Fundamental limits on the rate of bacterial cell division

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Abstract

Introduction

The range of bacterial growth rates can be enormous. In natural environments, some organisms might double only once per year, whereas in comfortable laboratory conditions growth can be rapid with several divisions per hour. This remarkable diversity illustrates the intimate relationship between environmental conditions and the rates at which cells convert nutrients into new cellular material. This relationship between the environment and cellular growth rate has remained a major topic of inquiry in bacterial physiology for over a century (***Jun et al., 2018***). In 1958, Schaecter, Malløe, and Kjeldgaard reported the discovery of a logarithmic relationship between the total cellular protein content and the cellular growth rate, revealing a fundamental relationship between the environment and the composition of the intracellular milieu (***Schaechter et al., 1958***).

Over the past decade, a remarkable body of work has reexamined this relationship with single-cell and single-protein resolution using modern methods of video microscopy (***Si et al., 2017***; ***Harris and Theriot, 2018***) and through advances in mass spectrometry and sequencing technologies (***Schmidt et al., 2016***; ***Li et al., 2014***). This has permitted quantitative insight into how bacteria like *E. coli* allocate their cellular resources under nutrient-limitation, and following genomic and pharmacological perturbations (***Scott et al., 2010***; ***Hui et al., 2015***; ***Basan et al., 2015***). This body of experimental data places us in the auspicious position to explore how the abundance of essential protein complexes are related to the growth rate of the population and interrogate what biological processes may set the speed limit of bacterial growth.

In this work, we seek to leverage a collection of proteomic data sets of *Escherichia coli* across 31 growth conditions (***Valgepea et al., 2013***; ***Li et al., 2014***; ***Peebo et al., 2015***; ***Hui et al., 2015***; ***Schmidt et al., 2016***) to quantitatively explore what biological processes may set the speed limit of bacterial growth. Broadly speaking, we entertain several classes of hypotheses as are illustrated in ***Figure 1***. First, we consider potential limits on the transport of nutrients into the cell. We address

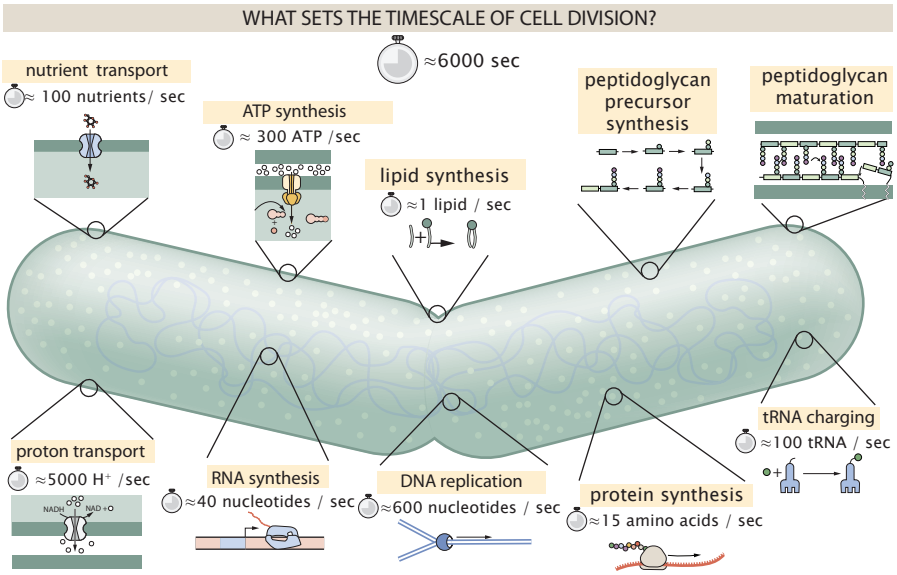

Figure 1. Transport and synthesis processes necessary for cell division. We consider an array of processes necessary for a cell to double its molecular components. Such processes include the transport of carbon across the cell membrane, the production of ATP, and fundamental processes of the central dogma namely RNA, DNA, and protein synthesis. A schematic of each synthetic or transport category is shown with an estimate of the rate per macromolecular complex. In this work, we consider a standard bacterial division time of ≈ 6000 sec.

this hypothesis by performing an order-of-magnitude estimate for how many carbon atoms needed to facilitate this requirement given a 6000 second division time. As a second hypothesis, we consider the possibility that there exists a fundamental limit on how quickly the cell can generate ATP. We approach this hypothesis from two angles, considering how many ATP synthase complexes must be needed to churn out enough ATP to power protein translation followed by an estimation of how many electron transport complexes must be present to maintain the proton motive force. Our third and final class of hypotheses centers on the synthesis of a variety of biomolecules. Our focus is primarily on the stages of the central dogma as we estimate the number of protein complexes needed for DNA replication, transcription, and protein translation.

With estimates in hand for each of these processes, we turn to our collection of data sets to assess the accuracy of our estimates. In broad terms, we find that the majority of our estimates are in line with experimental observations, with protein copy numbers apparently well-tuned for the task of cell doubling. This allows us to systematically scratch off the hypotheses diagrammed in ***Figure 1*** as setting possible speed limits. Ultimately, we find that protein translation (particularly the generation of new ribosomes) acts as 1) a rate limiting step for the *fastest* bacterial division, and 2) the major determinant of bacterial growth across all nutrient conditions we have considered under steady state, exponential growth. This perspective is in line with the linear correlation observed between growth rate and ribosomal content (usually quantified through the ratio of RNA to protein) for fast growing cells (***Scott et al., 2010***), but suggests a more prominant role for ribosomes in setting the doubling time across all conditions of nutrient limitation. Here we again leverage the quantitative nature of this data set and present a quantitative model of the relationship between the fraction of the proteome devoted to ribosomes and the speed limit of translation, revealing a fundamental tradeoff between the translation capacity of the ribosome pool and the maximal growth rate.

Energy Production

While the transport of nutrients is required to build new cell mass, the metabolic pathways involved in assimilation both consumes and generates energy in the form of NTPs. The high-energy

phosophodiester bonds of (primarily) ATP power a variety of cellular processes that drive biological systems away from thermodynamic equilibrium. Our next class of estimates will consider the energy budget of a dividing cell in terms of the synthesis of ATP from ADP and inorganic phosphate as well as maintenance of the electrochemical proton gradient which powers it.

ATP Synthesis

Hydrolysis of the terminal phosphodiester bond of ATP forming ADP and an inorganic phosphate is kinetic driving force in a wide array of biochemical reactions. One such reaction is the formation of peptide bonds during translation which requires ≈ 2 ATPs for the charging of an amino acid to the tRNA and ≈ 2 ATP equivalents for the formation of the peptide bond between amino acids. Together, these energetic costs consume ≈ 80% of the cells ATP budget (BNID: 107782; 106158; 101637; 111918, ***Milo et al.*** (***2010***)). The pool of ATP is produced by the F_1-F_0 ATP synthase – a membrane-bound rotary motor which under ideal conditions can yield ≈ 300 ATP per second (BNID: 114701; ***Milo et al.*** (***2010***); ***Weber and Senior*** (***2003***)).

To estimate the total number of ATP equivalents consumed during a cell cycle, we will make the approximation that there are $\approx 3 \times 10^6$ proteins per cell with an average protein length of ≈ 300 peptide bonds (BNID: 115702; 108986; 104877, ***Milo et al.*** (***2010***)). Together, we arrive at the estimate that the typical *E. coli* cell consumes $\approx 5 \times 10^9$ ATP per cell cycle on protein synthesis alone and $\approx 6 \times 10^9$ ATP in total. Assuming that the ATP synthases are operating at their fastest possible rate, we arrive at an estimate that ≈ 3000 ATP synthases are needed to keep up with the energy demands of the cell. This estimate and a comparison with the data are shown in ***Figure 2***(A). Despite our assumption of maximal ATP production rate per synthase and approximation of all NTP consuming reactions being the same as ATP, we find that an estimate of a few thousand complete synthases per cell to agree well with the experimental data.

Generating the Proton Electrochemical Gradient

In order to produce ATP, the F_1-F_0 ATP synthase itself must consume energy. Rather than burning through its own product, this intricate macromolecular machine has evolved to exploit the electrochemical potential established across the inner membrane through cellular respiration. This electrochemical gradient is manifest by the pumping of protons into the intermembrane space via the electron transport chains as they reduce NADH. In *E. coli*, this potential difference is ≈ −200 mV (BNID: 102120, ***Milo et al.*** (***2010***)). As estimated in the supporting information, this potential difference is generated by maintaining $\approx 2 \times 10^4$ protons in the intermembrane space.

However, the constant rotation of the ATP synthases would rapidly abolish this potential difference if it were not being actively maintained. To undergo a complete rotation (and produce a single ATP), the F_1-F_0 ATP synthase must shuttle ≈ 4 protons across the membrane into the cytosol (BNID: 103390, ***Milo et al.*** (***2010***)). With ≈ 3000 ATP synthases each generating 300 ATP per second, the 2×10^4 protons establishing the 200 mV potential would consumed in only a few milliseconds! This brings us to our next estimate: how many electron transport complexes are needed to support the consumption rate of the ATP synthases?

The electrochemistry of the electron transport complexes of *E. coli* have been the subject of intense biochemical and biophysical study over the past half century (***Ingledew and Poole, 1984***; ***Khademian and Imlay, 2017***; ***Cox et al., 1970***; ***Henkel et al., 2014***). A recent work (***Szenk et al., 2017***) examined the respiratory capacity of the *E. coli* electron transport complexes using structural and biochemical data revealing that each electron transport chain rapidly pumps protons into the intermembrane space at a clip of ≈ 5000 protons per second (BIND: 114704; 114687, ***Milo et al.*** (***2010***)). Using our estimate of the number of ATP synthases required per cell (***Figure 2***(A)), coupled with these recent measurements, we estimate that ≈ 1000 electron transport complexes would be necessary to facilitate the $\approx 4 \times 10^6$ protons per second diet of the celllular ATP synthases. This estimate is in agreement with the number of complexes identified in the proteomic datasets (plot in ***Figure 2***(B)).

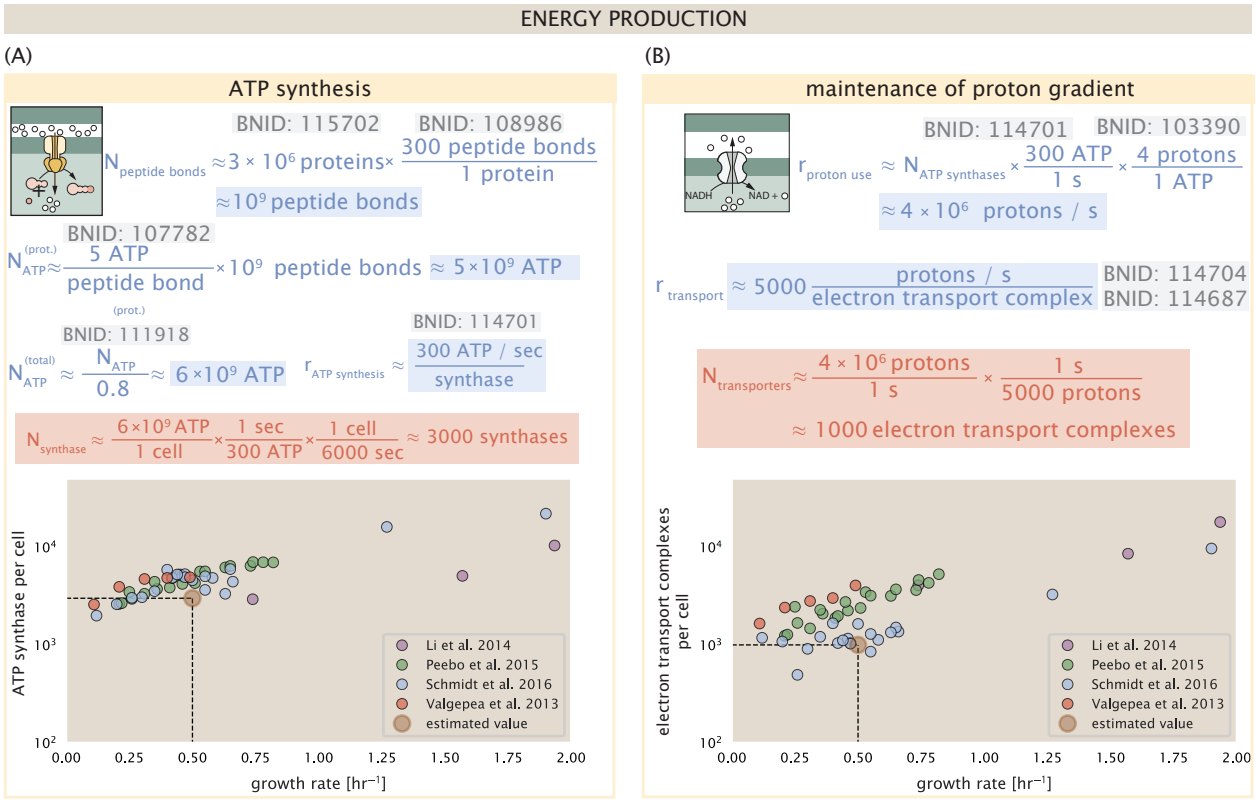

Figure 2. The abundance of F_1-F_0 ATP synthases and electron transport chain complexes as a function of growth rate.(A) Estimate of the number of F_1-F_0 ATP synthase complexes needed accommodate peptide bond formation and other NTP dependent processes Points in plot at bottom correspond to the mean number of complete F_1-F_0 ATP synthase complexes that can be formed given proteomic measurements and the subunit stoichiometry $[AtpE]_{10}[AtpF]_2[AtpB][AtpC][AtpH][AtpA]_3[AtpG][AtpD]_3$. (B) Estimate of the number of electron transport chain complexes needed to maintain a membrane potential of −200 mV given estimate of number of F_1-F_0 ATP synthases from (A). Points in plot correspond to the average number of complexes identified as being involved in aerobic respiratoion by the Gene Ontology identifier GO:0019646 that could be formed given proteomic observations. These complexes include cytochromes *bd1* ([CydA][CydB][CydX][CydH]), *bdII* ([AppC][AppB]), bo_3,([CyoD][CyoA][CyoB][CyoC]) and NADH:quinone oxioreducase I ([NuoA][NuoH][NuoJ][NuoK][NuoL][NuoM][NuoN][NuoB][NuoC][NuoE][NuoF][NuoG][NuoI]) and II ([Ndh]).

[GC: I think we really need to comment on the growth rate dependence here and see if we can make any sense of it with increasing surface area.]

Limitations to energy production in a crowded membrane.

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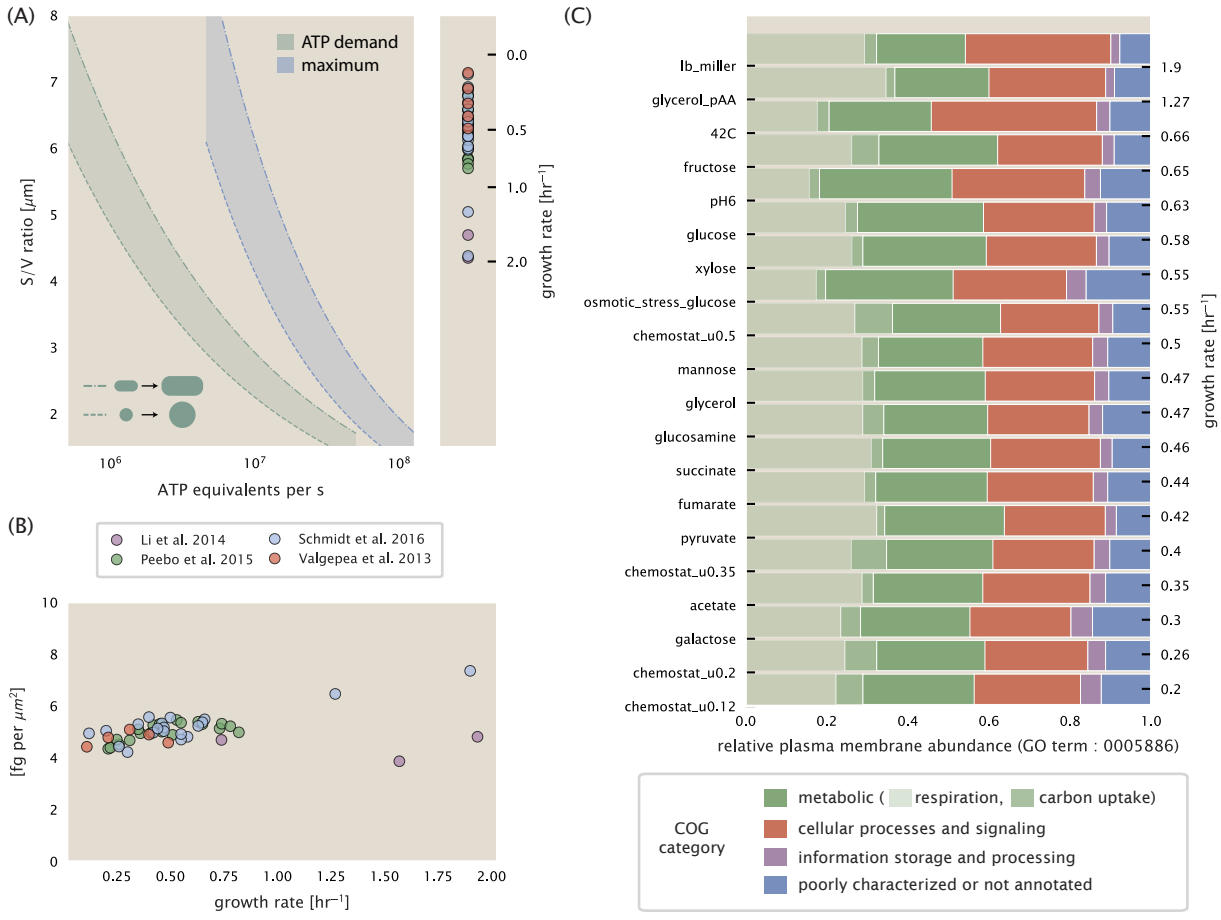
(A)
ATP demand
maximum
S/V ratio [μm]
ATP equivalents per s
growth rate [hr⁻¹]
(B)
Li et al. 2014
Peebo et al. 2015
Schmidt et al. 2016
Valgepea et al. 2013
[fg per μm²]
growth rate [hr⁻¹]
(C)
lb_miller
glycerol_pAA
42C
fructose
pH6
glucose
xylose
osmotic_stress_glucose
chemostat_u0.5
mannose
glycerol
glucosamine
succinate
fumarate
pyruvate
chemostat_u0.35
acetate
galactose
chemostat_u0.2
chemostat_u0.12
relative plasma membrane abundance (GO term : 0005886)
growth rate [hr⁻¹]
COG category
metabolic (respiration, carbon uptake)
cellular processes and signaling
information storage and processing
poorly characterized or not annotated

Figure 3. (A) (B) (C)

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