Growth rate variations establish distributions of generation times and division sizes in *E. coli*

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Escherichia coli **cells growing in a constant environment vary considerably in growth rates, generation times and division sizes. In spite of fluctuations, cells have to complete one round of replication in each cycle in order to retain its chromosome and it is unclear how this is accomplished. Using fluorescence microscopy to localize the replisome we find that initiation of replication occurs at a fixed origin to volume ratio independently of the time from division. In addition, a model of the bacterial cell cycle, where division occurs at a constant time after replication initiation, reproduces the variations in timing and sizes at division.**

I. BACKGROUND

For *E. coli* cells the time between two consecutive division events can, during fast growth, be substantially shorter than the time required to replicate the genome[1]. This is achieved by having parallel ongoing replication processes, which were initiated during the cell cycle of an individuals' mother or even grandmother[2]. In the classical description, initiation of DNA replication occurs at constant ratio of cell volume to number of origins independent of growth conditions[3]; a notion, which recently has been both questioned[4] and affirmed[5]. Isogenic *E. coli* cells living under constant growth conditions will vary considerably in their growth rates, cell cycle times and division sizes[6-7]. In spite of these fluctuations, a cell has to initiate and terminate one round of replication during each cycle in order to maintain its chromosome content. It has recently been suggested that, on average, adding a constant volume following cell division regulates the cell size at division[8-9]. It is, however, still unclear how chromosome replication is connected to cell division and how cells uphold one initiation and termination per generation given that Eukaryote-like cell cycle checkpoints are incompatible with overlapping rounds of replication.

II. EXPERIMENTAL METHODS

E. coli cells grow exponentially in a constant environment using a microfluidic device. Individual cells in the device can be localized and tracked over multiple generations using fully automated analysis methods. In addition, using single-molecule widefield fluorescence microscopy, we can detect and localize individual replisomes (DnaQ-Ypet) within individual cells.

III. RESULTS

We find that when the intracellular replisome localizations are aligned based on cell volume, the distribution of replisome localization for all cells is better defined than the corresponding distribution for cells aligned by time after division. This suggests that initiation of replication is size- rather than time-dependent. For two different growth rates we find a striking similarity in the ratio of number of origins to volume at initiation of replication. Based on these observations we construct and test a model where initiation of replication occurs at a constant ratio of number of origins per cell volume and that cell division occurs after a constant time-delay following initiation of replication. The model is parameterized based on our experimentally observed data. Using our observation of the variation in growth rates we can predict the variance in cell size at division, generation times and the correlation between these two parameters.

IV. CONCLUSIONS

We find that initiation of DNA replication is based on the ratio of origin numbers to cell volume rather than time after cell division and that the cell-to-cell variability in growth-rate accurately accounts for the variability in generation times, cell sizes and the correlations between the two.

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