Control and Inheritance of Division Time in *Caulobacter crescentus*

Dan Siegal-Gaskins [1], Sean Crosson [2,*]

1. Department of Physics, University of Chicago, Chicago, USA
2. Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, USA
*E-mail: scrosson@uchicago.edu

Extended Abstract

The population of a bacterial monoculture inoculated from a single colony is clonal, having very little or no genetic variation. Despite this genetic homogeneity, experimental observation of single-cells has shown a high level of variability in a number of fundamental cellular processes [1, 2, 3]. For example, the inter-division times of single *Escherichia coli* cells are broadly distributed, with no correlation in division time from one generation to the next [2]. This "noisy" behavior is masked in averaged, population-based measurements of cell division. Such results suggest that quantitative analyses of single cells will be required to produce a comprehensive understanding of cellular regulatory processes. Using the naturally-adherent freshwater bacterium *Caulobacter crescentus* as a model organism, we have developed a method to immobilize and monitor the growth and division of single bacterial cells over multiple generations on a chemically-inert glass surface (Fig. 1). Novel microfluidic devices have recently emerged as powerful tools for single-cell experiments [4, 5, 6]. The fluid mechanical properties of growth medium in our linear microfludic culture channel combined with the unique attachment properties of *C. crescentus* allow us to capture and image cell offspring without manipulation, permitting facile comparison of growth and division between mother and daughter cells. Under microfluidic culture conditions, the rate of *C. crescentus* cell division is more rapid than has been attained under any previously reported conditions, with select cell divisions approaching the theoretical maximal rate of chromosome replication ($\approx$ 1000 nt/s [7]). We monitored growth and division at the single-cell level in *C. crescentus* across 12 generations and show that cell division time (56.7 ± 9.8 minutes; a coefficient of variation of 17.3%) is more tightly controlled than that of *E. coli*, *B. cereus*, or *E. aerogenes* [8, 9]. Furthermore, we demonstrate that both cell generation time and cell death are highly correlated between mother and daughter cells compared to other cells within the monitoring field (Fig. 2). Our results suggest that non-genetic elements of the, *C. crescentus* cell division control network are inherited from mother to daughter, leading to persistence of cell division behavior over multiple generations.

References

[1] MB Elowitz, AJ Levine, ED Siggia, and PS Swain. Stochastic gene expression in a single cell. *SCIENCE*, 297(5584):1183 – 1186, 2002.

[2] Y Wakamoto, J Ramsden, and K Yasuda. Single-cell growth and division dynamics showing epigenetic correlations. *ANALYST*, 130(3):311 – 317, 2005.

[3] M Kollmann, L Lovdok, K Bartholome, J Timmer, and V Sourjik. Design principles of a bacterial signalling network. *NATURE*, 438(7067):504 – 507, 2005.

[4] A Groisman, C Lobo, HJ Cho, JK Campbell, YS Dufour, AM Stevens, and A Levchenko. A microfluidic chemostat for experiments with bacterial and yeast cells. *NATURE METHODS*, 2(9):685 – 689, 2005.

[5] S Cookson, N Ostroff, WL Pang, D Volfson, and J Hasty. Monitoring dynamics of single-cell gene expression over multiple cell cycles. *MOLECULAR SYSTEMS BIOLOGY*, page 2005.0024, 2005.

[6] C Hansen and SR Quake. Microfluidics in structural biology: smaller, faster... better. *CURRENT OPINION IN STRUCTURAL BIOLOGY*, 13(5):538 – 544, 2003.

[7] KJ Marians. Prokaryotic DNA replication. *ANNUAL REVIEW OF BIOCHEMISTRY*, 61:673 – 719, 1992.

[8] CD Kelly and O Rahn. The growth rate of individual bacterial cells. *JOURNAL OF BACTERIOLOGY*, 23(2):147 – 153, 1932.

[9] I Inoue, Y Wakamoto, and K Yasuda. Non-genetic variability of division cycle and growth of isolated individual cells in on-chip culture system. *PROCEEDINGS OF THE JAPAN ACADEMY SERIES B-PHYSICAL AND BIOLOGICAL SCIENCES*, 77(8):145 – 150, 2001.

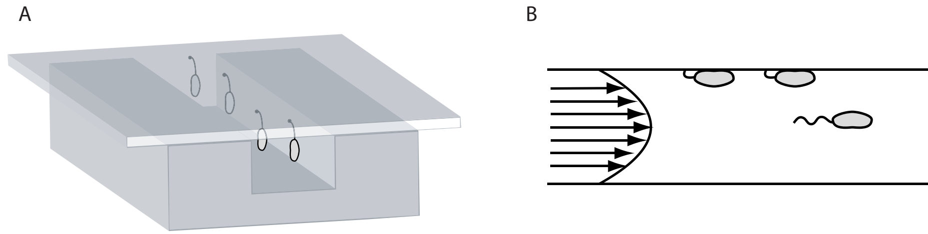

Figure 1: Schematic of the microfluidic single-cell imaging device. (A) Cells with a fully developed stalk readily adhere to the glass surface via the adhesive holdfast at the terminus of the stalk. (B) Fluid flowing in the channel at a constant rate and under laminar conditions causes the cells to lie flat against the glass. Upon cell division, daughter swarmer cells are most often carried away from their attached mother cells by medium flow in the channel.

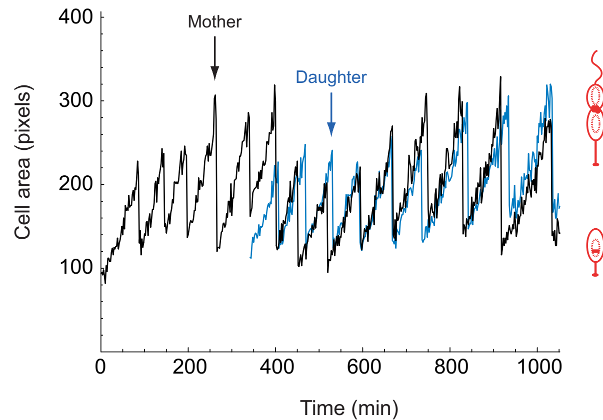

Figure 2: Plot of cell area versus time. The strength of holdfast attachment to the glass coverslip allows stalked cells to remain permanently attached within the channel over the course of an experiment, permitting imaging of single-cell growth and division across multiple generations. Though the majority of swarmer cells are flushed out of the microfluidic channel upon separation from their mother cell, the fluid mechanical properties of the channel lead to a finite probability of daughter cell attachment downstream of the mother cell following a division event. Growth of the mother (black trajectory) and daughter (blue trajectory) cells can then be simultaneously measured. A schematic of the average cell shape prior to and following division is shown in red.