Supplemental Information:

N. Belliveau, G. Chure, J. Theriot, R. Phillips

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1 Summary of Proteome Datasets.

Here we briefly summarize the datasets that were considered for the work of the main text. The goal of this section is to give an overview of each dataset considered, including the main experimental details, and to provide a more detailed look at how well each compares.

Table **??** provides an overview of the proteomic datasets that we found in the literature. These are predominately mass spectrometry-based, with the exception of the work from Li *et al.* (2014) which used ribosomal profiling, and the fluorescence-based counting done in Taniguchi *et al.* (2010). The general strategy taken in these works is to quantify fractional abundance of each protein and then to convert these to absolute abundance by multiplying these fractions by the bulk measured total cellular protein abundance. Note that the work of Peebo *et al.* (2014) did not perform any measurement of cell count or volume, and thus were only able to report cellular protein concentration.

Exceptions to this are found in Schmidt *et al.* and Taniguchi *et al.*. A key distinction in the work of Schmidt *et al.* is that in addition to determining relative abundance by mass spectrometry, they also selected 41 enzyme that cover over four orders of magnitude in cellular abundance to use in absolute protein quantification. Specifically, synthetic peptides were generated for each of these 41 enzymes and used to provide a calibration between measured mass spectrometry intensities and absolute protein abundances (using stable isotope dilution (SID) and selected reaction monitoring (SRM), though the details of this are beyond the scope of this section). In the work of Taniguchi *et al.*, the authored tagged each protein with a yellow fluorescent protein (YFP) and used fluorescence as readout of cellular expression.

Author	Method	Strain	N datasets	Reported Quantity	f
Taniguchi *et al.* (2010)	YFP-fusion, cell fluorescence			fg/copies per cell	
Valgepea *et al.* (2012)	Mass spectrometry			fg/copies per cell	
Peebo *et al.* (2014)	Mass spectrometry			fg/copies per fL	
Li *et al.* (2014)	Ribosomal profiling			protein synthesis rate	
Soufi *et al.* (2015)	Mass spectrometry			fg/copies per cell	
Schmidt *et al.* (2016)	Mass spectrometry			fg/copies per cell	
Caglar *et al.* (2017)	Mass spectrometry			relative abundance	

Figure **??** shows the distribution in reported protein abundance for a subset of the data.

An important consideration is whether the reported abundance per cell are correlated. while we expect some variability in expression of each protein due to growth rate, the

reported values are nonetheless expected to be correlated. Figure 2 compares each dataset to the copy numbers from Schmidt *et al.*, grown in M9 minimal media supplemented with glucose.

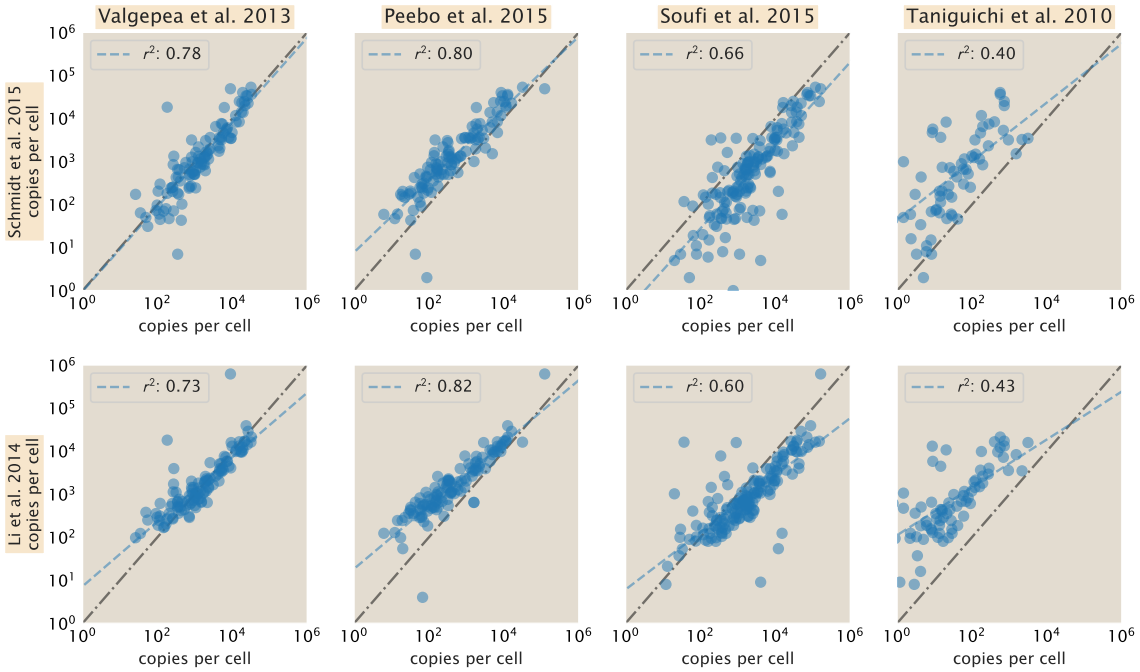

Figure 1

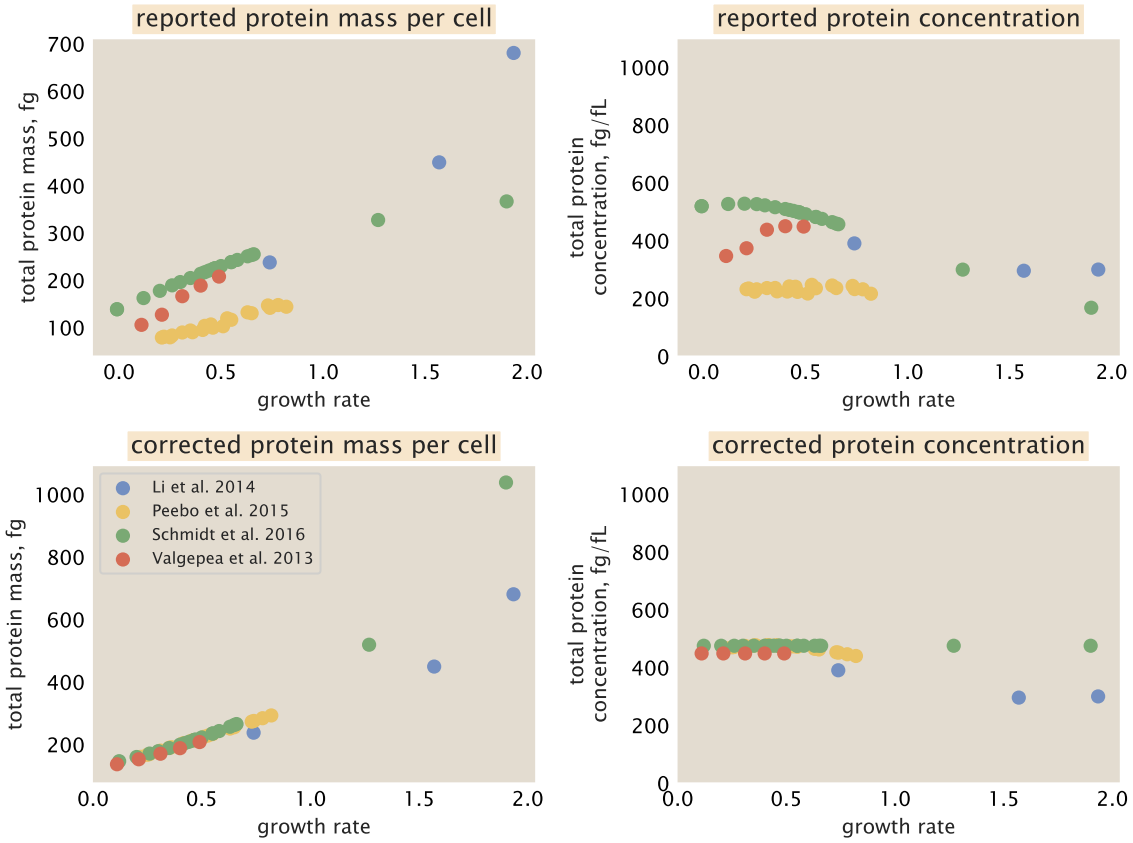

Figure 2

2 Adjustments to Copy Number Data.

The datasets encompass a wide range of bacterial growth conditions and different *e. coli* strains. In order to determine protein abundance on a cell basis, different strategies were taken to determine cell counts and cell volume. It was therefore important to consider if any obvious discrepancies exist across the data and whether these might be reasonably dealt with to make the compiled data set internally consistent. *a priori*, there are well-documented observations about how characteristics such as total protein mass per cell and cell volume should scale with growth rate. We were therefore inclined to only renormalize data in a way that was consistent with expectations. In this section we describe the data manipulations that were applied to those originally reported.

Figure **??**(A) compares the total protein mass reported as a function of growth rate for each of the original data sets, while in Figure **??**(B) we plot the final values that were used in this work. A .csv file containing protein copy numbers per cell and mass per cell across all data sets is available for download on our GitHub repository.

2.1 Adjustments to Schmidt *et al.* dataset

While the dataset from Schmidt *et al.* remains a heroic effort and has provided our lab with a resource that we continue to return to, there were several steps taken in their final calculation of protein copies per cell that needed to be reconsidered. In particular, the authors made an assumption of constant cellular protein concentration across all growth conditions and also used cell volume measurements that appear inconsistent with the expected exponential scaling of cell size with growth rate that has been well documented in *E. coli* [1, 2, 3]. This resulted in a similar non-exponential scaling of total cellular protein that is unexpected and in disagreement with other data [4].

We first consider the cell volume measurements that were used in their data adjustment, shown in blue in Figure 3. Figure [] we show the cell volumes reported from [, along with cell volumes reported in three other recent papers (part (A) shows the data with a linear y-axis, while part (B) shows it on a log scaled y-axis). The measurements from Taheri-Araghi *et al.* and Si *et al.* from from the lab of Suckjoon Jun, while those from Basan *et al.* come from the lab of Terence Hwa. Each of these additional measurements used microscopy and cell segmentation to determine the long and short axes, and then cell size was determined by treating the cell is a cylinder with two hemispherical ends. While there is a large discrepancy in cell size between the two research groups, Basan *et al.* shows in their supplemental material that this is specifically from uncertainty in determining the cell width, which is prone to error given the small cell size and optical resolution limits. Perhaps the more concerning point is that while these alternative measurements show the expected exponential increase in cell size as growth rate increases, the measurements used by Schmidt *et al.* appear to cause the total cellular protein to plateau toward some maximum value.

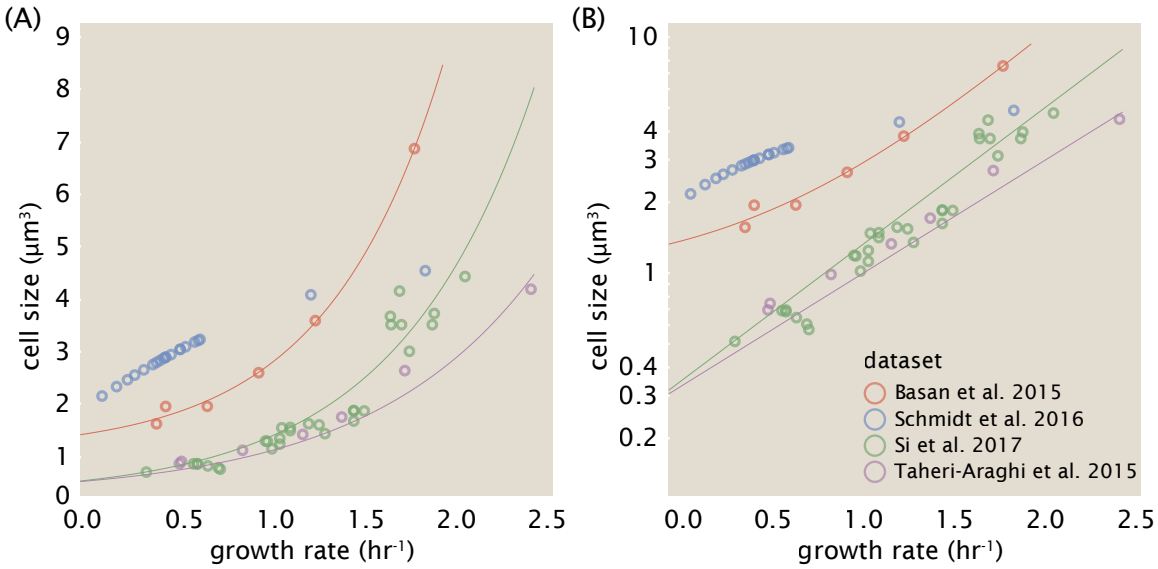

Figure 3: Measurements of cell size as a function of growth rate. (A) Plot of the reported cell sizes from several recent papers. The data in blue come from Volkmer and Heinemann, 2011 [5] and were used in the work of Schmidt *et al.*. Data from the lab of Terence Hwa are shown in red [4], while the two data sets shown in green and purple come from the lab of Suckjoon Jun [2, 3]. (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*.

in order to deal with potential loss during protein extraction, the authors first determined total protein independently with a BCA protein assay. With their flow cytometry cell counts, and a measurement of cell volume from earlier work of theirs, they calculated a cellular protein concentration. In personal communications, it was noted that determining reasonable total protein abundances by BCA across cells under the array of growth conditions was particularly troublesome. Instead, they made the assumption that cellular protein concentration remains constant across growth conditions. Using their previous cell volume measurements, which do vary with growth rate, they calculated the expected total protein mass at each growth condition and rescaled their absolute protein measurements to be consistent with these values.

In Figure [] we perform the total protein mass correction that Schmidt *et al.* used, but instead using the cell volume measurements from Si *et al.*. Following this adjustment, the total protein mass exibits an exponential increase in protein mass appears to be more consistent with expectations that the cell size should increease exponentially with growth rate.

As a quick estimate of protein concentration, if we assume a cellular mass density of 1.1 g/ml, 30% dry mass, and 55 % of the dry mass taken up by protein, the expected cellular protein concentration is given by 1.1 g/ml x 30 % x 60 %, which gives us about 200 fg / fL total protein.

We now turn to the assumption that cellular protein concentration is relatively independent of growth rate. One important experimental result that suggests some caution here is that the total RNA to protein ratio increases linearly as function of growth rate [].

This is due to substantial increase in rRNA as cells grow faster. While total dry mass was indeed found to increase linearly with cell size [], which itself suggests a constant dry mass concentration, it does not mean protein concentration will stay constant. In order to gain some intuition here, we will make use of experimental measurements of RNA to protein ratio from the recent work of Dai *et al.*, and DNA mass from Basan *et al.* These are shown in Figure []. Using this information, and the observation that the sum of protein, RNA, and DNA account for about 90 % of the dry mass [], we can predict the concentration of each of these as a function of growth rate. Indeed, given the increasing rRNA, we predict a significant decrease in cellular protein concentration at faster growth rates.

In the next three subsections we consider three different approaches to rescale the reported protein abundances from Schmidt *et al.* to make them consistent with our expectation that cell size and total protein should increase exponentially with growth rate. Figure [] summarizes Ultimately, we find that each approach leads to a similar result, with absolute abundances only changing substantially in the fastest growth conditions, in LB media and M9 minimal media + glycerol and amino acid supplement.

2.1.1 Schmidt *et al.* copy number correction, approach 1.

The first approach we consider is to only readjust the protein copy numbers such that the cell volumes used in the calculation of total protein grow exponentially with faster growth rates. Here we will allow that cellular protein concentrations remain constant with growth rate. In order to perform this calculation, we note that Schmidt *et al.* took growth in glucose as a reference condition, and measured a total mass of $P = 240$ fg per cell. Using their cell volume, reported as $V_{orig} = 2.84$ fL, we can calculate a cellular protein concentration of $\rho_{orig} = P/V_{orig} = 85$ fg/fL. If we instead use the cell volume predicted in the work of Si *et al.* which is found to be $V_{Si_{glu}} =$ [], the new cellular protein concentration becomes $\rho_{Si} = P/V_{Si_{glu}} =$ [] fg/fl. With the assumption that protein concentration remains constant, the rescaled total protein mass per cell for all conditions can be calculated from,

$$P = \rho_{Si} \cdot V_{Si_i} \tag{1}$$

where V_{Si_i} refers to the new volume prediction for each condition i, based on the predictions of Si *et al.*

2.1.2 Schmidt *et al.* copy number correction, approach 2.

In this next approach we instead rescale the total protein mass according to our prediction of cellular protein cocentration above. Specifically, we assume a cellular mass density of 1.1 g/ml, a 30% dry mass that remains constant with growth rate, and 90 % of the dry mass consisting of protein, DNA, and RNA. The total mass concentration of protein, DNA, and RNA will then be given by 1.1 g/ml x 30 % x 90 % or about $\rho = 300$ fg per fL. We again turn to the cell volume predictions from Si *et al.* to calculate the mass per cell. Finally, from the measured DNA mass, and RNA to protein ratio shown in Figure [], we calculate the total protein mass per cell. These are shown in Figure [].

2.1.3 Schmidt *et al.* copy number correction, approach 3.

In our last appoach we make no assumptions about protein concentration or cell volume. Instead we rescale the total protein according to the experimentally measured protein per cell that was reported in the work Basan *et al* and is reproduced in Figure []. While the strain of *E. coli* appears to differ from the strain used by Schmidt *et al.*, we note that the absolute protein abundances appear consistent with the values we estimated in the previous section and suggest this approach is reasonable valid. To estimate the total protein for each growth condition considered, we fit the data from Basan *et al.* to an exponential function and then use this to predict total protein mass for each of the measured growth rates. These are also plotted in Figure [].

One parameter that we do not expect to change substantially across growth conditions is cellular protein concentration. As a general rule of thumb, we expect an *e. coli* cell to have about 30% dry mass, with about 55% of this expected from protein. With a density of about 1.1 g/ml, we find that the protein concentration in a cell should be approximately 180 fg/fL. The cellular density and dry mass are essentially fixed, with the fraction of cellular protein varying from [X-Y; refs??]. Hence, this parameter provides a useful reference point that datasets should agree on. Indeed, out of concern over differences in protein extraction efficiency in growth phases like stationary phase, Schmidt *et al.* applied a correction to their measured protein abundances to ensure cellular protein concentrations were internally consistent.

From the work of Schmidt *et al.* they reported an ability to consistently get high protein yield from cells grown in M9 minimal media supplemented with glucose. In order to account any protein loss during extraction, they use their measured protein concentration from this sample as a reference for which total protein concentration in all other growth conditions should match. This is shown in Figure **??**A. One challenge in performing this calculation is that cell volume must be known; the authors use volumes that were measured by flow cytometry in previous work [cite]. These volumes are shown in Figure **??**B. While it is difficult to assess the accuracy of these numbers, we find them to be quite inconsistent with the expected scaling that is reported by Taheri-Araghi *et al.* (2015), carefully measured as a function of growth rate [and other work?].

In addition, since cell volume was not determined in all studies, and to be consistent throughout, we instead use the predicted cell volumes from Taheri-Araghi *et al.*. Dealing with each dataset seperately, we apply correction factors to correct for discrepencies in protein concentration across the different growth conditions considered [NB: I wonder if in these other datasets, the more appropriate thing to do is match to the average measured protein concentration]. Specifically, the scaling factor ϕ is given by,

$$\phi = \frac{P_i}{V_i} \cdot [P]_r \tag{2}$$

where P_i is the total protein mass in conditino i, V_i is the estimated cell volume, and $[P]_r$ is the reference protein concentration (i.e. growth in glucose for the Schmidt data).

2.2 Peebo *et al.*: Conversion from copies/ fL to copies per cell

In the work of Peebo *et al.*, the authors only report protein concentration. In order to determine protein per cell, we multiple these concentrations by expected cell volumes using the predictions from Taheri-Araghi *et al.* This is shown in Figure **??**A, where we see that reported mass is substantially lower than the other work considered here; as well as work from others [Sinauer, 1990].

Indeed, both Schmidt *et al.* and Li *et al.* reported a total protein mass of about 250 fg per cell at a growth rate of about $\lambda \approx 0.5hr^{-1}$ (M9 minimal media with glucose and MOPS minimal media, respectively). Given this discrepancy, in addition to requiring that cellular protein concentration be internally consistent across the growth conditions they reported on, we also required that total cellular mass be consistent with the work Schmidt *et al.* and Li *et al.* This amounted to performing a linear regression between total protein mass and growth rate, and using this to scale the Peebo *et al.* dataset according to this trend.

3 Translation-dependent limits on the rate of cell division.

Here we consider the hypothesis that the process of translation sets the speed limit of bacterial growth. We begin by considering the synthesis of the ribosome itself, finding that it sets a strict limit on division time, and then from there we consider how the remaining proteome further limits this achievable growth rate.

3.1 Maximum possible growth rate is set by the time to make a ribosome.

Ribosomes take a unique position among proteins due to their role in synthesizing the entire cellular proteome. In order for a cell to maintain its own pool of ribosomes during division into two daughter cells, a primary requirement is that the number of ribosomes must be doubled. Since the mass of a single ribosome is about 2.5 MDa, with about 2/3 RNA and 1/3 protein, each ribosome has to make about 800 kDa of protein. In *E. coli*, this corresponds to 7,459 amino acids. At a maximal translation rate of 20 amino acids per second, this would take just over 6 minutes. Growing any faster would result in a drop in the average number of ribosomes as the cell divides and highlights a strict time limit on how fast a cell can double itself. This result is irrespective of the absolute number of ribosomes, and contrasts with other proteins where the simple solution to making more proteins is to apparently devote more ribosomes to their synthesis.

NB: This time is longer if we need to add in e.g. elongation factors as a 'core' protein to replicate a ribosome. RP to NB: rRNA estimate can also go here.

3.2 The translation-limited growth rate is set by the fraction of ribosomal mass.

While the inability to parallelize ribosomal synthesis sets an inherent speed limit, this also represents a somewhat unachievable growth rate since ribosomes must spend some of their time doubling the remaining proteome. A translation-limited rate of growth is therefore set by the time to double the entire proteome. In order to understand the consequence of each ribosome having to duplicate itself, but also devote time to double the remaining proteome, we consider a hypothetical cell that consists of only two species of protein: ribosomes and non-ribosomal proteins. The cell is taken to contain R ribosomes per cell, and P non-ribosomal proteins per cell. The time τ needed to duplicate the entire proteome is simply given by,

$$\tau = \tau_R + \tau_P, \tag{3}$$

where τ_R is the time to double the ribosome copy number and τ_P is the time required to double the non-ribosomal proteins. While we found that τ_R is fixed at about 6 minutes, τ_P will depend on the number of ribosomes R available and can be approximated by,

$$\tau_P = \frac{N_{aa}}{r_t \cdot R}. \tag{4}$$

Here N_{aa} refers to the total number of amino acids (aa) that must be translated, while r_t refers to the elongation rate of translation. The translation-limited growth rate can then be calculated from,

$$\lambda_{\max} = \frac{ln(2)}{\tau}. \tag{5}$$

Using Equation 4 and 3, this becomes,

$$\lambda_{\max} = \frac{ln(2)}{\tau_R + \frac{N_{aa}}{r_t \cdot R}}. \tag{6}$$

We can see from Equation 6 that the only way to increase the translation-limited growth rate would be to make more ribosomes, or if it were possible, to decrease the number of non-ribosomal proteins. For now we will assume that the translation elongation rate is fixed at about 20 aa/s but will return to this assumption in a later section.

let's now use some representative values for R and N_{aa} to calculate $\lambda_{\max}$. From Schmidt *et al.*, cells grown in glucose were found to have 214 fg of non-ribosomal protein mass [6]. Taking the molecular weight of an average amino acid to be 110 g/mol and using Avogadro's number N_A, we can estimate $N_{aa} = 214$ x 10^{-15}g / (110 g/mol) x N_A, which corresponds to about 1x10^9 amino acids. Similarly, we can use their reported ribosomal mass of about 29 fg to estimate the ribosomal copy number, R. With a molecular weight of about 800 kg/mol as noted earlier, $R = 29$ x 10^{-18}kg / (800 kg/mol) x N_A, which we find to be about 22,000 per cell. Using Equation 6, this corresponds to a maximum growth rate of 0.8 hr^{-1}, versus the measured rate of 0.58 hr^{-1}, suggesting cells are growing slightly below their maximal rate.

Since the only way to divide faster than this limit set at 0.8 hr^{-1} would be for the cell to increase the number of ribosomes, we next consider how growth rate might vary as a function of ribosomal copy number. To keep our problem simple, lets first proceed with the simplifying assumption that our cell consists of 214 fg of non-ribosomal protein, and consider how $\lambda_{\max}$ varies as a function of the ribosome copy number R using Equation 6. While in reality we might expect other proteins to increase in proportion to the number of ribosomes, this calculation provides us with a bound on the maximum growth rate as a function of R. In Figure 4 we consider the range of experimentally observed values of R from about 10,000 copies per cell to 150,000 copies per cell. One observation is that the maximum growth rate is always less than that set by the synthesis time of a ribosome, at about 3 hr^{-1} when R is 150,000 ribosomes per cell. Indeed, while not shown, we find that R would need to be increased another 10 fold, to about one million copies per cell, to have a doubling time close to that set by the ribosome (with a 6 minute ribosome synthesis time corresponding to a growth rate of about 7 hr^{-1}).

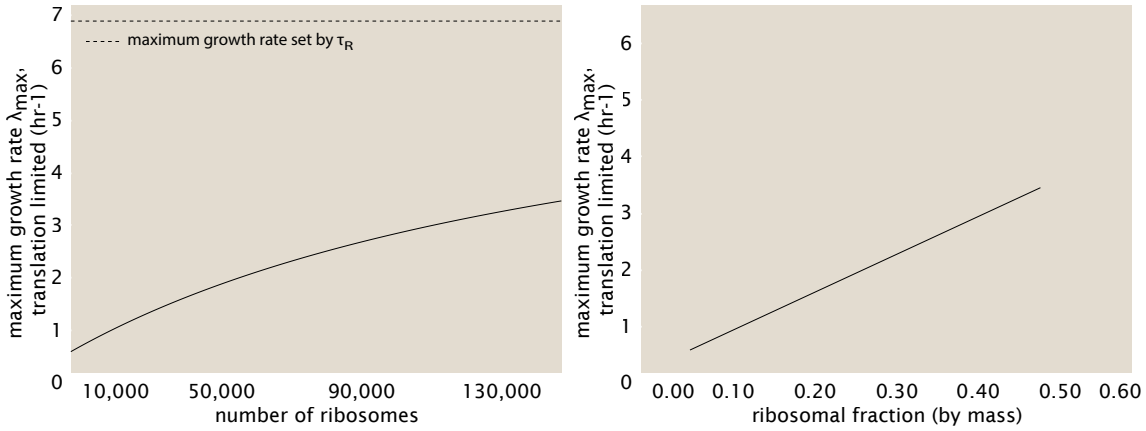

Figure 4: Expectations on the maximum growth rate as a function of ribosome abundance. A) Plot of the translation-limited growth rate in Equation 6, with $N_{aa} = 1.2$ x 10^9 amino acids, and R from about 10,000 to 150,000 copies per cell. B) Related to part A, but instead showing the translation-limited growth rate as a function of ribosomal mass fraction.

Given how many ribosomes a cell would need in order to double a cell in 6 minutes, it is also useful to consider what this might mean with respect to cell size. Note that cell volume will be proportional to cell mass. We can estimate a lower bound on the required cell volume as a function of the R by assuming a mass density of 1.1 g/ml, and a dry mass of 30% consisting of only protein and RNA. This is plotted in Figure 5, where we've extended the range of R up to about one million copies per cell. While we find cell volumes consistent with our expectation for *E. coli* for values of R less than about 100,000 per cell, the plot also highlights that a cell would need to be excessively largem with a minimal volume of about 25 fL, in order for $\lambda_{\max}$ to be close to the 6 minute doubling time set by the ribosome.

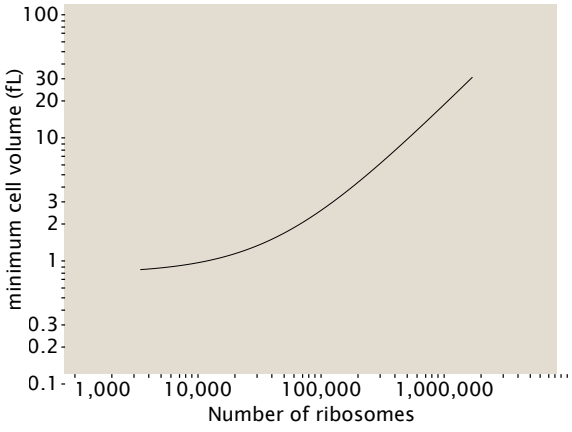

Figure 5: Estimated scaling of cell size with ribosomal copy number. As a first approximation, the cell mass it taken to consist of 214 fg non-ribosomal protein, and a ribosomal mass based on 1/3 corresponding to protein, and 2/3 corresponding to RNA. The cell volume is then calculated assuming a 30 % dry mass, and cell mass density of 1.1 g/ml.

As a last consideration, one additional observation from Figure 4B is an apparently

linear dependence between $\lambda_{\max}$ and the fraction of ribosomal mass. This, along with the scaling in ribosomal copy number, are particularly relevant to the phenomenological growth laws reported by others on how cell size and cell mass scale with growth rate in bacteria. The linear scaling appears to be a feature irrespective of the size of the non-ribosomal mass, as shown in Figure 6. Indeed, with a bit of algebra, we can re-write the translation-limited growth rate defined by Equation 6 as a function of ribosomal mass fraction, denoted by Φ_R, as,

$$\lambda_{\max} = \frac{ln(2)}{L_R} \cdot r_t \cdot \Phi_R. \tag{7}$$

L_R refers to the number of amino acids that make a single ribosome (L_R = 7,459 aa for a complete ribosome in *E. coli*). As a sanity check, we can quickly see that if $\Phi_R = 1$, we are once again limited only by the time required to double a ribosome L_R/r_t.

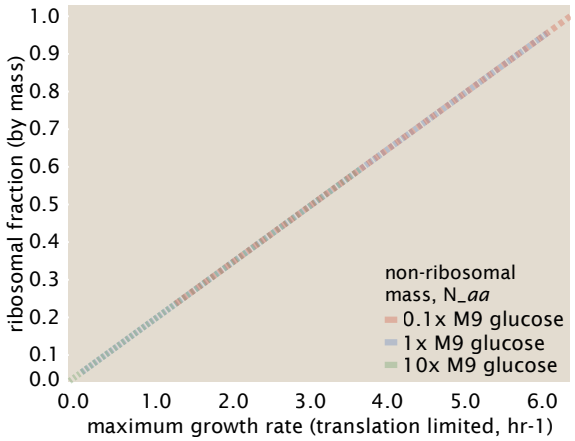

Figure 6: Effect of ribosomal mass fraction on translation-limited growth rate. Following the approach result from Figure 4B, we recalculate the maximum growth rate as the total non-ribosomal mass is either reduced or increased ten-fold (i.e. N_{aa} = [0.1xN_{aa}, N_{aa}, 10xN_{aa}]).

3.3 Growth only appears translation-limited in rich growth media.

With an expectation on the maximum growth rate achievable as a function of ribosomal content from our discussion above, lets now take a look at our experimental data. From Equation 7, we found that the translation-limited growth rate is simply determined by the fractional ribosomal mass Φ_R which we can easily calculate from our proteomic data. In Figure 7A we plot this maximal growth rate, $\lambda_{\max}$, against the measured growth rates, while in Figure 7B we plot the cell cycle or doubling time that would be associated with these growth rates. The shaded regions identify regions that should not be attainable with a translation elongation rate r_t of 20 aa/s. From these two plots, it appears that cells are only translation-limited in rich media (data points with growth rates greater than ≈ 1 hr^{-1} in Figure 7A)).

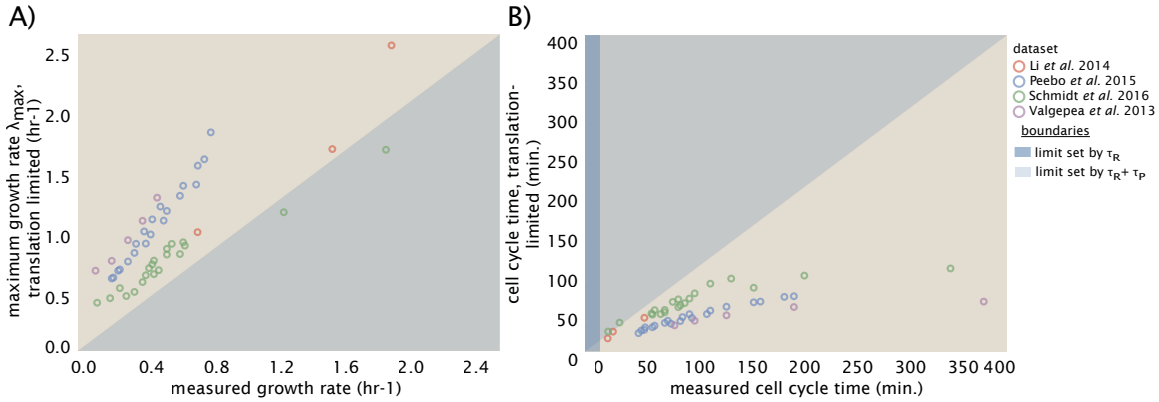

Figure 7: Comparison of translation-limited rate of growth to observed growth rates. A) Plot of maximum growth rates based on reported cell mass and calculated from Equation 6. B) Related to (A), but instead plotting the cell cycle time in minutes. The light shaded regions in (A) and (B) reflect boundaries where growth would not be possible due to a translation rate of 20 aa/s. The dark shaded region in (B) corresponds to the maximum division rate set by doubling a ribosome. (NB: There is something weird about the fraction of ribosomal protein in Peebo, Valgepea; it is higher, and also higher than that found in Scott *et al.* - is it real??)

3.4 The effect of a non-constant translation elongation rate.

From Figure 7B it is apparent that for cells with slower growth, the cell cycle time is indeed much longer than might have been expected under translation-limited growth. The remaining parameter we have yet to consider is the elongation rate r_t, which we have assumed to be 20 aa/s. Recent measurements of elongation rate from Dai *et al.* [7] across a wide range of growth rates found that it indeed varies with growth rate. In particular, they showed that the rate decreased to as low as 8 aa/s and exhibited a a Michaelis–Menten dependence on the ribosomal fraction. Here we use their result to further consider the consequence of a decreasing elongation rate r_t on the maximum predicted growth rate.

In the work of Dai et al. the authors propose that there may be a bottleneck in translation that arises due to lower availability of ternary complex (TC) that must bind the ribosome in order for translation to proceed. This complex consists of aminoacyl-tRNA, elongation factor Tu and guanosine triphosphate. To account for this bottleneck, they divide the elongation rate into two coarse-grained timescales: A) binding of the ternary complex to the ribosome, which will depend inversely on the effective TC concentration $[TC_{eff}]$, and B) other enzymatic processes that will not depend on TC concentration. Letting these two timescales be $1/(k_{on} \cdot [TC_{eff}])$ and $1/r_t$, the new elongation rate is given by,

NB: a better approach would be from point-of-view of biological rate-limiting steps. BUT the result suggests possibilities: aa-tRNA availability?, GTP?

$$\frac{1}{r'_t} = \frac{1}{k_{on} \cdot [TC_{eff}]} + \frac{1}{r_t} \tag{8}$$

where r_t/k_{on} is the binding constant of the TC with the ribosome. Further taking $[TC_{eff}]$ to be proportional to the RNA/protein ratio,

$$[TC_{eff}] = C \cdot (R_m/P_m), \tag{9}$$

they find that $r_t = 22$ aa/s, $k_{on} = 6.4\ \mu M^{-1}s^{-1}$, and $C = 31\ \mu M$.

Using the elongation rate calculated from Equation 9, we can now recalculate the translation-limited growth rate,

$$\lambda'_{\max} = \frac{ln(2)}{L_R} \cdot r'_t \cdot \Phi_R, \tag{10}$$

where we denote $\lambda'_{\max}$ as the translation-limited growth rate when elongation rates is no longer assumed to be fixed at 20 aa/s. Plugging in the translation rate r'_t given by Equation 8 along with the measured fraction of ribosomal mass Φ_R from each dataset, we find a further improvement in agreement between the measured and translation-limited growth rates. This is shown in Figure 8. This is particularly true with the data from Li *et al.* and Schmidt *et al.*, though we note that for the poorest nutrient conditions (i.e. the longest cell cycle time) a discrepancy still appears to exist.

This assumption of proportionality is something that will need some more consideration.

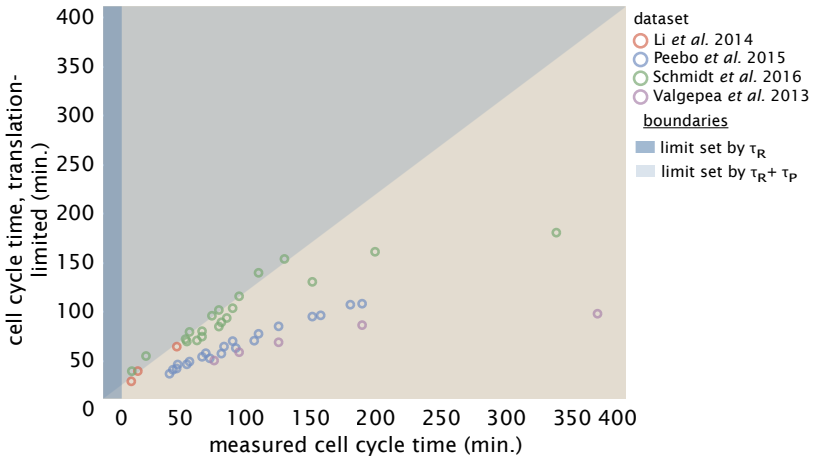

Figure 8: Comparison of translation-limited rate of growth to observed growth rates using the predicted elongation rate from Dai *et al.*. Predicted cell cycle time, calculated from Equation 7, is plotted against the measured doubling time. The light shaded region reflect a boundary where growth would not be possible given the predicted translation rate r'_t in Equation 8, which varies from about 8 aa/s to about 20 aa/s. The dark shaded region corresponds to the maximum division rate set by the synthesis of a ribosome. To calculate the RNA/ protein ratio R_m/P_m we assume it is proportional to the fraction of ribosomal mass Φ_R, which empirically was found to be $R_m/P_m = \Phi_R/0.411$ [7].

4 Nutrient-dependent limits on the rate of cell division.

In the preceeding section we identified limitations on the speed of cell division that reflect inherent limits on the maximum translation elongation rate and the necessity to double both the pool of ribosomes and the cell's remaining proteome. We next consider the conseqences of nutrient limtations on the maximal growth rate of the cell.

Here it is helpful to point out three notable experimental observations. The first is that in the limit of zero growth, the ribosomal fraction of *E. coli* converges toward nonzero value (5-10 % by mass). In the context of poorer and poorer nutrient conditions, there must be a point in which cells have more ribosomes than they can utilize. The next point, which is related to this, is that cells actually appear to reduce the fraction of ribosomes that are actively translating when growing at a growth rate less than 0.7 hr^{-1}. Lastly, below this growth rate, the cell's elongation rate also begins to decrease, with a minimum value of about 8 aa/s measured in stationary phase.

4.1 Nutrient limitation does not explain increasing ribosomal content.

We begin by considering the consequence of nutrient limitation on the ribosomal elongation rate. In the work of Dai *et al.* it was suggested that perhaps there is a bottleneck in the availability of ternary complex, referring to the assembly of aminoacyl-tRNA, elongation factor Tu and guanosine triphosphate (GTP) that is needed for translation. If cells are indeed reducing their fraction of actively translating ribosomes, it is difficult to rationalize the possibility that a protein like Tu might be limiting. If it were limiting, for example, a simple solution seems to be for the cell to make fewer of the unused ribosomes and increase the number of Tu. In contrast, at least in the limit of poorer nutrient conditions, the possibility of limitations on more basic building blocks like amino acids and GTP seem more reasonable. Here we consider the possibility that the synthesis rate of amino acids, and therefore the cellular concentration of amino acids $[aa]$ is limiting. An important finding from our analysis below is that while we are able to account for both the change in translation rate and the apparent fraction of ribosomes that are needed for a specific growth rate, it provides no basis to explain why ribosomal content continues to increase as growth rate increases.

In order to consider that the amino acid synthesis rate is limiting, we can follow a similar approach to that employed by Dai et al., which was to divide the elongation rate into two coarse-grained timescales. Here we assume that the translation rate depends on A) binding of a ternary complex, which we propose depends on a rate-limiting concentration of $[aa]$ and, 2) other enzymatic processes that will not depend on $[aa]$. The effective elongation rate is given by the inverse timescales associated with each step,

$$\frac{1}{r_t'} = \frac{1}{k_{on} \cdot [aa]} + \frac{1}{r_t}. \tag{11}$$

where r_t' is the measured elongation rate, r_t is the maximum elongation rate, and r_t/k_{on} is the binding constant K_d of the ternary complex with the ribosome. Alternatively, we can re-write this in terms of the binding constant,

$$r'_t = r_t \cdot \frac{1}{1 + K_d/[aa]}. \tag{12}$$

If we consider only consumption of amino acids by ribosomes, during steady state growth $[aa]$ will be depend on the amino acid synthesis rate r_{aa}, consumption rate by ribosomes, $R \cdot r'_t$, and the cell volume V,

$$[aa] = \tau \cdot \frac{r_{aa} - R \cdot r'_t}{V}. \tag{13}$$

Here τ refers to the doubling time of the cell in seconds. If we plug this into Equation 12, we find that

$$r'_t = r_t \cdot \frac{1}{1 + K_d \cdot V/(\tau \cdot (r_{aa} - R \cdot r'_t))}. \tag{14}$$

This brings us to a somewhat confusing result. If ribosomes are in excess of the available amino acids, ribosomes will deplete the supply of amino acids and translation will grind to a halt. This apparent conundrum seems to be similarly present if we were to instead consider that the supply of tRNA or GTP is limiting. This may provide some rationalization for why a cell would regulate its fraction of active ribosomes.

In order to proceed, we will take for granted that the cell actively regulates its fraction of active ribosomes, and make an assumption that it does so to support a net positive concentration of amino acids in the cell. Specifically, we are interested in how the elongation rate might depend on a positive increase in the synthesis rate of amino acids. Here we re-write Equation 14 as,

$$r'_t = r_t \cdot \frac{1}{1 + K'_d/r'_{aa}}, \tag{15}$$

where r'_{aa} is the effective rate in which amino acid are being supplied to each of the active ribosomes, and K'_d is the apparent rate when r'_t is half maximal. Here $K'_d = K_d \cdot V/\tau$, while $r'_{aa} = r_{aa} - R \cdot r'_t$.

With a maximal elongation rate of about 17 aa/s, we find that for any K'_d less than 8.5 aa/s, the time to double the cell's proteome will be limited by r'_{aa}, and not the elongation rate r_t. Said differently, if the total number of amino acids consumed to double the cell is $N_a a$, it should take $\tau = N_a a/r'_{aa}$ to double the cell, which is less than the time required if all ribosomes were constantly synthesizing proteins at their rate of r'_t. Under such a scenario, the fraction of available ribosomes that are needed would just be given by the ratio of r'_{aa}/r'_t.

In Figure 9(A) we consider such a scenario and consider the growth rate of cells as the value of r'_t is increased from 2 - 50 aa/(s ribosome). Here we have selected a value of K'_d less than 8.5 aa/(s ribosome), with a cell containing 7% ribosome by mass. Since we are considering cells growing in steady state, and we assume that the cell has some mechanism in place to match a specific supply of amino acids defined by r'_{aa}, the fraction of available ribosomes needed to double the cell will be given by r'_{aa}/r'_t, up to a maximum value of 1. This is plotted in Figure 9(B).

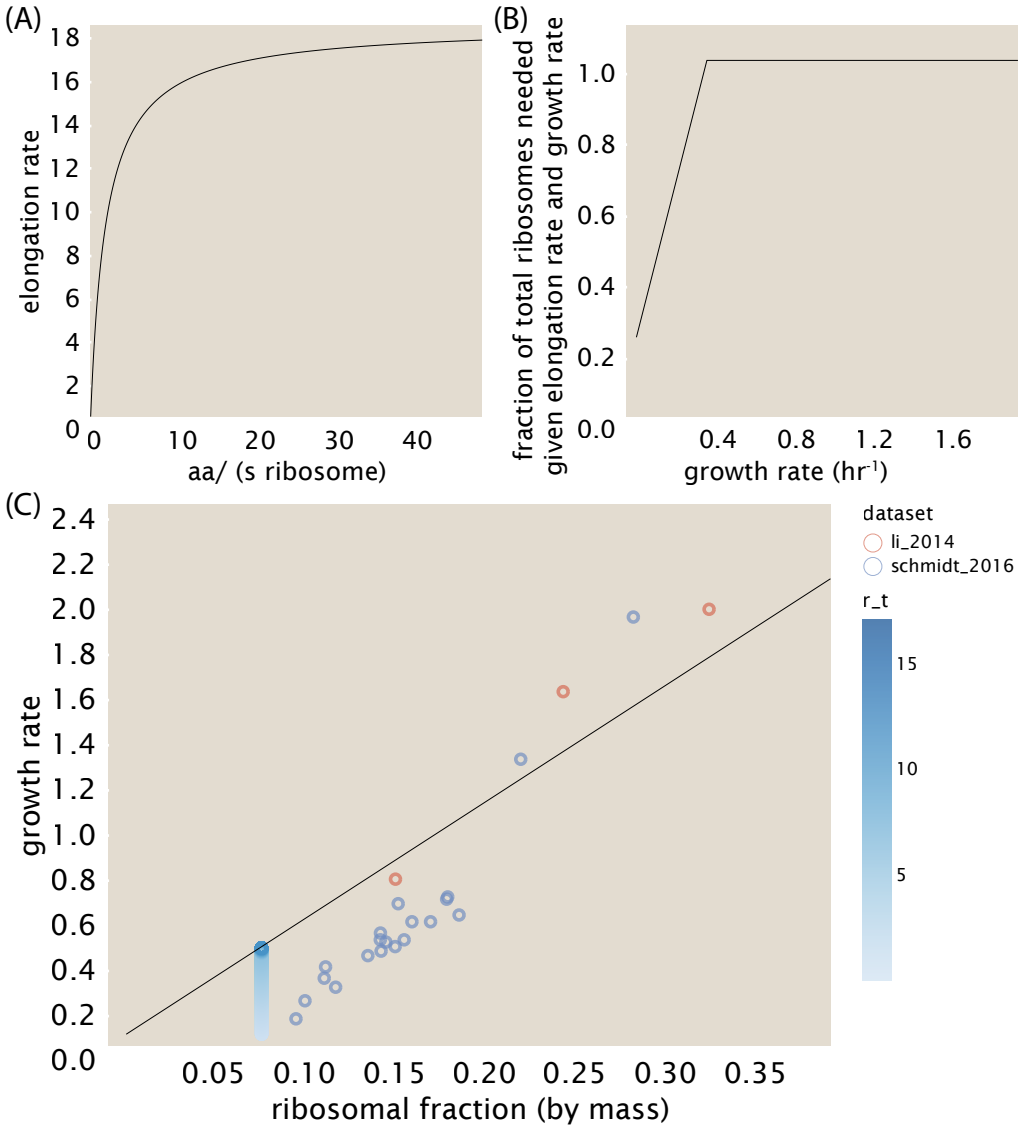

Figure 9: Expectations on cell growth in a nutrient-limited regime. (A) Plot of elongation rate r_t using Equation 15, with K'_d less than 8.5 aa/(s ribosome). (B) The apparent fraction of ribosomes that would be needed given that the supply of amino acids r'_{aa} is less than the rate with which ribosomes are using them. We assume that cells are growing in steady state, and that the cell is able to regulate its fraction of ribosomes in order to maintain a constant supply of amino acids r'_{aa} . (C) Plot of growth rate versus ribosomal fraction. Blue line refers to a cell whose ribosomal fraction in limiting growth is 7 %, with the color indicating the elongation rate as production rate of amino acids r'_{aa} is increased over the range shown in part (A).

Importantly, while this provides some perspective on why elongation might decrease in the nutrient limit, and can be consistent with apparent regulation of the active pool of ribosomes. However, as shown in Figure 9(C) it does so without requiring any increase in ribosomal content as a function of growth rate. This suggests that something else must be considered in order to explain the observedtrend in ribosomal present that is still observed

in the nutrient-limit.

4.2 Nutrient limitations provide another boundary on the rate of bacterial growth.

As an *E. coli* cell encounters more nutrient rich conditions, the data in Figure XA shows that the measured steady state growth rates follows a well-defined path as a function of ribosomal content. This reflects the so-called growth law that has been observed for *E. coli* and some other bacterium. However, we might have naively expected alternative paths to reach the translation-limited growth boundary to be equally plausable. In order to make progress it useful to highlight that experimentally, from work by Dai *et al* and others, the elongation rate is expected to increase gradually from about 8 aa/s to a maximum in rich media of about 17 aa/s.

To understand the consequence of this, we consider the hypothetical situation illustrated in Figure Z. Here a cell is initially growing at a rate of about X hr^{-1}, with an elongation rate of X aa/s. As nutrient conditions improve and the apparent elongation rate increases, the maximum growth rate becomes defined by this new elongation rate. The first naieve scenario is that the bacterium keeps its proteome unchanged, including the number of available ribosomes. Such a situation would correspond to a cell whose size and total contents can remain unchanged and it can just double itself faster. The second scenario is that the cell takes advantage of its apparent increase in protein synthesizing capacity, and somehow bias its protein production to make more ribosomes. By doing so, the cell is able to grow faster given its new elongation rate.

We next attempt to estimate the additional ribosomes than might be made from this additional capacity. This can be determined from the relative increase in elongation rate and the number of available ribosomes. The additional amino acids available to put toward making more proteins is given by,

$$N'_{aa} = (r_{t16} - r_{t14}) \cdot \tau \cdot R, \tag{16}$$

where τ is the doubling time of the cell. The maximum additional ribosomes than can then be made is simply given by,

$$R' = N'_{aa}/L_R, \tag{17}$$

where again, L_R refers to the total number of amino acids that make a ribosome. This scenario results in a cell with larger ribosomal fraction given by,

$$\Phi' = \frac{R + R'}{R + R' + P'}. \tag{18}$$

The maximal growth rate at this elongation rate will then be equal to

$$\lambda = \frac{ln(2)}{L_R} \cdot r_{t16} \cdot \Phi'. \tag{19}$$

While this treatment is somewhat artificial and assumes that the cell continues to double its core proteome, as measured in the slow-growth limit, it provides us with a convenient way to rationalize the constantly increasing ribosomal fraction for growth rates below the traslation-limited growth rate. Indeed, since cells do indeed making more ribosomes and

get more massive with growth rate, this perspective provides an a potential way to connect to the well-characterized cell size scaling observed in *E. coli*.

We can repeat the same thought process for the entire range of measured elongation rates, from about 8 aa/s to 17 aa/s, which is plotted in Figure Z. Here we now identify a nutrient-limited boundary. This boundary represents the scenario where any increase in cell protein mass has been devoted to making more ribosomes.

Here it is worth noting that this boundary isn't fundamentally as restricted as the translation-limited boundary. For example, there are many examples from the Hwa lab where the addition of an antibiotic like chloramphenicol shift the observed ribsomal fraction and growth rates into this region.

4.3 Nutrient-limited building blocks and biased ribosome production.

Here we include our proposed dependence of translation elongation on the production rate of amino acids. Specifically, we look at how the growth rate increases as a function of amino acid production rate using our proposed scheme above.

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