Fundamental limits on the rate of bacterial cell division

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Abstract   This will be written next eventually

Translation and Ribosomal Synthesis

Lastly, we turn our attention to the process of synthesizing new proteins, translation. This process stands as a good candidate for potentially limiting growth since the synthesis of new proteins relies on the generation of ribosomes, themselves proteinaceous molecules. As we will see in the coming sections of this work, this poses a "chicken-or-the-egg" problem where the synthesis of ribosomes requires ribosomes in the first place.

We will begin our exploration of protein translation in the same spirit as we have in previous sections – we will draw order-of-magnitude estimates based on our intuition and available literature, and then compare these estimates to the observed data. In doing so, we will estimate both the absolute number of ribosomes necessary for replication of the proteome as well as the synthesis of amino-acyl tRNAs. From there we consider the limitations on ribosomal synthesis in light of our estimates on both the synthesis of ribosomal proteins and our earlier results on rRNA synthesis.

tRNA Synthetases

We begin by first estimating the number of tRNA synthetases in *E. coli* needed to convert free amino-acids to polypeptide chains. 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 the 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 $\approx$ 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 1*(A), 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 1*(B)), 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 synthases. 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.

Protein Synthesis

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

While the rate at which ribosomes translates is well known to have a growth rate dependence *Dai et al.* (*2018*) and is a topic which we discuss in detail in the coming sections. However, for the purposes of our order-of-magnitude estimate, we can make the approximation that translation occurs at a rate of $\approx$ 15 amino acids per second per ribosome (BNID: 100233). Under this approximation and assuming a division time of 5000 s, we can arrive at an estimate of $\approx 10^4$ ribosomes are needed to replicate the cellular proteome, shown in *Figure 1*(B). This point estimate, while glossing over important details such as chromosome copy number and growth-rate dependent translation rates, proves to be notably accurate when compared to the experimental observations (*Figure 1*(B)).

PROTEIN SYNTHESIS

(A) tRNA charging

$$N_{\text{amino-acyl tRNA}} \approx 3{\times}10^6 \text{ proteins} \times \frac{300 \text{ amino acids}}{1 \text{ protein}} \times \frac{1 \text{ amino-acyl tRNA}}{1 \text{ amino acid}}$$
$$\approx 10^9 \text{ amino-acyl tRNAs}$$

$$r_{\text{consumption}} \approx \frac{N_{\text{amino-acyl tRNA}}}{t_{\text{division}}} = \frac{10^9 \text{ amino-acyl tRNAs}}{5{,}000 \text{ sec}} \approx \frac{2 \times 10^5 \text{amino-acyl tRNAs}}{1 \text{ sec}}$$

$$r_{\text{tRNA charging}} \approx 20 \text{ amino-acyl tRNA / tRNA synthetase} \times \text{sec} \quad \text{BNID: 105279}$$

$$N_{\text{tRNA synthetase}} \approx \frac{r_{\text{tRNA supply}}}{r_{\text{tRNA charging}}} \approx 10^4 \text{ tRNA synthetases}$$

(B) translation

$$N_{\text{peptide bonds}} \approx 3{\times}10^6 \text{ proteins} \times \frac{300 \text{ amino acids}}{1 \text{ protein}} \times \frac{1 \text{ peptide bond}}{1 \text{ amino acid}}$$
$$\approx 10^9 \text{ peptide bonds}$$

$$r_{\text{translation}} \approx 15 \text{ peptide bonds / ribosome} \times \text{sec} \quad \text{BNID: 109043}$$

$$N_{\text{ribosomes}} \approx \frac{N_{\text{peptide bonds}}}{r_{\text{translation}} \times t_{\text{division}}} \approx 1.5{\times}10^4 \text{ ribosomes}$$
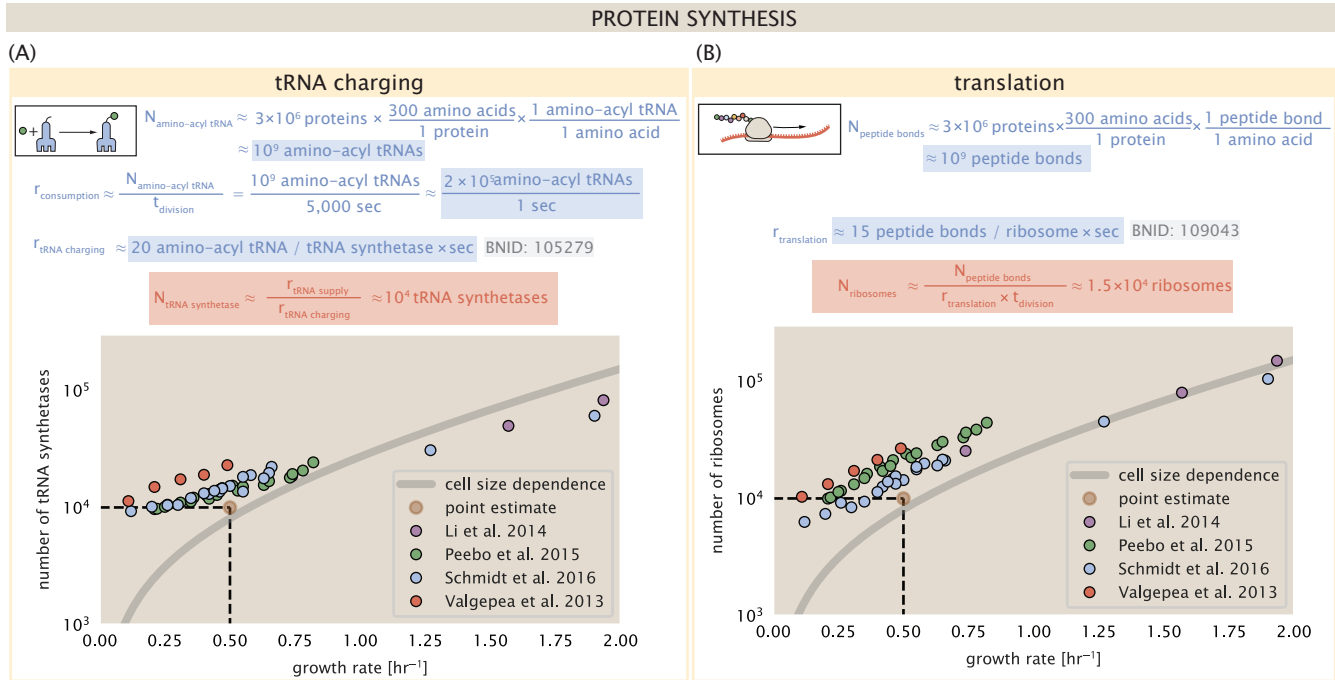
Figure 1. Estimation of the required tRNA synthetases and ribosomes. (A) Estimation for the number of tRNA synthetases that will supply the required amino acid demand. The sum of all tRNA synthetases copy numbers are plotted as a function of growth rate ([ArgS], [CysS], [GlnS], [GltX], [IleS], [LeuS], [ValS], [AlaS]$_2$, [AsnS]$_2$, [AspS]$_2$, [TyrS]$_2$, [TrpS]$_2$, [ThrS]$_2$, [SerS]$_2$, [ProS]$_2$, [PheS]$_2$[PheT]$_2$, [MetG]$_2$, [lysS]$_2$, [HisS]$_2$, [GlyS]$_2$[GlyQ]$_2$). (B) Estimation of the number of ribosomes required to synthesize 10^9 peptide bonds with an elongation rate of 15 peptide bonds per second. The average abundance of ribosomes is plotted as a function of growth rate. Our estimated values are shown for a growth rate of 0.5 hr^{-1}. Grey lines correspond to the estimated complex abundance calculated at different growth rates. See Supplemental Information XX for a more detail description of this calculation.

References

Dai, X., Zhu, M., Warren, M., Balakrishnan, R., Okano, H., Williamson, J. R., Fredrick, K., and Hwa, T. (2018). Slowdown of Translational Elongation in *Escherichia coli* under Hyperosmotic Stress. - PubMed - NCBI. *mBio*, 9(1):281.