subunit } i}^{(\text{measured})}}{m_{\text{subunit } i}}. \quad (6)$$

Here, $p_{\text{subunit } i}^{(\text{measured})}$ refers to the measured protein copy number of species i , and m refers to the number of monomers present for that protein in the complex. For example, the 70S mature ribosome complex has 55 protein components, all of which are present in a single copy except L4 (RplL), which is present in 4 copies ($m = 4$). For each ribosomal protein, we then calculate the maximum number of complexes that could be formed using [Equation 6](#). This example, along with example from 5 other macromolecular complexes, can be seen in [Figure A9](#).

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

$$\langle N_{\text{complex}} \rangle = \frac{1}{N_{\text{subunits}}} \sum_i^{N_{\text{subunits}}} \frac{p_i^{(\text{measured})}}{m_{\text{subunit } i}}. \quad (7)$$

in [Figure A9](#), we show this mean value as a grey line for a variety of different complexes. Additionally, we have built an interactive figure accessible on the [paper website](#) where the validity of this approach can be examined for any complex with more than two subunits (thus, excluding monomers and dimers).

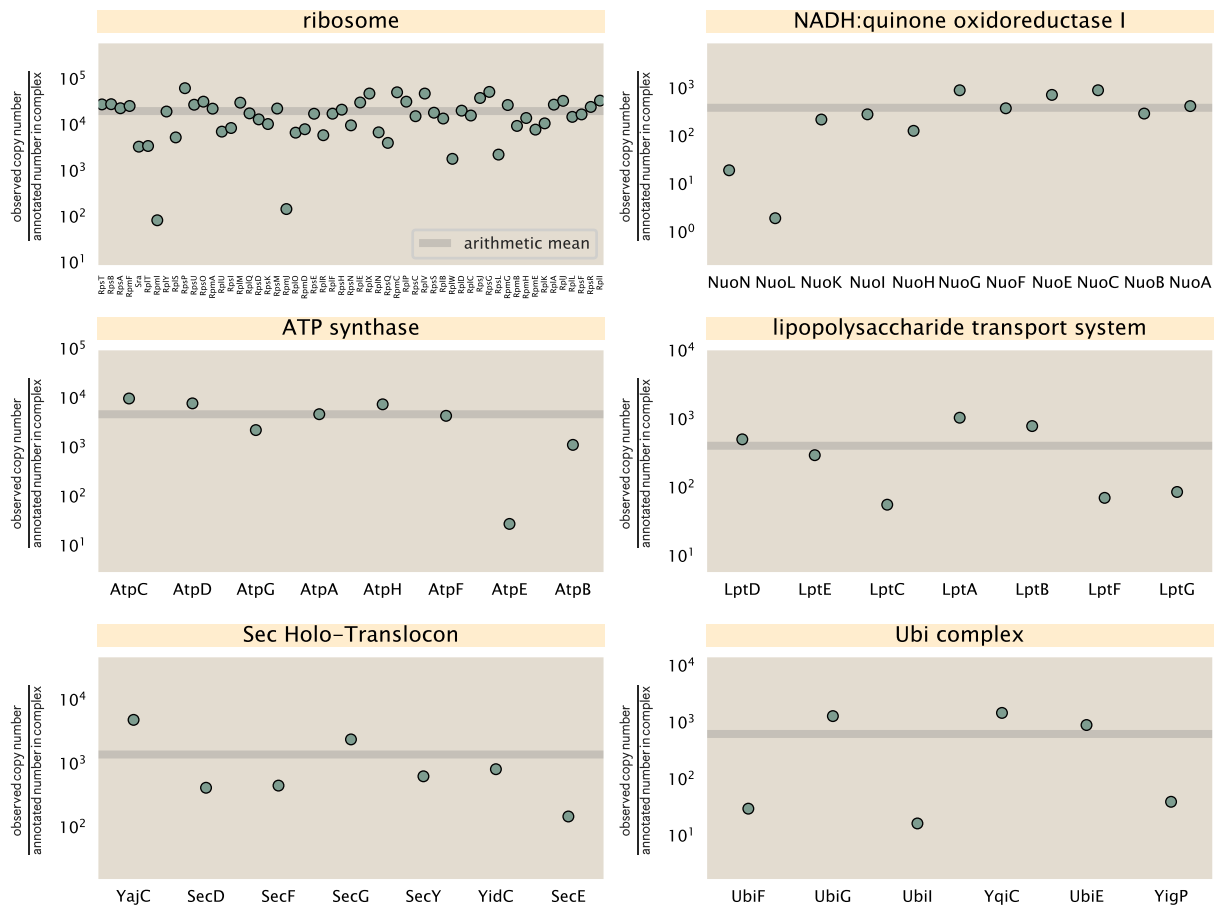


Figure A9. Calculation of the mean complex abundance from measurements of single subunits. Six of the largest complexes (by number of subunits) in *E. coli*. Points correspond to the maximum number of complexes that can be formed given measurement of that individual protein. Solid grey line corresponds to the arithmetic mean across all subunits. These data correspond to measurements from *Schmidt et al. (2016)* in a glucose-supplemented minimal growth medium.

Extending Estimates to a Continuum of Growth Rates

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

Estimation of the total cell mass

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

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

$$m_{\text{cell}} \approx \rho V(\lambda) \approx \rho a e^{\lambda b} \quad (8)$$

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

Complex Abundance Scaling With Cell Volume

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

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

$$m_X \approx 0.3 \times \rho V(\lambda) \theta_X, \quad (9)$$

where we have used $\rho V(\lambda)$ as an estimate of the total cell mass, defined in *Equation 8*. To convert this to the number of units N_X of substance X in the cell, we can use the formula weight w_X of a single unit of X in conjunction with *Equation 9*,

$$N_X \approx \frac{m_X}{w_X}. \quad (10)$$

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

$$N_{\text{transporters}}(\lambda) = \frac{0.3 \times \rho V(\lambda) \theta_X}{w_X r_X \tau}. \quad (11)$$

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

Equation 11 is a very general relation for processes where the cell volume is the "natural variable" of the problem. This means that, as the cell increases in volume, the requirements for substance X also scale with volume rather than scaling with surface area,

for example. So long as the rate of the process, the fraction of the dry mass attributable to the substance, and the formula mass of the substance is known, **Equation 11** can be used to compute the number of complexes needed. For example, to compute the number of ATP synthases per cell, **Equation 11** can be slightly modified to the form

$$N_{\text{ATP synthases}}(\lambda) = \frac{0.3 \times \rho V(\lambda) \theta_{\text{protein}} N_{\text{ATP}}}{w_{\text{AA}} r_{\text{ATP}} \tau}, \quad (12)$$

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

A Relation for Complex Abundance Scaling With Surface Area

In our estimation for the number of complexes needed for lipid synthesis and peptidoglycan maturation, we used a particular estimate for the cell surface area ($\approx 5 \mu\text{m}^2$, BNID: 101792) and the fraction of dry mass attributable to peptidoglycan ($\approx 3\%$, BNID: 101936). Both of these values come from glucose-fed *E. coli* in balanced growth. As we are interested in describing the scaling as a function of the growth rate, we must also consider how these values scale with cell surface area, which is the natural variable for these types of processes. In the coming paragraphs, we highlight how we incorporate a condition-dependent surface area into our calculation of the number of lipids and murein monomers that need to be synthesized and crosslinked, respectively.

Number of Lipids

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

$$N_{\text{lipids}}(\lambda) \approx \frac{4 \text{ leaflets} \times 0.4 \times \eta \pi \left(\frac{\eta \pi}{4} - \frac{\pi}{12} \right)^{-2/3} V(\lambda)^{2/3}}{A_{\text{lipid}}} \quad (13)$$

where η is the length-to-width aspect ratio and V is the cell volume.

Number of Murein Monomers

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

$$N_{\text{murein monomers}}(\lambda) \approx \frac{\rho_{\text{pg}} w \eta \pi \left(\frac{\eta \pi}{4} - \frac{\pi}{12} \right)^{-2/3} V(\lambda)^{2/3}}{m_{\text{murein}}}, \quad (14)$$

where ρ_{pg} is the density of peptidoglycan.

Complex Abundance Scaling With Number of Origins, and rRNA Synthesis

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

$$\langle \# \text{ori} \rangle \approx 2^{t_{\text{cyc}}^2 / \ln 2} \quad (15)$$

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

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

$$D_{\text{rRNA}}(\lambda) \approx N_{\text{rRNA operons}} \times 2^{t_{\text{cyc}} \lambda / \ln 2}. \quad (16)$$

This makes the approximation that *all* rRNA operons are localized around the origin. In reality, the operons are some distance away from the origin, making **Equation 16** an approximation (**Dennis et al., 2004**).

In the main text, we stated that at a growth rate of 0.5 hr^{-1} , there is ≈ 1 chromosome per cell. While a fair approximation, **Equation 15** illustrates that is not precisely true, even at slow growth rates. In estimating the number of RNA polymerases as a function of growth rate, we consider that regardless of the number of rRNA operons, they are all sufficiently loaded with RNA polymerase such that each operon produces one rRNA per second. Thus, the total number of RNA polymerase as a function of the growth rate can be calculated as

$$N_{\text{RNA polymerase}}(\lambda) \approx L_{\text{operon}} D_{\text{rRNA}} \rho_{\text{RNA polymerase}}, \quad (17)$$

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

Calculation of active ribosomal fraction.

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

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

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

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

In **Figure A10(A)** and **(B)** we plot the measured t_C and t_{cyc} values versus the doubling time from **Si et al. (2017)**. The authors estimated t_C by marker frequency analysis using qPCR, while $t_{\text{cyc}} = t_C + t_D$ were inferred from t_C and τ . In the plots we see that both t_C and t_{cyc} reach a minimum at around 40 and 75 minutes, respectively. For a C period of 40 minutes, this would correspond to a maximum rate of elongation of about 1,000 bp/sec. Since we lacked a specific model to describe how each of these parameters vary with growth condition, we assumed that they were linearly dependent on the doubling time. For each parameter, t_C and t_{cyc} , we split them up into two domains corresponding to poorer nutrient conditions and rich nutrient conditions (cut off at $\tau \approx 40$ minutes where chromosomal replication becomes nearly constant). The fit lines are shown as solid black lines. In **Figure A10(C)** and **(D)** we also show t_C and t_{cyc} as a function of growth rate λ along with our piecewise linear fits, which match the plots in the main text.

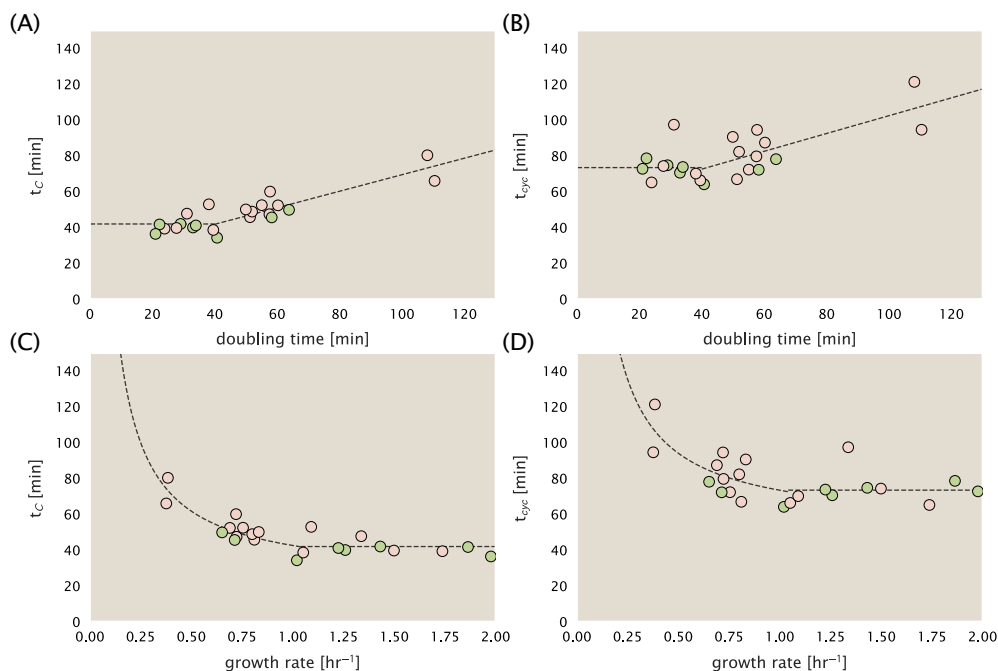


Figure A10. Estimation of $\langle \#ori \rangle / \langle \#ter \rangle$ and $\langle \#ori \rangle$ using data from Si *et al.* (2017). (A) and (B) plot the reported t_C and t_{cyc} as a function of cell doubling time τ , respectively. The dashed lines show a piecewise fit to the data. For short doubling times (rich media), t_C and t_{cyc} are assumed constant ($t_C = 42$ minutes, $t_{cyc} = 73$ minutes). At the transition, taken to occur at 40 minutes, the dashed line corresponds to an assumed proportional increase in each parameter as a function of the doubling time ($t_C = 0.46 \tau + 23.3$ minutes, $t_{cyc} = 0.50 \tau + 52.7$ minutes). (C) and (D) plot the same data as in (A) and (B), but as a function of growth rate, given by $\lambda = \ln(2)/\tau$.

Derivation of Minimal Model for Nutrient-Mediated Growth Rate Control

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

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

In our model, we need to determine the rate of peptide elongation r_i , which we consider as simply depending on the supply of amino acids (and, therefore, also amino-acyl tRNAs) through a parameter r_{AA} in units of AA per second, and the rate of amino acid consumption by protein synthesis ($r_i \times R \times f_a$). The balance between these two rates will determine the effective amino acid concentration in the cell $[AA]_{eff}$. An important premise for this formulation is growing evidence that cells are able to modulate their biosynthesis activity according to nutrient availability (i.e. extent of chromosomal replication, transcriptional, and translation activity) through secondary-messenger molecules like (p)ppGpp (Hauryliuk *et al.*, 2015; Zhu and Dai, 2019; Kraemer *et al.*, 2019; Fernández-Coll *et al.*, 2020; Büke *et al.*, 2020). Given our observation that protein synthesis and energy production are not limiting, we assume that other molecular players required by ribosomes like elongation factors and GTP are available in sufficient abundance. In addition, experimentally, the relative number of tRNA and elongation factor EF-Tu per ribosome have been found to increase in poorer nutrient conditions (Pedersen (1978); Dong *et al.* (1996); Klumpp *et al.* (2013)).

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

$$\frac{1}{r_t} = \frac{1}{k_{on}\alpha[AA]_{eff}} + \frac{1}{r_t^{max}}. \quad (18)$$

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

$$K_D = \frac{r_t^{max}}{\alpha k_{on}}, \quad (19)$$

where the elongation rate r_t is now given by

$$r_t = \frac{r_t^{max}}{1 + K_D/[AA]_{eff}}. \quad (20)$$

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

$$\int_0^t \frac{d[AA]_{eff}}{dt} dt = \int_0^t ([r_{AA}] - [r_t \times R \times f_a]) dt. \quad (21)$$

This considers the net change in amino acid concentration over a time from 0 to t , with the square brackets indicating concentrations per unit time. Integrating Equation 21 yields,

$$[AA]_{eff} = t([r_{AA}] - [r_t \times R \times f_a]). \quad (22)$$

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

$$[AA]_{eff} = \frac{t(r_{AA} - r_t \times R \times f_a)}{V \times N_A}, \quad (23)$$

where r_{AA} is in units of AA per unit time and r_t is in units of AA per unit time per ribosome. N_A refers to Avogadro's number and is needed to convert between concentration and absolute numbers per cell. With an expression for $[AA]_{eff}$ in hand, we can now solve Equation 20 for r_t , which is a quadratic function with a physically-meaningful root of

$$r_t = \frac{t(r_{AA} + r_t^{(max)} R f_a) + K_D V N_A - \sqrt{(r_{AA} t + r_t^{(max)} R f_a t + K_D V N_A)^2 - 4(R f_a t)(r_t^{(max)} r_{AA} t)}}{2 R f_a t}. \quad (24)$$

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

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

The second and last point we note is that the recent works from *Dai et al. (2016)* and *Klumpp et al. (2013)* also employ a similar coarse-graining of translation elongation as we've considered above. Here, however, a notable distinction is that the authors consider the entire ternary complex (i.e. the complex of amino-acyl tRNA, EF-Tu, and GTP) as rate limiting. Further, through an assumed proportionality between ternary complex and ribosome abundance, they arrive at a formulation of elongation rate r_t that exhibits a Michaelis-Menten dependence on the ribosomal fraction Φ_R . They demonstrate that all their measurements of elongation rate, even upon addition of sublethal doses of chloramphenicol (which cause an increase in both r_t and Φ_R), can be collapsed onto a single curve described by this Michaelis-Menten dependence. There is always a benefit to increase their ribosomal fraction Φ_R on growth rate when nutrient conditions allow (see Section "Maximum Growth Rate is Determined by the Ribosomal Mass Fraction" on the main text), and this trend in the data in part follows from the tendency for cells to increase Φ_R and better maximize r_t as nutrient conditions improve. In addition, it does not account for the decrease in the fraction of actively translating ribosome f_a that was strikingly apparent at slow growth rates or in sublethal doses of chloramphenicol in the work of *Dai et al. (2016)*. Through **Equation 24** we also account for changes in the fraction of actively translating ribosomes. Ultimately, we find that cells are able to maximize both Φ_R , r_t , and their growth rate only to the extent allowed by the nutrient conditions (i.e. via r_{AA}) and through the maintenance of the cellular pool of amino acids $[AA]_{\text{eff}}$, amino-acyl tRNA, GTP, as well as the synthesis of other key molecular constituents like EF-Tu.

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