DnaA and the timing of chromosome replication in *E.coli*

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1. Introduction

The theoretical foundations for understanding chromosome replication in *E. coli* were set by Cooper and Helmstetter when they showed that the time taken for a single chromosome to be replicated (C period) and the time period between completion of chromosome replication and the following cell division (D period) were approximately constant for a cell doubling time of less than one hour[1]. In this paper they also introduced the idea of overlapping rounds of chromosome replication where a round of replication can be initiated while an existing round of replication is still going on (Figure 2). This explained the mechanism by which *E. coli* could grow with a doubling time faster than the time it takes to copy its genome.

We denote X as the time at which the replication of the chromosome starts, τ as the cell doubling time and n as the number of overlapping replication rounds. Cooper and Helmstetter's work implied that X was uniquely determined for a given τ, satisfying the equation:

$$X + C + D = (n+1)\tau$$

The question then arises of how the timing of DNA replication initiation is determined in the cell and how this process is coupled to the growth rate.

It was then proposed that an initiating factor accumulated during the cell cycle[2], reaching a threshold which triggered initiation.

2. The DnaA Protein

The DnaA protein has been shown to be required for replication of the chromosome to be initiated at *oriC* (the origin of chromosome replication). Moreover:

- DnaA binds to *oriC* and induces DNA melting required for assembly of the replication forks[3].
- DnaA gene expression is growth-rate dependent and negatively auto-regulated, resulting in a constant concentration of protein[4].
- When DnaA is over-expressed, initiation of DNA replication occurs earlier in the cell cycle, and when DnaA is depleted it results in delayed initiation[5].
- DnaA exists under two forms, ATP or ADP bound. Only the first of these can initiate replication at the origin.
- The hydrolysis of the ATP in a DNA-replication dependent manner results in a decrease in the levels of the protein after DNA replication initiation has taken place, preventing further initiations[6]. This process is known as RIDA.

We propose that DnaA-ATP/genome length is the best candidate for an initiation factor. DnaA-ATP can bind at a large number of non-specific sites (proportional to the length of the genome) on the chromosome and DnaA-ATP bound at these sites forms the reservoir of available DnaA-ATP. We propose that it is only when the number of DnaA-ATP per non-specific site reaches a certain threshold value that DnaA-ATP can bind at the origin and initiate replication.

3. The Model

The form of the model is shown in Figure 3. It is a statistical physics approach. The form of the promoter term (circled in blue) is a statistical physics representation of the probability that RNA polymerase is bound to the DnaA promoter, with DnaA-ATP acting as an auto-repressor. This represents newly synthesised DnaA-ATP.

We have equations for the growth of the chromosome and the increase in DnaA-ADP. These are both proportional to the number of pairs of replication forks. In the latter case, this is because RIDA (mentioned above) happens at the replication forks.

We first solve the equation when the parameters in the model are constant. This can't explain a constant threshold at initiation across all growth rates (see Figure 4).

We then impose a threshold at initiation upon the model using a mathematical transformation. We ask what implications this has on the parameters of the model. Are these implication consistent with existing results?

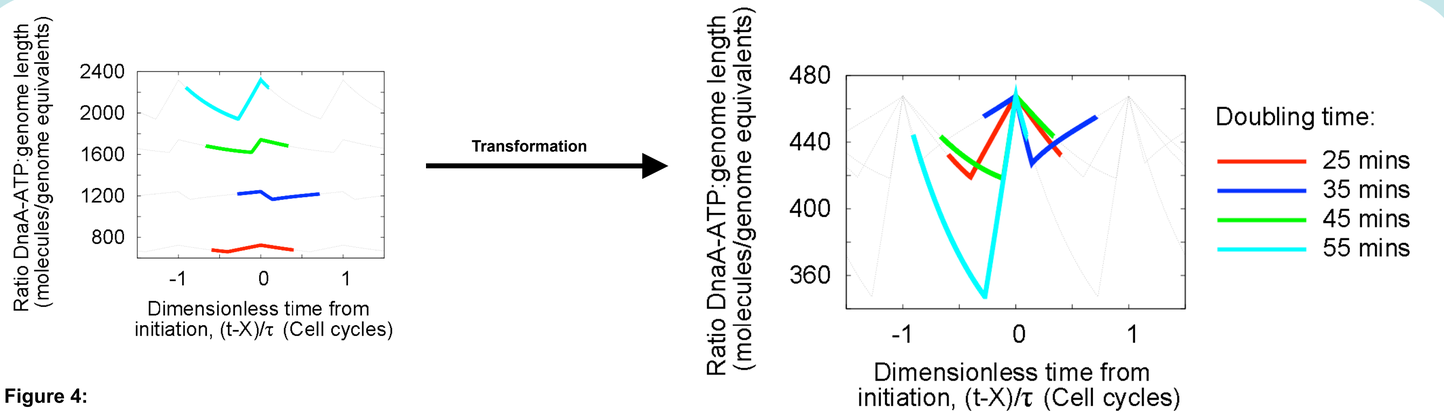


Figure 4:

A mathematical transformation can fix a threshold reached at initiation. Initially, we keep the parameters in the model constant. We see that the ratio DnaA-ATP:genome length takes a different value at initiation (t=X) for the different growth rates (left panel). We impose a mathematical transformation on the model which fixes this ratio at initiation across the different growth rates (right panel). We ask what implications this has for the parameters in the model.

4. Results and Discussion

The transformation gives two possible scenarios that can achieve a fixed threshold across all growth rates.

1. The binding affinity of DnaA-ATP to its auto-repression sites increases with growth rate, as does the binding affinity of RNAP to the DnaA promoter. Furthermore, the transcription rate, k_A, increases with growth rate, as do the levels of RNA polymerase in the cell.
2. The RIDA rate decreases with growth rate. As in the previous scenario, the transcription rate, k_A, increases with growth rate, as do the levels of RNA polymerase in the cell.

In the first of these scenarios, the trend of RNAP with growth rate can be chosen to match values in existing literature[7].

In both scenarios, the expression of the DnaA promoter matches data from existing experiments well[8]. A possible mechanism by which the RIDA rate could vary is through changes in the levels of Hda (a protein that mediates RIDA)[9]. A possible mechanism by which the binding affinities could change is through changes in supercoiling[10]. Experiments to directly measure these parameters will help to demonstrate which scenario might be occurring in the cell.

5. Acknowledgements:

Thanks to support from the Human Frontier Science Program Organization and EPSRC. Thanks also to Avelino Javer Godinez whose images are used in Figure 1.

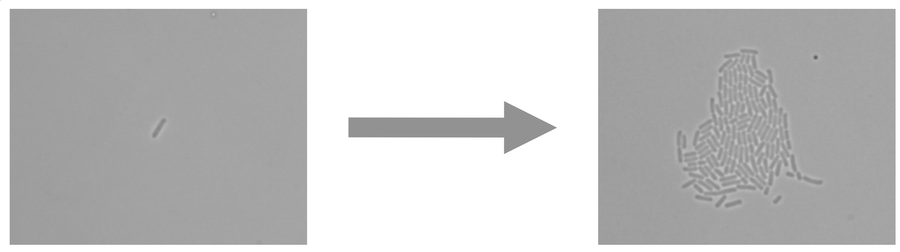


Figure 1:

***E.coli* grows and divides for survival**. At every generation, each bacterium must replicate its genome so that when it divides there is one copy for each of the daughter cells. All the genetic information is contained on one circular chromosome. We call the time at which the replication of the chromosome starts, t=X.

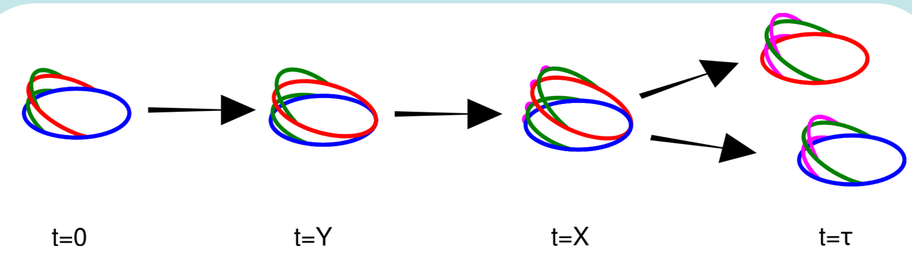


Figure 2:

***E.coli* prepares chromosomes for its grandchildren**. It takes 40 minutes for the bacterium to copy the chromosome (C period) and a further 20 minutes for the cell to divide (D period). *E.coli* overlaps replication rounds, enabling it to replicate with doubling times faster than 60 minutes. In fact, *E.coli* can grow with doubling times as fast as 20 minutes.

$$\frac{\partial A_-}{\partial t} = \frac{\Theta k_A}{1 + \frac{c_1 \Lambda}{P} + \frac{c_2 A_-}{P}} - k_R F$$

$$\frac{\partial A_+}{\partial t} = k_R F$$

$$\frac{\partial \Lambda}{\partial t} = k_\Lambda F$$

RIDA

Auto-regulation of DnaA promoter

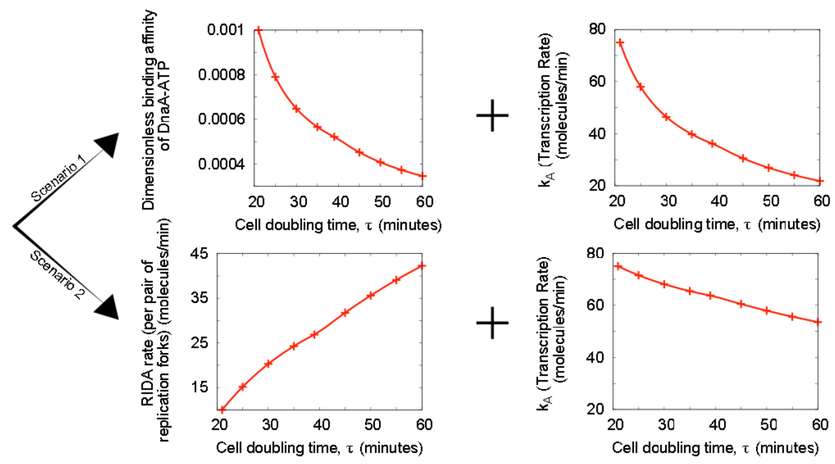
Parameters:

A_-	Number of DnaA-ATP (molecules)
A_+	Number of DnaA-ADP (molecules)
Λ	Total length of genome
F	Pairs of replication forks
P	Number of RNAP (molecules)
Θ	Number of copies of DnaA gene
k_A	Transcription rate (molecules/min)
k_R	Hydrolysis rate (molecules/min)
c_1	Boltzman weight
c_2	Number of DnaA-ATP

Figure 3:

The mathematical form of the model. There is an equation for DnaA-ATP, DnaA-ADP and the length of the genome. The source term for DnaA-ATP represents the probability that RNA polymerase is bound to the DnaA promoter. The model is discussed further in section 3.

Consequences of the Scenarios:



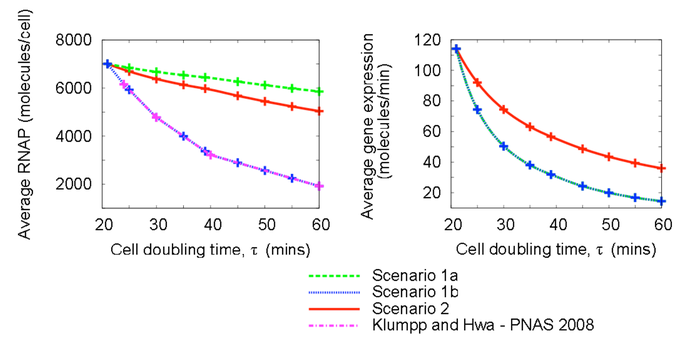


Figure 5:

The parameters of the model must vary to achieve a constant threshold across the growth rates. Two different scenarios can achieve this. In scenario 1 the binding affinity varies and in scenario 2 the RIDA rate varies. Both scenarios are compatible with existing literature[7,8].

6. References:

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