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]**, Jané Kondev**[6]**, Julie Theriot**[1, 7]**, Rob Phillips**[1, 8, †]

[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]Allen Institute for Cell Science, Seattle, WA, USA; [8]Department of Physics, California Institute of Technology, Pasadena, CA, USA; [†]Address correspondence to phillips@pboc.caltech.edu; [*]Contributed equally

*For correspondence:

*These authors contributed equally to this work

Abstract

Introduction

The range of bacterial growth rates can be enormous. In natural environments, doublings occur approximately 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 strong, linear dependence of the total cellular protein content on the 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 examined this relationship with single-protein resolution using modern methods of mass spectrometry (*Valgepea et al., 2013*; *Peebo et al., 2015*; *Schmidt et al., 2016*) and ribosomal profiling (*Li et al., 2014*) which permit a quantitative investigation of the relationship between gene expression and growth rate. This body of experimental data places us in the auspicious position to explore how the abundance of fundamental 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 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 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 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
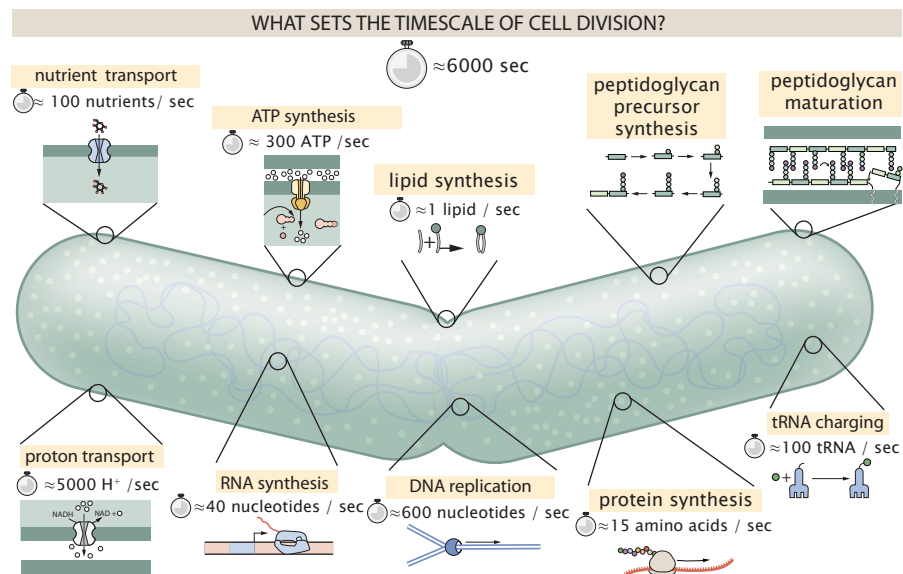
Figure 1. Transport and synthesis processes necessary for cell division. We consider an array of processes necessary for a cell to double its molecular components. Such processes include the transport of carbon across the cell membrane, the production of ATP, and fundamental processes of the central 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.

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 exceeded by the experimental observations, allowing us to systematically scratch off the hypotheses diagrammed in *Figure 1* as setting the speed limit. Ultimately, we find that protein translation (particularly the generation of new ribosomes) acts as the rate limiting step of bacterial division. 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 cellular growth rate.

Nutrient Transport

In order to build new cellular mass, the molecular and elemental building blocks must be scavenged from the environment in different forms. Carbon, for example, is acquired via the transport of carbohydrates and sugar alcohols with some carbon sources receiving preferential treatment in their consumption (*Monod, 1947*). Phosphorus, sulfur, and nitrogen, on the other hand, are harvested primarily in the forms of inorganic salts, namely phosphate, sulfate, and ammonia (*Jun et al., 2018*; *Assentoft et al., 2016*; *Stasi et al., 2019*; *Antonenko et al., 1997*; *Rosenberg et al., 1977*; *Willsky et al., 1973*). All of these compounds have different permeabilities across the cell membrane and most require some energetic investment either via ATP hydrolysis or through the proton electrochemical gradient to bring the material across the hydrophobic cell membrane. Given the diversity of biological transport mechanisms and the vast number of inputs needed to build a cell, we begin by considering transport of elemental requirements as a possible rate-limiting step

68 of bacterial cell division.

69 The elemental composition of *E. coli* has received much quantitative attention over the past
70 half century (*Neidhardt et al., 1991*; *Taymaz-Nikerel et al., 2010*; *Heldal et al., 1985*; *Bauer and*
71 *Ziv, 1976*), providing us a starting point for estimating the copy numbers of various transporters.
72 While there is some variability in the exact elemental percentages (with different uncertainties), we
73 can estimate that the dry mass of a typical *E. coli* cell is $\approx$ 45% carbon (BNID: 100649, *Milo et al.*
74 (*2010*)), $\approx$ 15% nitrogen (BNID: 106666, *Milo et al.* (*2010*)), $\approx$ 3% phosphorus (BNID: 100653, *Milo*
75 *et al.* (*2010*)), and 1% sulfur (BNID: 100655, *Milo et al.* (*2010*)). In the coming paragraphs, we will
76 examine how many transporters and/or channels must be present to maintain these elemental
77 compositions with a moderate doubling time of 6000 s.

Carbon Transport

79 We begin with the most abundant element by mass, carbon. Using $\approx$ 0.3 pg as the typical *E. coli*
80 dry mass (BNID: 103904, *Milo et al.* (*2010*)), we estimate that $\approx 10^{10}$ carbon atoms must be brought
81 into the cell in order to double all of the carbon-containing molecules (*Figure 2*(A, top))). Typical
82 laboratory growth conditions, such as those explored in the aforementioned proteomic data sets,
83 provide carbon as single class of sugar such as glucose, galactose, or xylose to name a few. *E.*
84 *coli* has evolved myriad mechanisms by which these sugars can be transported across the cell
85 membrane. One such mechanism of transport is via the PTS system which is a highly modular
86 system capable of transporting a diverse range of sugars (*Escalante et al., 2012*). The glucose-
87 specific component of this system transports $\approx$ 200 glucose molecules per second per channel
88 (BNID: 114686, *Milo et al.* (*2010*)). Making the assumption that this is a typical sugar transport rate,
89 coupled with the need to transport 10^{10} carbon atoms, we arrive at the conclusion that on the
90 order of 1000 transporters must be expressed in order to bring in enough carbon atoms to divide
91 in 6000 s, diagrammed in the top panel of *Figure 2*(A). This estimate, along with the observed
92 average number of carbohydrate transporters present in the proteomic data sets (*Schmidt et al.,*
93 *2016*; *Peebo et al., 2015*; *Valgepea et al., 2013*; *Li et al., 2014*), is shown in *Figure 2*(A). While we
94 estimate 1000 transporters are needed, the data reveals that at a division time of ≈ 6000 s there is
95 nearly a ten-fold excess of transporters. Furthermore, the data illustrates that the average number
96 of carbohydrate transporters present is largely-growth rate independent.

97 The estimate presented in *Figure 2*(A) neglects any specifics of the regulation of carbon trans-
98 port system and presents a data-averaged view of how many carbohydrate transporters are present
99 on average. Using the diverse array of growth conditions explored in the proteomic data sets,
100 we can explore how individual carbon transport systems depend on the population growth rate.
101 In *Figure 2*(B), we show the total number of carbohydrate transporters specific to different car-
102 bon sources. A striking observation, shown in the top-left plot of *Figure 2*(B), is the constancy
103 in the expression of the glucose-specific transport systems (the PtsG enzyme of the PTS system
104 and the glucose-transporting ManXYZ complex). Additionally, we note that the total number of
105 glucose-specific transporters is tightly distributed $\approx 10^4$ per cell, an order of magnitude beyond
106 the estimate shown in *Figure 2*(A). This illustrates that *E. coli* maintains a substantial number of
107 complexes present for transporting glucose which is known to be the preferential carbon source
108 (*Monod, 1947*; *Liu et al., 2005*; *Aidelberg et al., 2014*).

109 It is now understood that a large number of metabolic operons are regulated with dual-input
110 logic gates that are only expressed when glucose concentrations are low (mediated by cyclic-AMP
111 receptor protein CRP) and the concentration of other carbon sources are elevated (*Gama-Castro*
112 *et al., 2016*; *Zhang et al., 2014b*). A famed example of such dual-input regulatory logic is in the regu-
113 lation of the *lac* operon which is only natively activated in the absence of glucose and the presence
114 of allolactose, an intermediate in lactose metabolism (*Jacob and Monod, 1961*), though we now
115 know of many other such examples (*Ireland et al., 2020*; *Gama-Castro et al., 2016*; *Belliveau et al.,*
116 *2018*). This illustrates that once glucose is depleted from the environment, cells have a means to
117 dramatically increase the abundance of the specific transporter needed to digest the next sugar
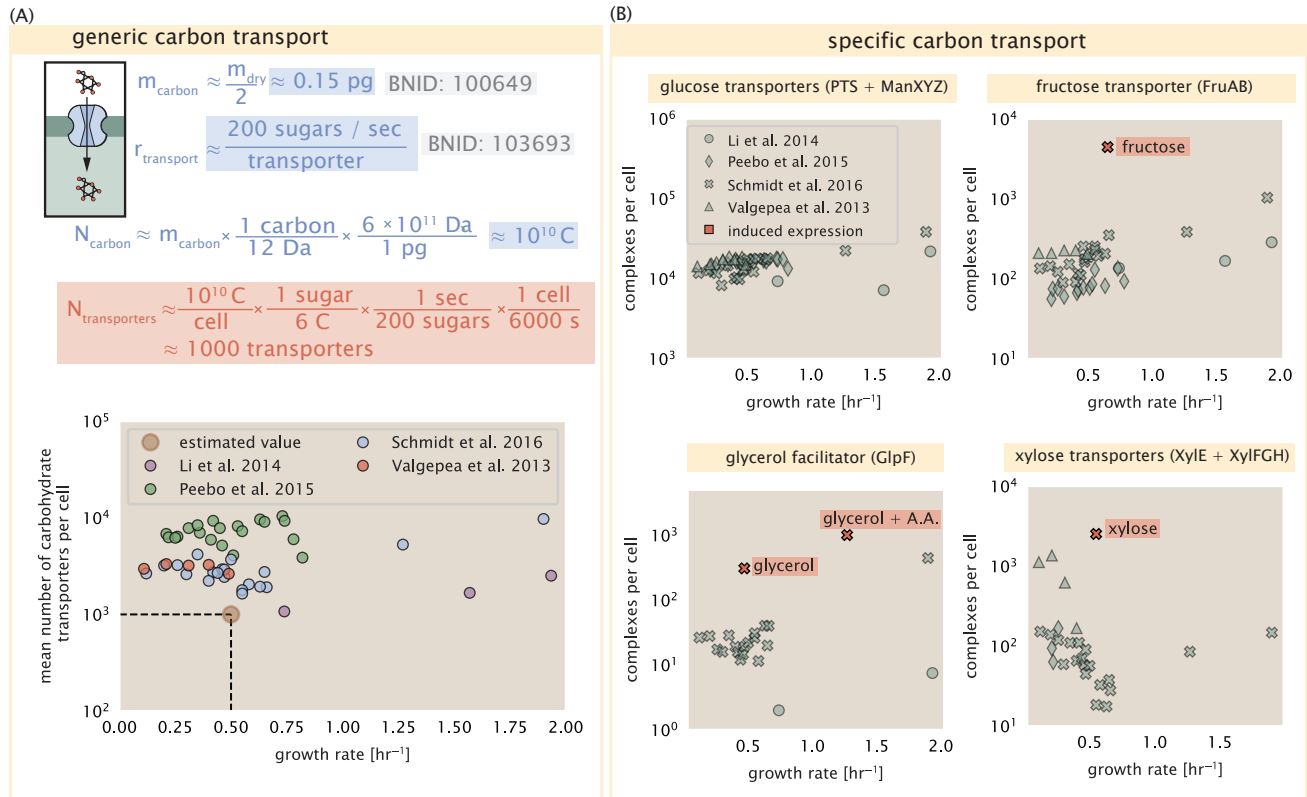
Figure 2. The abundance of carbon transport systems across growth rates. (A) A simple estimate for the minimum number of generic carbohydrate transport systems (top) assumes $\approx 10^{10}$ C are needed to complete division, each transported sugar contains ≈ 6 C, and each transporter conducts sugar molecules at a rate of ≈ 200 per second. Bottom plot shows the estimated number of transporters needed at a growth rate of ≈ 0.5 per hr (light-brown point and dashed lines). Colored points correspond to the mean number of carbohydrate transporters for different growth conditions across different published datasets. (B) The abundance of various specific carbon transport systems plotted as a function of the population growth rate. Red points and red-highlighted text indicate conditions in which the only source of carbon in the growth medium induces expression of the transport system.

118 that is present. Several examples of induced expression of a specific carbon-source transporters
119 are shown in *Figure 2*(B). Points colored in red (labeled by red text-boxes) correspond to growth
120 conditions in which the specific carbon source (glycerol, xylose, or fructose) is present. These plots
121 show that, in the absence of the particular carbon source, expression of the transporters is main-
122 tained on the order of $\sim 10^2$ per cell. However, when induced, the transporters become highly-
123 expressed and are present on the order of $\sim 10^4$ per cell, which exceeds the generic estimate given
124 in *Figure 2*(A). Together, this generic estimation and the specific examples of induced expression
125 suggest that transport of carbon across the cell membrane, while critical for growth, is not the
126 rate-limiting step of cell division.

127 **Phosphorus and Sulfur Transport**
128 We now turn our attention towards other essential elements, namely phosphorus and sulfur. Phos-
129 phorus is critical to the cellular energy economy in the form of high-energy phosphodiester bonds
130 making up DNA, RNA, and the XTP energy pool as well as playing a critical role in the post-translational
131 modification of proteins and defining the polar-heads of lipids. In total, phosphorus makes up
132 $\approx$3% of the cellular dry mass which in typical experimental conditions is in the form of inorganic
133 phosphate. The cell membrane has remarkably low permeability to this highly-charged and critical
134 molecule, therefore requiring the expression of active transport systems. In *E. coli*, the proton elec-
135 trochemical gradient across the inner membrane is leveraged to transport inorganic phosphate
136 into the cell *Rosenberg et al.* (*1977*). Proton-solute symporters are widespread in *E. coli Ramos and*
137 *Kaback* (*1977*); *Booth et al.* (*1979*) and can have rapid transport rates of 50 molecules per second
138 for sugars and other solutes (BNID: 103159; 111777, *Milo et al.* (*2010*)). In *E. coli* the PitA phosphate
139 transport system has been shown to very tightly coupled with the proton electrochemical gradient
140 with a 1:1 proton:phosphate stoichiometric ratio (*Harris et al., 2001*; *Feist et al., 2007*). Illustrated
141 in *Figure 3*(A), we can estimate that $\approx$ 300 phosphate transporters are necessary to maintain an
142 $\approx$ 3% dry mass with a 6000 s division time. This estimate is again satisfied when we examine the
143 observed copy numbers of PitA in proteomic data sets (plot in *Figure 3*(A)). While our estimate is
144 very much in line with the observed numbers, we emphasize that this is likely a slight over estimate
145 of the number of transporters needed as there are other phosphorous scavenging systems, such
146 as the ATP-dependent phosphate transporter Pst system which we have neglected.

147 Satisfied that there are a sufficient number of phosphate transporters present in the cell, we
148 now turn sulfur transport as another potentially rate limiting process. Similar to phosphate, sulfate
149 is highly-charged not particularly membrane permeable, requiring active transport. While there ex-
150 ists a H+/sulfate symporter in *E. coli*, it is in relatively low abundance and is not well characterized
151 (*Zhang et al., 2014a*). Sulfate is predominantly acquired via the ATP-dependent ABC transporter
152 CysUWA system which also plays an important role in selenium transport (*Sekowska et al., 2000*;
153 *Sirko et al., 1995*). While specific kinetic details of this transport system are not readily available,
154 generic ATP transport systems in prokaryotes are on the order of 1 to 10 molecules per second
155 (BNID: 109035, *Milo et al.* (*2010*)). Combining this generic transport rate, measurement of sulfur
156 comprising 1% of dry mass, and a 6000 second division time yields an estimate of $\approx$ 1000 CysUWA
157 complexes per cell (*Figure 3*(B)). Once again, this estimate is in notable agreement with proteomic
158 data sets, suggesting that there are sufficient transporters present to acquire the necessary sulfur.
159 In a similar spirit of our estimate of phosphorus transport, we emphasize that this is likely an over-
160 estimate of the number of necessary transporters as we have neglected other sulfur scavenging
161 systems that are in lower abundance.

162 **Nitrogen Transport**
163 Finally, we turn to nitrogen transport as the last remaining transport system highlighted in *Fig-*
164 *ure 1*. Unlike phosphate, sulfate, and various sugar molecules, nitrogen in the form of ammonia
165 can readily diffuse across the cell membrane and has a permeability on par with water ($\approx 10^5$ nm/s,
166 BNID:110824 *Milo et al.* (*2010*)). In particularly nitrogen-poor conditions, *E. coli* expresses a trans-

(A)

phosphorus transport

$m_{phosphorus} \approx 0.03 \times m_{dry}$ $\approx 10\ fg$ BNID: 100653

$N_{phosphorus} \approx m_{phosphorus} \times \dfrac{1\ P}{30\ Da} \times \dfrac{6 \times 10^8\ Da}{1\ fg}$

$\approx 2 \times 10^8\ P$

$r_{transport} \approx \dfrac{100\ phosphate\ /\ s}{transporter}$

$N_{transporters} \approx \dfrac{2 \times 10^8\ P}{cell} \times \dfrac{1\ phosphate}{1\ P} \times \dfrac{1\ s}{10^2\ phosphate} \times \dfrac{1\ cell}{6000\ s}$

$\approx 300\ transporters$

(B)

sulfur transport

$m_{sulfur} \approx 0.01 \times m_{dry}$ $\approx 3\ fg$ BNID: 109665

$N_{sulfur} \approx m_{sulfur} \times \dfrac{1\ S}{32\ Da} \times \dfrac{6 \times 10^8\ Da}{1\ fg}$ $\approx 5 \times 10^7\ S$

$r_{transport} \approx \dfrac{10\ sulfate\ /\ s}{transporter}$ BNID: 109035

$N_{transporters} \approx \dfrac{5 \times 10^7\ S}{cell} \times \dfrac{1\ sulfate}{1\ S} \times \dfrac{1\ s}{10\ sulfate} \times \dfrac{1\ cell}{6000\ s}$
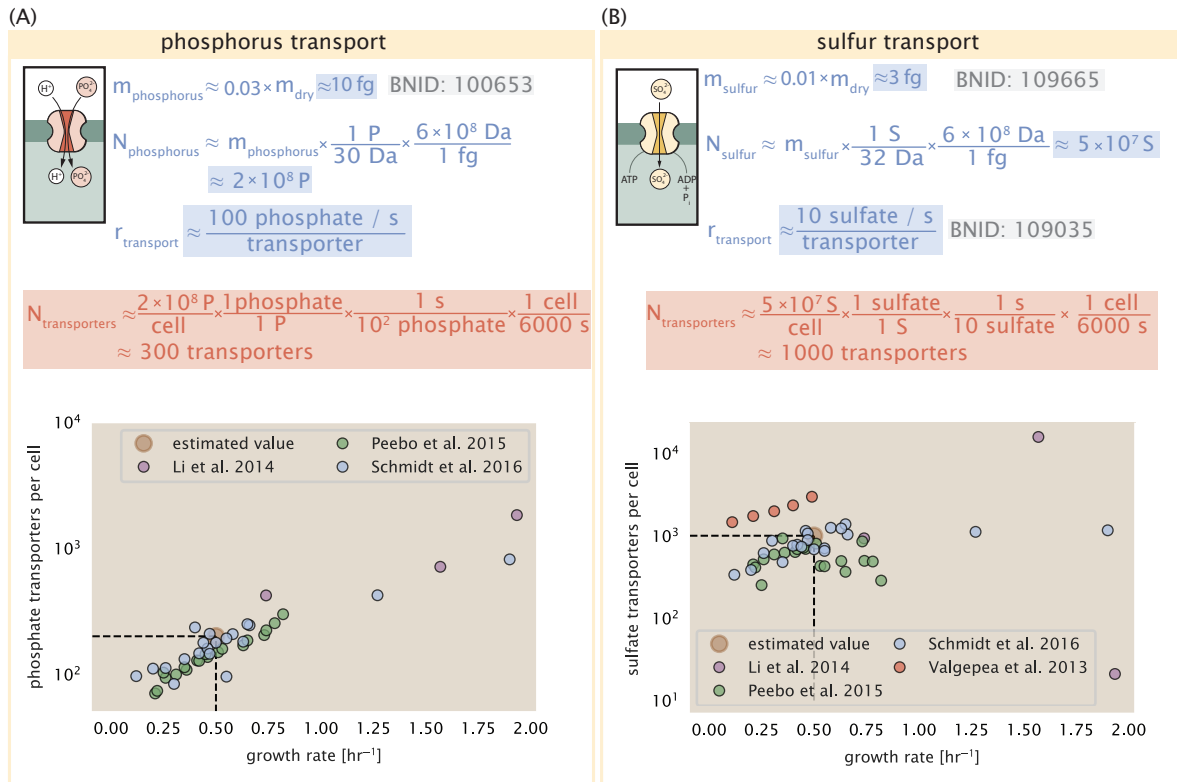
$\approx 1000\ transporters$

Figure 3. Estimates and measurements of phosphate and sulfate transport systems as a function of growth rate. (A) Estimate for the number of PitA phosphate transport systems needed to maintain a 3% phosphorus *E. coli* dry mass. Points in plot correspond to the the total number of PitA transporters per cell. (B) Estimate of the number of CysUWA complexes necessary to maintain a 1% sulfur *E. coli* dry mass. Points in plot correspond to average number of CysUWA transporter complexes that can be formed given the transporter stoichiometry [CysA]$_2$[CysU][CysW][Sbp/CysP].

167 porter (AmtB) which appears to aid in nitrogen assimilation, though the mechanism and kinetic
168 details of transport is still a matter of debate (*van Heeswijk et al., 2013*; *Khademi et al., 2004*). Be-
169 yond ammonia, another plentiful source of nitrogen come in the form of glutamate, which has it's
170 own complex metabolism and scavenging pathways. However, nitrogen is plentiful in the growth
171 conditions examined in this work, permitting us to neglect nitrogen transport as a potential rate
172 limiting process in cell division.

References

174 Aidelberg, G., Towbin, B. D., Rothschild, D., Dekel, E., Bren, A., and Alon, U. (2014). Hierarchy of non-glucose
175   sugars in Escherichia coli. *BMC Systems Biology*, 8(1):133.

176 Antonenko, Y. N., Pohl, P., and Denisov, G. A. (1997). Permeation of ammonia across bilayer lipid membranes
177   studied by ammonium ion selective microelectrodes. *Biophysical Journal*, 72(5):2187–2195.

178 Assentoft, M., Kaptan, S., Schneider, H.-P., Deitmer, J. W., de Groot, B. L., and MacAulay, N. (2016). Aquaporin 4
179   as a NH3 Channel. *Journal of Biological Chemistry*, 291(36):19184–19195.

180 Bauer, S. and Ziv, E. (1976). Dense growth of aerobic bacteria in a bench-scale fermentor. *Biotechnology and*
181   *Bioengineering*, 18(1):81–94. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/bit.260180107.

182 Belliveau, N. M., Barnes, S. L., Ireland, W. T., Jones, D. L., Sweredoski, M. J., Moradian, A., Hess, S., Kinney, J. B.,
183   and Phillips, R. (2018). Systematic approach for dissecting the molecular mechanisms of transcriptional
184   regulation in bacteria. *Proceedings of the National Academy of Sciences*, 115(21):E4796–E4805.

185 Booth, I. R., Mitchell, W. J., and Hamilton, W. A. (1979). Quantitative analysis of proton-linked transport systems.
186   The lactose permease of Escherichia coli. *Biochemical Journal*, 182(3):687–696.

187 Escalante, A., Salinas Cervantes, A., Gosset, G., and Bolívar, F. (2012). Current knowledge of the Escherichia coli
188   phosphoenolpyruvate–carbohydrate phosphotransferase system: Peculiarities of regulation and impact on
189   growth and product formation. *Applied Microbiology and Biotechnology*, 94(6):1483–1494.

190 Feist, A. M., Henry, C. S., Reed, J. L., Krummenacker, M., Joyce, A. R., Karp, P. D., Broadbelt, L. J., Hatzimanikatis,
191   V., and Palsson, B. Ø. (2007). A genome-scale metabolic reconstruction for Escherichia coli K-12 MG1655 that
192   accounts for 1260 ORFs and thermodynamic information. *Molecular Systems Biology*, 3(1):121.

193 Gama-Castro, S., Salgado, H., Santos-Zavaleta, A., Ledezma-Tejeida, D., Muñiz-Rascado, L., García-Sotelo, J. S.,
194   Alquicira-Hernández, K., Martínez-Flores, I., Pannier, L., Castro-Mondragón, J. A., Medina-Rivera, A., Solano-
195   Lira, H., Bonavides-Martínez, C., Pérez-Rueda, E., Alquicira-Hernández, S., Porrón-Sotelo, L., López-Fuentes,
196   A., Hernández-Koutoucheva, A., Moral-Chávez, V. D., Rinaldi, F., and Collado-Vides, J. (2016). RegulonDB
197   version 9.0: High-level integration of gene regulation, coexpression, motif clustering and beyond. *Nucleic*
198   *Acids Research*, 44(D1):D133–D143.

199 Harris, R. M., Webb, D. C., Howitt, S. M., and Cox, G. B. (2001). Characterization of PitA and PitB from *Escherichia*
200   *coli*. *Journal of Bacteriology*, 183(17):5008–5014.

201 Heldal, M., Norland, S., and Tumyr, O. (1985). X-ray microanalytic method for measurement of dry matter and
202   elemental content of individual bacteria. *Applied and Environmental Microbiology*, 50(5):1251–1257.

203 Ireland, W. T., Beeler, S. M., Flores-Bautista, E., Belliveau, N. M., Sweredoski, M. J., Moradian, A., Kinney, J. B.,
204   and Phillips, R. (2020). Deciphering the regulatory genome of *Escherichia coli*, one hundred promoters at a
205   time. *bioRxiv*.

206 Jacob, F. and Monod, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular*
207   *Biology*, 3(3):318–356.

208 Jun, S., Si, F., Pugatch, R., and Scott, M. (2018). Fundamental principles in bacterial physiology - history, recent
209   progress, and the future with focus on cell size control: A review. *Reports on Progress in Physics*, 81(5):056601.

210 Khademi, S., O'Connell, J., Remis, J., Robles-Colmenares, Y., Miercke, L. J. W., and Stroud, R. M. (2004). Mechanism
211   of Ammonia Transport by Amt/MEP/Rh: Structure of AmtB at 1.35 Å. *Science*, 305(5690):1587–1594.

212 Li, G.-W., Burkhardt, D., Gross, C., and Weissman, J. S. (2014). Quantifying absolute protein synthesis rates
213   reveals principles underlying allocation of cellular resources. *Cell*, 157(3):624–635.

214 Liu, M., Durfee, T., Cabrera, J. E., Zhao, K., Jin, D. J., and Blattner, F. R. (2005). Global Transcriptional Programs
215   Reveal a Carbon Source Foraging Strategy by *Escherichia coli*. *Journal of Biological Chemistry*, 280(16):15921–
216   15927.

217 Milo, R., Jorgensen, P., Moran, U., Weber, G., and Springer, M. (2010). BioNumbers—the database of key num-
218   bers in molecular and cell biology. *Nucleic Acids Research*, 38(suppl_1):D750–D753.

219 Monod, J. (1947). The phenomenon of enzymatic adaptation and its bearings on problems of genetics and
220   cellular differentiation. *Growth Symposium*, 9:223–289.

221 Neidhardt, F. C., Ingraham, J., and Schaechter, M. (1991). *Physiology of the Bacterial Cell - A Molecular Approach*,
222   volume 1. Elsevier.

223 Peebo, K., Valgepea, K., Maser, A., Nahku, R., Adamberg, K., and Vilu, R. (2015). Proteome reallocation in *Es-*
224   *cherichia coli* with increasing specific growth rate. *Molecular BioSystems*, 11(4):1184–1193.

225 Ramos, S. and Kaback, H. R. (1977). The relation between the electrochemical proton gradient and active trans-
226   port in Escherichia coli membrane vesicles. *Biochemistry*, 16(5):854–859.

227 Rosenberg, H., Gerdes, R. G., and Chegwidden, K. (1977). Two systems for the uptake of phosphate in Es-
228   cherichia coli. *Journal of Bacteriology*, 131(2):505–511.

229 Schaechter, M., Maaløe, O., and Kjeldgaard, N. O. (1958). Dependency on medium and temperature of cell size
230   and chemical composition during balanced growth of *Salmonella typhimurium*. *Microbiology*, 19(3):592–606.

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

234 Sekowska, A., Kung, H.-F., and Danchin, A. (2000). Sulfur Metabolism in Escherichia coli and Related Bacteria:
235   Facts and Fiction. *Journal of Molecular Microbiology and Biotechnology*, 2(2):34.

236 Sirko, A., Zatyka, M., Sadowy, E., and Hulanicka, D. (1995). Sulfate and thiosulfate transport in Escherichia coli K-
237   12: Evidence for a functional overlapping of sulfate- and thiosulfate-binding proteins. *Journal of Bacteriology*,
238   177(14):4134–4136.

239 Stasi, R., Neves, H. I., and Spira, B. (2019). Phosphate uptake by the phosphonate transport system PhnCDE.
240   *BMC Microbiology*, 19.

241 Taymaz-Nikerel, H., Borujeni, A. E., Verheijen, P. J. T., Heijnen, J. J., and van Gulik, W. M. (2010).
242   Genome-derived minimal metabolic models for Escherichia coli MG1655 with estimated in
243   vivo respiratory ATP stoichiometry. *Biotechnology and Bioengineering*, 107(2):369–381. _eprint:
244   https://onlinelibrary.wiley.com/doi/pdf/10.1002/bit.22802.

245 Valgepea, K., Adamberg, K., Seiman, A., and Vilu, R. (2013). *Escherichia coli* achieves faster growth by increasing
246   catalytic and translation rates of proteins. *Molecular BioSystems*, 9(9):2344.

247 van Heeswijk, W. C., Westerhoff, H. V., and Boogerd, F. C. (2013). Nitrogen Assimilation in *Escherichia coli*: Putting
248   Molecular Data into a Systems Perspective. *Microbiology and Molecular Biology Reviews*, 77(4):628–695.

249 Willsky, G. R., Bennett, R. L., and Malamy, M. H. (1973). Inorganic Phosphate Transport in Escherichia coli:
250   Involvement of Two Genes Which Play a Role in Alkaline Phosphatase Regulation. *Journal of Bacteriology*,
251   113(2):529–539.

252 Zhang, L., Jiang, W., Nan, J., Almqvist, J., and Huang, Y. (2014a). The Escherichia coli CysZ is a pH dependent
253   sulfate transporter that can be inhibited by sulfite. *Biochimica et Biophysica Acta (BBA) - Biomembranes*,
254   1838(7):1809–1816.

255 Zhang, Z., Aboulwafa, M., and Saier, M. H. (2014b). Regulation of **crp** Gene Expression by the Catabolite Repres-
256   sor/Activator, Cra, in **Escherichia coli**. *Journal of Molecular Microbiology and Biotechnology*, 24(3):135–141.