Fundamental limits on the rate of bacterial cell division

Nathan M. Belliveau[†, 1], **Griffin Chure**[†, 2, 3], **Christina L. Hueschen**[4], **Hernan G. Garcia**[5], **Jane Kondev**[6], **Daniel S. Fisher**[7], **Julie A. Theriot**[1, 8], **Rob Phillips**[2, 9, *]

***For correspondence:**

[†]These authors contributed equally to this work

[1]Department of Biology, University of Washington, Seattle, WA, USA; [2]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA; [3]Department of Applied Physics, California Institute of Technology, Pasadena, CA, USA; [4]Department of Chemical Engineering, Stanford University, Stanford, CA, USA; [5]Department of Molecular Cell Biology and Department of Physics, University of California Berkeley, Berkeley, CA, USA; [6]Department of Physics, Brandeis University, Waltham, MA, USA; [7]Department of Applied Physics, Stanford University, Stanford, CA, USA; [8]Allen Institute for Cell Science, Seattle, WA, USA; [9]Department of Physics, California Institute of Technology, Pasadena, CA, USA; [*]Contributed equally

Abstract This will be written next (promise).

Relationship Between Cell Size and Growth Rate

The relationship between cell size and growth rate has long been of interest in the study of bacterial physiology, particularly following the now six decade-old observation that cell volume appears to increase exponentially with growth rate; known as Schaechter's growth law (***Schaechter et al., 1958***; ***Taheri-Araghi et al., 2015***). However, the mechanism that governs this relationship, and even the question of whether the change in average cell size is truly exponential has remained under debate (***Harris and Theriot, 2018***). Given the importance of cell size in determining the total protein mass that must be doubled and in setting other parameters like the surface-area-to-volume ratio, we examine the proteomic data presented thus far data to consider cell size and growth rate.

At moderate growth rates (above about 0.5 hr^{-1}), cells grow at a near-maximal rate near given their ribosomal mass fraction Φ_R (**??**(B)). This means that in order to grow any faster, cells must increase Φ_R further. A naïve strategy following the constraint of **??** is simply that cells should make additional ribosomes. In reality, however, large swaths of the proteome increase in absolute protein abundance as cells grow faster (Supplemental Figure X), and the ability to add additional ribosomes is likely constrained by other factors including crowding due to their large size (***Delarue et al., 2018***; ***Soler-Bistué et al., 2020***). Instead, it is well-documented that *E. coli* cells add a constant volume per origin of replication, which is robust to a remarkable array of cellular perturbations (***Si et al., 2017***). To consider this in the context of the proteomic data, we used the measurements from ***Si et al.*** (***2017***) for wild-type *E. coli* cells grown in different nutrient conditions (***Figure 1***(A)) to estimate the average number of origins per cell ⟨# ori⟩ across the data. Indeed, we find an approximately linear trend between protein copy number and ⟨# ori⟩, and in ***Figure 1***(B) plot this for ribosomal copy numbers.

The average number of origins ⟨# ori⟩ is set by how often replication must be initiated per cell doubling under steady-state growth, and can be quantified via

$$\langle \#\ \text{ori} \rangle = 2^{\tau_{cyc}/\tau} = 2^{\tau_{cyc}\lambda/ln(2)}, \tag{1}$$

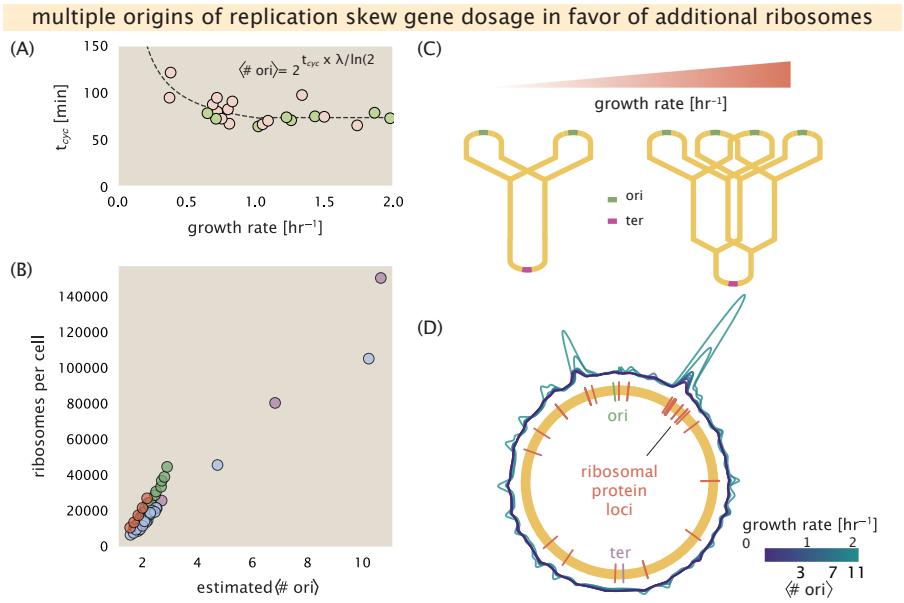

Figure 1. Multiple replication initiations bias protein synthesis in favor of more ribosome. (A) Experimental data from Si *et al.* (2017). Dashed line shows fit to the data, which were used to estimate ⟨# ori⟩. t_{cyc} was assumed to vary in proportion to τ for doubling times great than 40 minutes, and then reach a minimum value of [fill in] minutes below this (see Supplemental Appendix X for additional details). Red data points correspond to measurements in strain MG1655, while light green points are for strain NCM3722. (B) Plot of the ribosome copy number estimated from the proteomic data against the estimated ⟨# ori⟩. (C) Schematic shows the expected increase in replication forks (or number of ori regions) as *E. coli* cells grow faster. (D) A running Gaussian average (20 kbp st. dev.) of protein copy number is calculated for each growth condition considered by (***Schmidt et al., 2016***). Since total protein abundance increases with growth rate, protein copy numbers are median-subtracted to allow comparison between growth conditions. ⟨# ori⟩ are estimated using the data in (A) and Equation 1. [still looking into how best to use this type of analysis]

where t_{cyc} is the cell cycle time (referring to the time from replication initiation to cell division), and τ is the cell doubling time. For a constant cell cycle time, observed at growth rates above about 0.5 hr^{-1} (***Helmstetter and Cooper, 1968***), ***Equation 1*** says that ⟨# ori⟩ will increase exponentially with the growth rate.

Insight into why cells add a constant volume per ⟨# ori⟩, and how this relates to growth, however, requires us to consider the changes in the proteome, and in particular ribosomal proteins across the different growth conditions. In ***Figure 1***(D) we consider the position-dependent protein expression across the chromosome for each of the growth conditions from ***Schmidt et al.*** (***2016***). Here we calculated a running Gaussian average of protein copy number (20 kbp st. dev. averaging window) based on each gene's transcriptional start site, which were then median-subtracted to account for the differences in total protein abundance with each growth condition. Importantly, we find that the major deviations in protein copy number are largely restricted to regions of ribosomal protein genes, with substantially higher deviations observed for cells with high ⟨# ori⟩ (teal), as compared to those with low ⟨# ori⟩ (purple). This is particularly apparent for genes closer to the origin, where the majority of ribosomal proteins are found. This suggests that in addition to the linear scaling between protein abundance and ⟨# ori⟩, cells are also varying their relative ribosomal abundance in proportion to ⟨# ori⟩. Since growth rate depends specifically on the ribosomal fraction Φ_R, this result suggests that cells are changing their size as a way to tune Φ_R to match the available nutrient conditions.

While this dependence between cell size and ribosomal abundance is apparent across moderate to fast growth rates, it is worth noting that this scaling is likely to change at slow growth rates (below $\lambda \approx 0.5\,\mathrm{hr}^{-1}$). Here, the number of ribosomes R no longer reflects the cell's protein synthesis

capacity (***Dai et al., 2016***), so far taken to be $r_t \times R$, and instead, cells have an excess number of ribosomes. Additional regulatory control through the small-molecule alarmones such as guanosine pentaphosphate [(p)ppGpp] reduce the fraction of actively translating ribosomes at these slower growth rates. This overabundance of ribosomes provides different challenges on the ability of the cell to maintain steady-state growth under limiting nutrient conditions, and in Supplemental Section XX we consider this slow growth regime further.

As a final comment, it has recently been shown that growth in a (p)ppGpp null strain also lacked both the condition-dependent changes in ⟨# ori⟩ as well as changes in cell size across different growth condition. Instead, cells always exhibited a high ratio of ⟨# ori⟩ to ⟨# ter⟩, irrespective of growth rate, and a cell size that was more consistent with a fast growth state where (p)ppGpp levels are normally low (***Fernández-Coll et al., 2020***) and ribosomal fraction is high (***Zhu and Dai, 2019***). There is also evidence that this may be achived through inhibition of DNA replication initiation (***Kraemer et al., 2019***). These observations raise the possibility that (p)ppGpp may be playing a causal role in tuning ⟨# ori⟩ and cell size, which ultimately allows the cell to vary its ribosomal content according to nutrient availability.

[This last paragraph may be better placed in the discussion]

References

Dai, X., Zhu, M., Warren, M., Balakrishnan, R., Patsalo, V., Okano, H., Williamson, J. R., Fredrick, K., Wang, Y.-P., and Hwa, T. (2016). Reduction of translating ribosomes enables *Escherichia coli* to maintain elongation rates during slow growth. *Nature Microbiology*, 2(2):16231.

Delarue, M., Brittingham, G. P., Pfeffer, S., Surovtsev, I. V., Pinglay, S., Kennedy, K. J., Schaffer, M., Gutierrez, J. I., Sang, D., Poterewicz, G., Chung, J. K., Plitzko, J. M., Groves, J. T., Jacobs-Wagner, C., Engel, B. D., and Holt, L. J. (2018). mTORC1 Controls Phase Separation and the Biophysical Properties of the Cytoplasm by Tuning Crowding. *Cell*, 174(2):338–349.e20.

Fernández-Coll, L., Maciag-Dorszynska, M., Tailor, K., Vadia, S., Levin, P. A., Szalewska-Palasz, A., Cashel, M., and Dunny, G. M. (2020). The Absence of (p)ppGpp Renders Initiation of *Escherichia coli* Chromosomal DNA Synthesis Independent of Growth Rates. *mBio*, 11(2):45.

Harris, L. K. and Theriot, J. A. (2018). Surface Area to Volume Ratio: A Natural Variable for Bacterial Morphogenesis. *Trends in microbiology*, 26(10):815–832.

Helmstetter, C. E. and Cooper, S. (1968). DNA synthesis during the division cycle of rapidly growing Escherichia coli Br. *Journal of Molecular Biology*, 31(3):507–518.

Kraemer, J. A., Sanderlin, A. G., and Laub, M. T. (2019). The Stringent Response Inhibits DNA Replication Initiation in E. coliby Modulating Supercoiling of oriC. *mBio*, 10(4):822.

Schaechter, M., MaalØe, O., and Kjeldgaard, N. O. (1958). Dependency on Medium and Temperature of Cell Size and Chemical Composition during Balanced Growth of *Salmonella typhimurium*. *Microbiology*, 19(3):592–606.

Schmidt, A., Kochanowski, K., Vedelaar, S., Ahrné, E., Volkmer, B., Callipo, L., Knoops, K., Bauer, M., Aebersold, R., and Heinemann, M. (2016). The quantitative and condition-dependent *Escherichia coli* proteome. *Nature Biotechnology*, 34(1):104–110.

Si, F., Li, D., Cox, S. E., Sauls, J. T., Azizi, O., Sou, C., Schwartz, A. B., Erickstad, M. J., Jun, Y., Li, X., and Jun, S. (2017). Invariance of Initiation Mass and Predictability of Cell Size in *Escherichia coli*. *Current Biology*, 27(9):1278–1287.

Soler-Bistué, A., Aguilar-Pierlé, S., Garcia-Garcerá, M., Val, M.-E., Sismeiro, O., Varet, H., Sieira, R., Krin, E., Skovgaard, O., Comerci, D. J., Eduardo P. C. Rocha, and Mazel, D. (2020). Macromolecular crowding links ribosomal protein gene dosage to growth rate in Vibrio cholerae. *BMC Biology*, 18(1):1–18.

Taheri-Araghi, S., Bradde, S., Sauls, J. T., Hill, N. S., Levin, P. A., Paulsson, J., Vergassola, M., and Jun, S. (2015). Cell-size control and homeostasis in bacteria. - PubMed - NCBI. *Current Biology*, 25(3):385–391.

Zhu, M. and Dai, X. (2019). Growth suppression by altered (p)ppGpp levels results from non-optimal resource allocation in *Escherichia coli*. *Nucleic Acids Research*, 47(9):4684–4693.