Single-cell measurements imply that replication licenses division during chemostatic growth in *E. coli*

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Cell division and DNA replication are tightly coupled processes. In bacteria, however, the study of their temporal relationship is extremely difficult, even in synchronized populations, because the phase between replication and division is noisy at the single-cell level. These inherent fluctuations complicate the analysis of a causal link between replication and division. To limit the effect of these fluctuations, we use a newly developed single-cell chemostat to track cellular lineages and to acquire long time-series of division and replication events. We measure the synthesis rate of a constitutive transcriptional reporter, *gfp,* inserted in the chromosome as a proxy for tracking the timing of replication. Each additional copy of chromosomal DNA increases proportionally the synthesis rate of GFP. This information allows us to measure whether variations in the timing of newly replicated DNA are reflected in the timing of division or vice-versa. We find that delays in the replication process positively correlate with delays in the next division. Therefore, our study supports a model in which replication licenses cell division.

***Keywords*—Single-cell experiments, chromosome replication, cell division, single-cell chemostat.**

I. BACKGROUND In all organisms, DNA replication and cell division are processes that have a tight temporal coupling to ensure that every daughter cell gets a copy of the genome. In eukaryotes, the prevailing "licensing model" states that the cell division process starts a chain of events that leads to chromosome replication [1]. This implies that a new replication round cannot start if cell division has not occurred. In prokaryotes, the relationship between cell division and DNA replication is still an issue of debate. There is evidence for a eukaryotic-like model in which cell division licenses replication [2, 3]. There is also evidence favoring the opposite model, where replication licenses cell division [4]. *Escherichia coli* is the main prokaryotic model for the study of cell division and replication mainly because it is possible to synchronize the cell division of liquid cultures [5]. This synchronization method was essential to development of the first model of DNA replication in bacteria [6]. However, the synchronization of *E. coli* populations is not complete because the replication and cell division processes are intrinsically noisy. This intrinsic dephasing between these two processes makes it difficult to establish a causal link between DNA replication and cell division.

II. METHODS We propose an alternative method for examining the relationship between DNA replication and cell division, which allows us to circumvent the problem associated with the synchronization method. We use a single-cell chemostat [7] and a set of single-cell quantification techniques to track single cells from birth to division and to record the moment when the chromosome is replicated, without physiologically perturbing the cell. In the chemostat, we grow linear colonies of *E. coli* under chemostatic conditions for dozens of generations. Our quantification methods automatically track the lineage (including division time) of hundreds of cells. Also, we measure the activity of a chromosomally inserted promoter controlling the expression of a fluorescent protein in order to monitor DNA replication. We count each two-fold increase in the transcription rate of the fluorescent reporter as a proxy for a chromosome replication event.

III. RESULTS In log phase, *E. coli* cultures double their number and their DNA mass at a constant rate. At the single cell level, we find that *E. coli,* on average, doubles its mass and its DNA content every time it divides. We find that delays in the replication process positively correlate with delays in the next division. By contrast, delays in the previous division do not correlate with deleays in the replication process.

IV. CONCLUSION Our results, at the single cell level, support a model in which replication licenses cell division in *E. coli*. These findings suggest that the prokaryotic and eukaryotic cell cycle controls might be fundamentally different. For future directions, we think it would be interesting to uncover both the molecular and evolutionary mechanisms underlying this difference.

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