Revealing Responsive and Stochastic Switching in Bacteria

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Abstract:
Microorganisms respond to environment changes by switching their behavior and phenotype between different states. This phenotypic switching can either be stochastic in nature or purely deterministic. Here, we developed a microfluidic device to culture and monitor small populations of bacteria for hundreds of generations and use it to study the responsive and stochastic switching of E. coli bacteria under fluctuating environmental conditions.

Summary of Research:
Living organisms have to respond to environmental changes in order to survive and maintain their ability to reproduce. Microbial species can adapt to new environmental conditions by using different strategies; the optimal use of these strategies, however, strongly depends on the type of change faced [1, 2]. For instance, a cell may respond to a new environment such as a change in nutrient source by expressing a different set of genes — a process called responsive switching. On the other hand, it may also be beneficial to acquire new phenotypes by stochastically responding to changes through genetic change. The acquisition of mutations that confer antibiotic resistance is an example of a stochastic switch. Each of these strategies (responsive and stochastic switching) are used by microorganisms in nature and a better understanding of type of switching that allows bacteria to resist and adapt to harmful environments — such as antibiotic treatment — is essential to the scientific understanding of pathogens and microbial diversity.

Here, we use microfluidic technologies to continuously culture and monitor small populations of bacteria (~ 100) at the single cell level in fluctuating environments. Our goal is to characterize the type of switching used by E. coli as it undergoes environmental changes. A schematic representation of the device used is shown in Figure 1. The design is based on a similar one developed to study bacterial aging [3], where a main flow channel brings nutrients to cells growing inside chambers on each side of the channel. As cells grow in length, they push on each other and this “peer” pressure helps maintain cells near the end of the growth chamber (see Figure 2). As a result, the population inside each chamber is fixed in size and can be monitored for hundreds of generations.

Figure 1: Representation of the microfluidic device used to culture E. coli bacteria. The height of the chambers (1 micron) is chosen such that it matches the diameter of the cell, thereby restricting cells to grow as a monolayer. Cells are pushed into the flow channel and removed from the device as the population grows and divides.

Figure 2: Fluorescence micrograph of E. coli cells growing inside the device. We use the green fluorescent protein intensity to identify each cell and track its lineage over time.
As opposed to conventional culture methods (i.e., test tubes or chemostat), we can use our device to induce rapid and reversible changes in the environment. Indeed, due to the inherently small volumes involved in our microfluidic device, we have the ability to instantaneously switch between two (or more) medium. For instance, we are able to study the dynamics of the lac operon by alternating minimal media containing either glucose or lactose as the sole carbon source. We observe that the response of cells to a glucose-to-lactose transition only occurs after the environment has changed (i.e., responsive switching). Figure 3 shows fluorescence intensity of the cells during a few transitions: we see that a rapid environment changes result in momentary decreases in fluorescence intensity, which itself is tied to protein production through the *rpsL* promoter.

We can also study stochastic switching by monitoring the occurrence of genetic changes for cells inside our device. Most genetic changes occur at very low frequencies, but we use engineered cells which turn on the production of a specific gene, in this case green fluorescent proteins, when a promoter region undergoes methylation changes [4]. Since these type of epigenetic change occur at a rate more than 1000 faster than DNA mutations, we are able to observe how stochastic changes occur and spread within a small population. Figure 4 shows an example of stochastic switching where a single gene is turned on in a population.

Future work will use these type of epigenetic switches to stochastically trigger the expression beneficial genes in order to study the dynamics of stochastic switches within a population.

References: