Fundamental limits on the rate of bacterial cell division

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Abstract This will be written next (promise).

Introduction

The range of bacterial growth rates is enormously diverse. In natural environments, some microbial organisms might double only once per year while in comfortable laboratory conditions, growth can be rapid with several divisions per hour. This six order-of-magnitude difference illustrates the intimate relationship between environmental conditions and the rates at which cells convert nutrients into new cellular material – a relationship that has remained a major topic of inquiry in bacterial physiology for over a century (***Jun et al., 2018***). As was noted by Jacques Monod, "the study of the growth of bacterial cultures does not constitute a specialized subject or branch of research, it is the basic method of Microbiology." Those words ring as true today as they did when they were written 70 years ago. Indeed, the study of bacterial growth has undergone a molecular resurgence since many of the key questions addressed by the pioneering efforts in the middle of the last century can be revisited by examining them through the lens of the increasingly refined molecular census that is available for bacteria such as the microbial workhorse *Escherichia coli*. Several of the outstanding questions that can now be studied about bacterial growth include: what sets the fastest time scale that bacteria can divide, and how is growth rate tied to the quality of the carbon source. In this paper, we address these two questions from two distinct angles. First, as a result of an array of high-quality proteome-wide measurements of the *E. coli* proteome under a myriad of different growth conditions, we have a census that allows us to explore how the number of key molecular players change as a function of growth rate. This census provides a window onto whether the processes they mediate such as molecular transport into the cells and molecular synthesis within cells can run faster. Second, because of our understanding of the molecular pathways responsible for many of the steps in bacterial growth, we can also make order-of-magnitude estimates to infer the copy numbers that would be needed to achieve a given growth rate. In this paper, we pass back and forth between the analysis of a variety of different proteomic datasets and order-of-magnitude estimations to determine possible molecular bottlenecks that limit bacterial growth and to see how

the growth rate varies in different carbon sources.

Specifically, we leverage a combination of *E. coli* proteomic data sets collected over the past decade using either mass spectrometry (***Schmidt et al., 2016***; ***Peebo et al., 2015***; ***Valgepea et al., 2013***) or ribosomal profiling (***Li et al., 2014***) across 31 unique growth conditions. Broadly speaking, we entertain several classes of hypotheses that relate to cell growth and are illustrated in ***Figure 1***. Throughout our esimates we consider a a 5000 second division time since these is where most data sets overlap, but we also consider how these values will vary at other growth rates due to changes in cell size and surface area (***Taheri-Araghi et al., 2015***). First, we consider potential limits on the transport of nutrients into the cell. We address this hypothesis by performing an order-of-magnitude estimate for how many carbon, phosphorous, and sulfur atoms are needed to facilitate this requirement. 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. A third class of estimates considers the need to maintain the size and shape of the cell through the construction of new lipids for the cell membranes as well as the glycan polymers which make up the rigid peptidoglycan. Our 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.

In broad terms, we find that for the majority of these estimates, the required molecular copy numbers are in close agreement with the 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 potential growth bottlenecks. 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) a major determinant of bacterial growth across the nutrient conditions we have considered under steady state, exponential growth. This perspective is consistent with the linear correlation observed between growth rate and ribosomal content (typically quantified through the ratio of RNA to protein) for fast growing cells (***Scott et al., 2010***), but also suggest a more prominent role for ribosomes in governing the changes in cell size and doubling time across all conditions of nutrient limitation.

Synthesis of the Cell Envelope

The subjects of our estimates thus far have been localized to the periphery of the cell, embedded within the hydrophobic lipid bilayer of the inner membrane. As outlined in **??**, cells could in principle increase the expression of the membrane-bound ATP synthases and electron transport chains to support a larger energy budget across a wide range of cell volumes and membrane surface areas. This ability, however, is contingent on the ability of the cell to expand the surface area of the cell by synthesizing new lipids and peptidoglycan for the cell wall. In this next class of estimates we will turn our focus to these processes and consider the copy numbers of the relevant enzymes.

Lipid Synthesis

The cell envelopes of gram negative bacteria (such as *E. coli*) are composed of an inner and outer phospholipid bilayer membrane separated by a ≈ 10 nm periplasmic space (BNID: 100016, ***Milo et al.*** (***2010***)). As mentioned in our discussion of the surface area to volume constraints on energy production, *E. coli* is a rod-shaped bacterium with a 4:1 length-to-width aspect ratio. At modest growth rates, such as our stopwatch of 5000 s, the total cell surface area is ≈ 5 μm^2 (BNID: 101792, ***Milo et al.*** (***2010***)). As there are two membranes, each of which composed of two lipid leaflets, the total membrane area is ≈ 20μm^2, a remarkable value compared to the $\approx$ 2μm length of the cell.

While this represents the total area of the membrane, this does not mean that it is composed entirely of lipid molecules. Rather the dense packing of the membrane with proteins means that

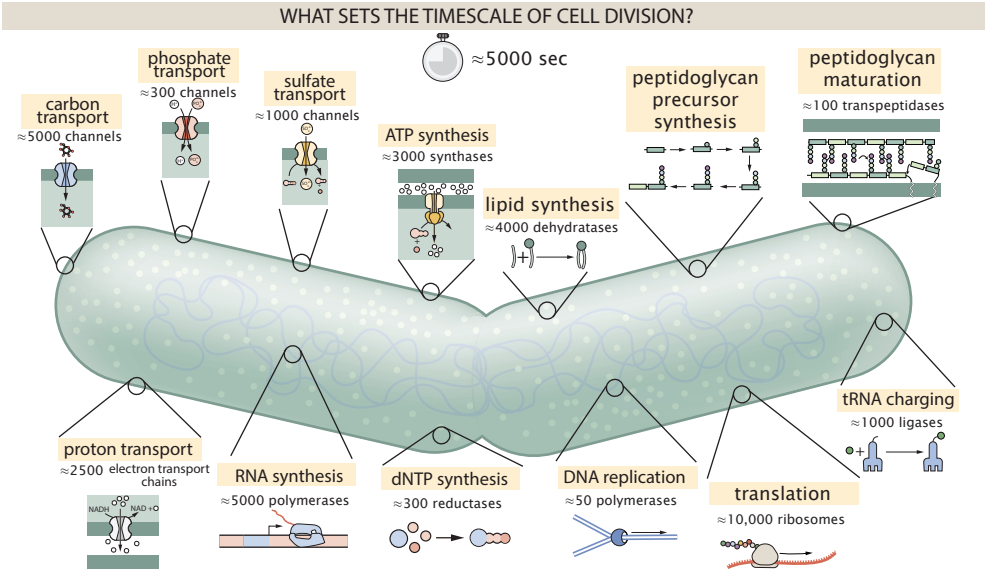

Figure 1. Transport and synthesis processes necessary for cell division. We consider an array of processes necessary for a cell to double its molecular components. Such processes include the transport of carbon across the cell membrane, the production of ATP, and fundamental processes of the central dogma namely, RNA, DNA, and protein synthesis. A schematic of each synthetic or transport category is shown with an approximate measure of the complex abundance at a growth rate of 0.5 per hour. In this work, we consider a standard bacterial division time of ≈ 5000 sec.

only ≈ 40 % of the membrane area is occupied by lipids (BNID: 100078, ***Milo et al.*** (***2010***)). Using a rule-of-thumb of 0.5 nm^2 as the surface area of the typical lipid (BNID: 106993, ***Milo et al.*** (***2010***)), we arrive at an estimate of $\approx 2 \times 10^7$ lipids per cell, an estimate in close agreement with experimental measurements (BNID: 100071, 102996; ***Milo et al.*** (***2010***)).

The membranes of *E. coli* are composed of a variety of different lipids, each of which unique in their structures and biosynthetic pathways (***Sohlenkamp and Geiger, 2016***). With such diversity in biosynthesis, it becomes difficult to identify which step(s) may be the rate-limiting reactions. This objective is further complicated by sparsity of *in vivo* kinetic data. Recently, a combination of stochastic kinetic modeling (***Ruppe and Fox, 2018***) and *in vitro* kinetic measurements (***Ranganathan et al., 2012***; ***Yu et al., 2011***) have revealed remarkably slow steps in the fatty acid synthesis pathways which may serve as the rate limiting reactions. One such step is the removal of hydroxyl groups from the fatty-acid chain by ACP dehydratase, leading to the formation of carbon-carbon double bonds. This reaction, catalyzed both by proteins FabZ and FabA in *E. coli* (***Yu et al., 2011***), have been estimated to have kinetic turnover rates of ≈ 1 dehydration per second per enzyme (***Ruppe and Fox, 2018***). Combining this rate estimate, our previous estimates for the number of lipids to be formed, and a 5000 second division yields and estimate that the cell requires ≈ 4000 ACP dehydratases. This estimate is in reasonable agreement with the experimentally observed copy numbers of FabZ and FabA (***Figure 2***(A)). Furthermore, we can extend this estimate to account for the change in membrane surface area as a function of the growth rate (grey line in ***Figure 2***(A)), which captures the observed growth rate dependent expression of these two enzymes.

Despite the slow catalytic rate of the FabZ and FabA enzymes, we argue that the generation of fatty acids is not a bottleneck in cell division and is not the key process responsible for setting the bacterial growth rate. Experimental evidence has shown that the rate of fatty-acid synthesis can be drastically increased *in vitro* by increasing the concentration of FabZ ***Yu et al.*** (***2011***). Stochastic simulations of the complete fatty acid synthesis pathway of *E. coli* further supports this experimental observation ***Ruppe and Fox*** (***2018***). Thus, if this step was the determining factor in cell division, increasing growth rate could be as simple as increasing the number of ACP dehydratases per cell.

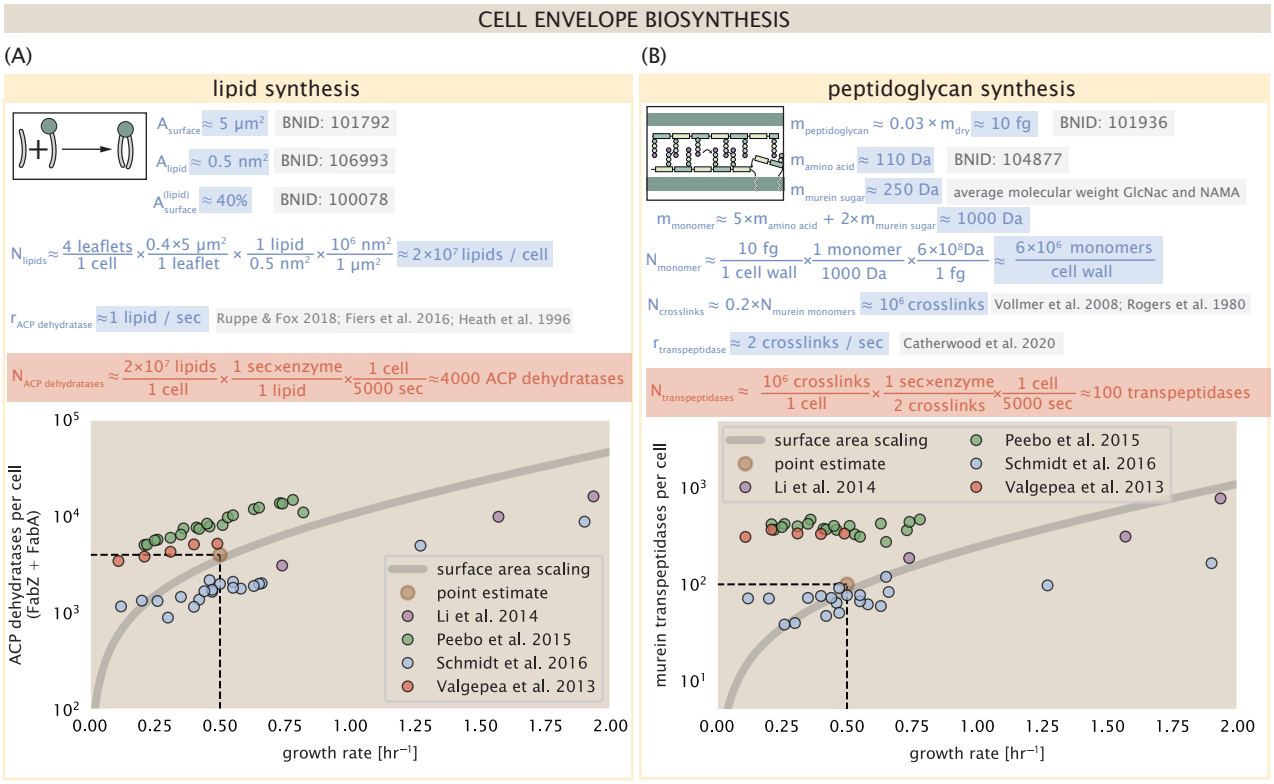

Figure 2. Estimation of the key components involved in cell envelope biosynthesis. (A) Top panel shows a schematic estimation for the number of ACP dehydratases necessary to form functional phospholipids. The rate of ACP dehydratases was inferred from experimental measurements via a stochastic kinetic model described in ***Ruppe and Fox* (*2018*)**. Bottom panel shows the experimentally observed complex copy numbers using the stoichiometries $[FabA]_2$ and $[FabZ]_2$. (B) An estimate for the number of peptidoglycan transpeptidases needed to complete maturation of the peptidoglycan. The mass of the murein monomer was estimated by approximating each amino acid in the pentapeptide chain as having a mass of 110 Da and each sugar in the disaccharide having a mass of ≈ 250 Da. The *in vivo* rate of transpeptidation rein *E. coli* was taken from recent analysis by ***Catherwood et al.* (*2020*)**. The bottom panel shows experimental measurements of the transpeptidase complexes in *E. coli* following the stoichiometries $[MrcA]_2$, $[MrcB]_2$, $[MrdA]_1$, and $[MrdB]_1$. Grey curves in each plot show the estimated number of complexes needed to satisfy the synthesis requirements scaled by the surface area as a function of growth rate. We direct the reader to the supplemental information for a more detailed discussion of this estimate.

With a proteome size of ≈ 3×10^6 proteins, a drastic increase in expression from 4000 to 40,000 ACP dehydratases would result in a ≈ 1% increase in the proteome pool. As other proteins such as ribosomes and tRNA synthetases are in much larger abundance than 4000 per cell (as we will see in the coming sections), it is unlikely that expression of ACP dehydratases couldn't be increased to facilitate faster growth.

Peptidoglycan Synthesis

While variation in cell size as a function growth rate can be large, bacterial cells demonstrate exquisite control over their cell shape. The maintenance of cell shape is due primarily to the intricate meshwork of the cell wall, a shell of polymerized discaccharides interspersed with short peptide crosslinks termed the peptidoglycan. In gram negative bacteria, such as *E. coli*, this enormous peptidoglycan molecule is a few nanometers thick and resides within the periplasmic space between the inner and outer membrane. The formation of the peptidoglycan is an intricate process, involving the bacterial actin homolog MreB (***Shi et al., 2018***) along with a variety of membrane-bound and periplasmic enzymes (***Morgenstein et al., 2015***). The coordinated action of these components result in a highly-robust polymerization framework which maintains cell shape even in the face of large-scale perturbations and can restore rod-shaped morphology even after digestion of the peptidoglycan (***Harris and Theriot, 2018***; ***Shi et al., 2018***).

In glucose-supported balanced growth, the peptidoglycan alone comprises ≈ 3% of the cellular dry mass (BNID: 101936, **Milo et al.** (**2010**)), making it the most massive molecule in *E. coli*. The polymerized unit of the peptidoglycan is a N-acetylglucosamine and N-acetylmuramic acid disaccharide, the former of which is functionalized with a short pentapeptide. With a mass of ≈ 1000 Da, this unit, which we refer to as a murein monomer, is polymerized to form long strands in the periplasm which are attached to eachother via the peptide linkers. Using the aforementioned measurement that ≈ 3% of the dry mass is peptidoglycan, it can be estimated that the peptidoglycan is composed of $\approx 6\times10^6$ murein monomers.

In principle, each one of these murein monomers can be crosslinked to another glycan strand via the pentapeptide. In some species, such as in gram-positive bacterium *Staphylococcus aureus*, the extent of crosslinking can be large with > 90% of pentapeptides forming a connection between glycan strands. In *E. coli*, however, a much smaller proportion (≈ 20%) of the peptides are crosslinked, resulting in a weaker and more porous cell wall **Vollmer et al.** (**2008**); **Rogers et al.** (**1980**). The formation of these crosslinks primarily occur during the polymerization of the murein monomers and is facilitated by a family of enzymes called transpeptidases. In *E. coli*, there are four primary transpeptidases that are involved in lateral and longitudinal extension of the peptidoglycan. These transpeptidases have only recently been quantitatively characterized *in vivo* via liquid chromatography mass spectrometry (**Catherwood et al., 2020**), which revealed a kinetic turnover rate of $\approx 1-2$ crosslinking reactions formed per second per enzyme.

Pulling these measurements together permits us to make an estimate that on the order of ≈ 100 transpeptidases are needed for complete maturation of the peptidoglycan, given a division time of ≈ 5000 seconds, a value that is closely aligned with the experimental observations (**Figure 2**(B)). Expanding this estimate to account for the changing volume of the peptidoglycan as a function of growth rates (grey line in **Figure 2**(B)) also qualitatively captures the observed dependence in the data, though systematic disagreements between the different data sets makes the comparison more difficult.

Much as in the case of fatty acid synthesis, we find it unlikely that the formation of peptidoglycan is a rate limiting step in bacterial cell division. The estimate we have presented considers only the transpeptidase enzymes that are involved lateral and longitudinal elongation of the peptidoglycan (proteins MrdA, MrdB, MrcA, and MrcB). This neglects the presence of other transpeptidases that are present in the periplasm that are involved in remodeling and maturation of the peptidoglycan. It is therefore possible that if this was setting the speed limit for cell division, the simple expression of more maturation transpeptidases may be sufficient to maintain the structural integrity of the cell wall.

Furthermore, it has been shown experimentally that, while critical for cell wall formation, there are components needed beyond the transpeptidases to maintain cell shape and permit cell division. For example, the bacterial actin homolog MreB polymerizes laterally along the inner membrane and facilitates the longitudinal expansion of the peptidoglycan. Inhibition of MreB through the addition of small molecules results in loss of the cell shape, though formation of the peptidoglycan is not significantly hindered **Shi et al.** (**2018**). This suggests that in considering the development of the cell envelope, proper assembly may be a more important property to consider beyond synthesis of the appropriate number of glycan polymers.

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