Fundamental limits on the rate of bacterial cell division

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Abstract This will be written next eventually

Function of the Central Dogma

Up to this point, we have considered a variety of transport and biosynthetic processes that are critical to acquiring and generating new cell mass. While there are of course many other metabolic processes we could consider and perform estimates of (such as the components of fermentative versus aerobic respiration), we now turn our focus to some of the most central processes which *must* be undertaken irrespective of the growth conditions – the processes of the central dogma.

DNA

Most bacteria (including *E. coli*) harbor a single, circular chromosome and can have extra-chromosomal plasmids up to $\sim$ 100 kbp in length. We will focus our quantitative thinking solely on the chromosome of *E. coli* which harbors $\approx$ 5000 genes and $\approx 5 \times 10^6$ base pairs. To successfully divide and produce viable progeny, this chromosome must be faithfully replicated and segregated into each nascent cell. We again rely on the near century of literature in molecular biology to provide some insight on the rates and mechanics of the replicative feat as well as the production of the required starting materials, dNTPs.

dNTP synthesis

We begin our exploration DNA replication by examining the production of the deoxyribonucleotide triphosphates (dNTPs). The four major dNTPs (dATP, dTTP, dCTP, and dGTP) are synthesized *de novo* in separate pathways, requiring different building blocks. However, a critical step present in all dNTP synthesis pathways is the conversion from ribonucleotide to deoxyribonucleotide via the removal of the 3' hydroxyl group of the ribose ring (***Rudd et al., 2016***). This reaction is mediated by a class of enzymes termed ribonucleotide reductases, of which *E. coli* possesses two aerobically active complexes (termed I and II) and a single anaerobically active enzyme. Due to their peculiar formation of a radical intermediate, these enzymes have received much biochemical, kinetic, and

structural characterization. One such work (***Ge et al., 2003***) performed a detailed *in vitro* measurement of the steady-state kinetic rates of these complexes, revealing a turnover rate of ≈ 10 dNTP per second.

Since this reaction is central to the synthesis of all dNTPs, it is reasonable to consider the abundance of these complexes is a measure of the total dNTP production in *E. coli*. Illustrated schematically in ***Figure 1*** (A), we consider the fact that to replicate the cell's genome, on the order of $\approx 10^7$ dNTPs must be synthesized. Assuming a production rate of 10 per second per ribonucleotide reductase complex and a cell division time of 5000 seconds, we arrive at an estimate of ≈ 200 complexes needed per cell. As shown in the bottom panel of ***Figure 1*** (A), this estimate agrees with the experimental measurements of these complexes abundances within $\approx 1/2$ an order of magnitude. Extension of this estimate across a continuum of growth rate, including the fact that multiple chromosomes can be replicated at a given time, is shown as a grey transparent line in ***Figure 1***(A). Similarly to out point estimate, this refinement agrees well with the data, accurately describing both the magnitude of the complex abundance and the dependence on growth rate.

Recent work has revealed that during replication, the ribonucleotide reductase complexes coalesce to form discrete foci colocalized with the DNA replisome complex (***Sánchez-Romero et al., 2011***). This is particularly pronounced in conditions where growth is slow, indicating that spatial organization and regulation of the activity of the complexes plays an important role.

DNA Replication

We now turn our focus to the integration of these dNTP building blocks into the replicated chromosome strand via the DNA polymerase. Replication is initiated at a single region of the chromosome termed the *oriC* locus at which a pair of DNA polymerases bind and begin their high-fidelity replication of the genome in opposite directions. Assuming equivalence between the two replication forks, this means that the two DNA polymerase complexes (termed replisomes) meet at the midway point of the circular chromosome termed the *ter* locus. The kinetics of the five types of DNA polymerases (I – V) have been intensely studied, revealing that DNA polymerase III performs the high fidelity processive replication of the genome with the other "accessory" polymerases playing auxiliary roles (***Fijalkowska et al., 2012***). *In vitro* measurements have shown that DNA Polymerase III copies DNA at a rate of ≈ 600 nucleotides per second (BNID: 104120, ***Milo et al.*** (***2010***)). Therefore, to replicate a single chromosome, two replisomes (containing two DNA polymerase III each) moving at their maximal rate would copy the entire genome in ≈ 4000 s. Thus, with a division time of 5000 s (our "typical" growth rate for the purposes of this work), there is sufficient time for a pair of replisomes complexes to replicate the entire genome. However, this estimate implies that 4000 s would be the upper-limit time scale for bacterial division which is at odds with the familiar 20 minute (1200 s) doubling time of *E. coli* in rich medium.

It is well known that *E. coli* can parallelize its DNA replication such that multiple chromosomes are being replicated at once, with as many as 10 - 12 replication forks at a given time (***Bremer and Dennis, 2008***; ***Si et al., 2017***). Thus, even in rapidly growing cultures, we expect only a few polymerases (≈ 10) are needed to replicate the chromosome per cell doubling. However, as shown in ***Figure 1***(B), DNA polymerase III is nearly an nearly an order of magnitude more abundant. This discrepancy can be understood by considering its binding constant to DNA. DNA polymerase III is highly processive, facilitated by a strong affinity of the complex to the DNA. *In vitro* biochemical characterization has quantified the K_D of DNA polymerase III holoenzyme to single-stranded and double-stranded DNA to be 50 and 200 nM, respectively (***Ason et al., 2000***). The bottom plot in ***Figure 1*** (B) shows that the concentration of the DNA polymerase III across all data sets and growth conditions is within this range. Thus, while the copy number of the DNA polymerase III is in excess of the strict number required to replicate the genome, its copy number appears to vary such that its concentration is approximately equal to the dissociation constant to the DNA. While the processes regulating the initiation of DNA replication are complex and involve more than just the holoenzyme, these data indicate that the kinetics of replication rather than the explicit copy number of the DNA

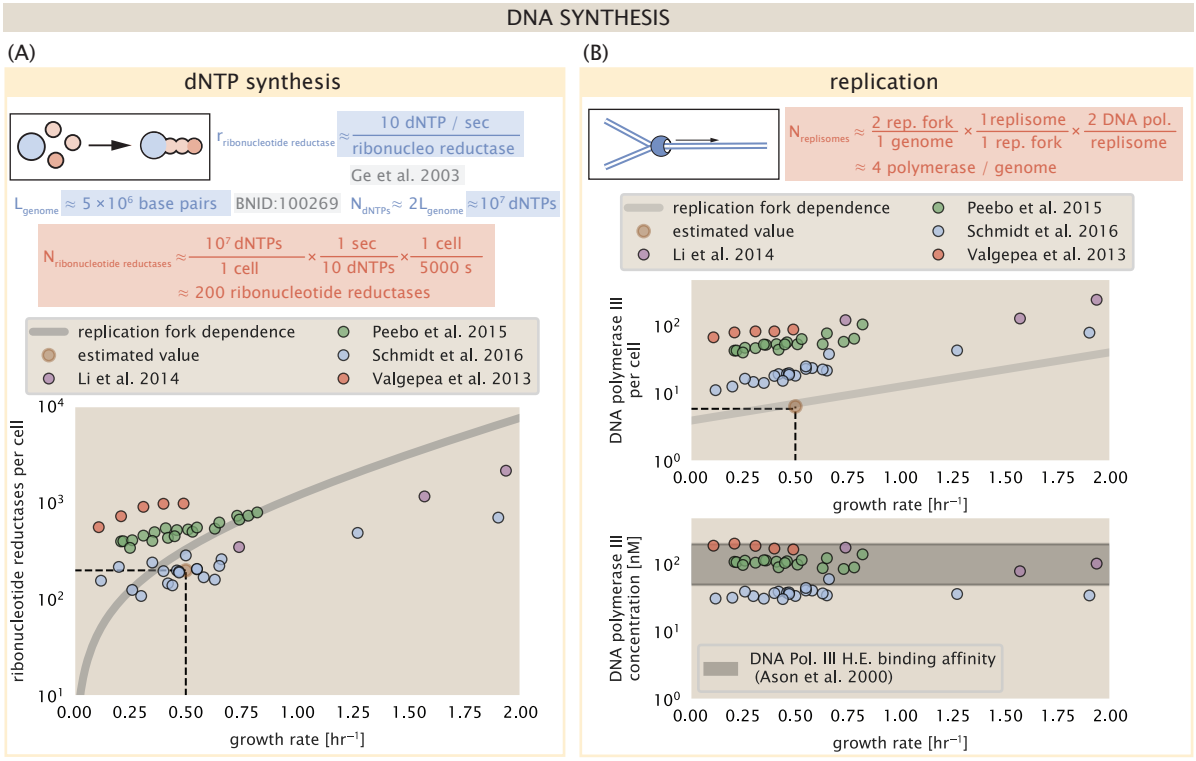

Figure 1. Complex abundance estimates for dNTP synthesis and DNA replication. (A) Estimate of the number of ribonucleotide reductase enzymes needed to facilitate the synthesis of $\approx 10^7$ dNTPs over the course of a 5000 second generation time. Points in the plot correspond to the total number of ribonucleotide reductase I ($[NrdA]_2[NrdB]_2$) and ribonucleotide reductase II ($[NrdE]_2[NrdF]_2$) complexes. (B) An estimate for the minimum number of DNA polymerase holoenzyme complexes needed to facilitate replication of a single genome. Points in the top plot correspond to the total number of DNA polymerase III holoenzyme complexes ($[DnaE]_3[DnaQ]_3[HolE]_3[DnaX]_5[HolB][HolA][DnaN]_4[HolC]_4[HolD]_4$) per cell. Bottom plot shows the effective concentration of DNA polymerase III holoenzyme given cell volumes calculated from the growth rate as in ***Si et al.*** (***2019***) (See Supplemental Information Section 4). Grey lines in (A) and top panel of (B) show the estimated number of complexes needed as a function of growth, the details of which are described in the Supplemental Information.

polymerase III holoenzyme is the more relevant feature of DNA replication to consider. In light of this, the data in ***Figure 1***(B) suggests that for bacteria like *E. coli*, DNA replication does no that represent a rate-limiting step in cell division. However, it is worth noting that for bacterium like *C. crescentus* whose chromosomal replication is initiated only once per cell cycle (***Jensen et al., 2001***), the time to double their chromosome likely represents an upper limit to their growth rate.

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