A microfluidic system for studying single-cell behavior

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A lab-on-a-chip-type system employs optical tweezers to study changes in the response of single yeast cells to variations in the immediate environment.

A cell lives in a dynamically changing environment and adapts to a new one by changing its behavior and the expression of various proteins that are needed at certain times. Traditional studies of cell behavior are carried out using ensemble-averaging techniques that study entire populations of cells at the same time. A disadvantage of these approaches is that the heterogeneous and dynamic behavior of individual cells is lost. New techniques are needed that make it possible to follow single cells in real time. Here we describe a project that focuses on the actions of single yeast cells in response to metabolic perturbations.

Yeast cells are the simplest eukaryotic cells (similar to human cells), and are thus eminently suitable for study. We have adopted two different strategies to developing single-cell techniques, both of which employ optical tweezers and fluorescence imaging within a microfluidic channel that is amenable to environmental manipulation. In the first strategy, the optical tweezers are used to capture and hold a single yeast cell and move it within a microfluidic flow cell. The latter is constructed of polydimethylsiloxane (PDMS) and consists of two channels that merge into one. The channels contain different media flowing adjacently, which creates a distinct concentration profile (see Figure 1). Proteins of interest are tagged with green fluorescent protein (GFP), and the movement of the fluorescent signal is followed as the cells respond to their changing environment. In this case, the protein is called Mig1 and it represses the expression of proteins that digest a secondary energy source (other than glucose, which is the primary energy source). When glucose is present in the environment of the yeast cell, Mig1-GFP remains in the nucleus. But when the cell is deprived of glucose, Mig1-GFP moves out of the nucleus and into the cytosol (intracellular fluid).1,2

Figure 1. The two arms of the microfluidic channels are 100µm wide. After the junction, the channel becomes 200µm wide. Optical tweezers are used to trap and move a cell between two different media, one from each channel. The microfluidic system allows a distinct concentration profile to be created between the two different media.

Figure 2. The microfluidic system allows cells to be trapped and moved to a measurement area where the environment around the cell can be manipulated to induce a response.

This first strategy has limited throughput since only one cell can be followed at a time. The second strategy that has been developed within this project has solved the throughput problem by using optical tweezers to select cells and position them on the glass surface, where they are imaged while their environment is changed instead of moving the cells themselves. The change in environment is effected using a three-channel microfluidic flow cell (see Figure 2).

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Figure 3. In the cellular array, yeast cells are placed on the bottom surface within the microfluidic flow system shown in Figure 2. (left) The cells are in a glucose environment and the Mig1-GFP repressor remains in the nucleus. (right) The cells are deprived of glucose and the Mig1-GFP repressor moves out into the cytosol.

The cells are placed in the measurement area by the optical tweezers. Creating an array and discarding cell clusters and dead cells enables automated analysis of the fluorescent and brightfield images much more easily than is possible with methods based on random cell sedimentation. Figure 3 shows a cellular array.3

These two strategies have different advantages and drawbacks when it comes to technical issues. The first approach suffers from low throughput, and the yeast cells need to be held in the laser trap for a long time, which could potentially damage them. When studying fast cellular response, however, this strategy is the better choice since the environment changes more rapidly and precisely. The second strategy has a higher throughput, and environmental changes occur within 2s, which is still good enough given that many cell-signaling responses take minutes rather than seconds. But surface immobilization of the cells requires surface preparation.

In summary, we have created a platform suitable for studying various types of behavior in single yeast cells in real time, including protein migration between the cell nucleus and the cytosol, and size changes on alterations in osmotic pressure. The changes to the cell surroundings in this system are reversible. Moreover, the fluorescent signal can be followed during several cycles of protein migration in and out of the nucleus. In the future, this experimental system should make it possible to investigate single-cell behavior using various types of signaling pathways within the cell and possible to discover heterogeneous cell responses. This platform will be used for detailed investigations of the behavior of the Mig-1 protein in single yeast cells in response to metabolic changes. In addition, we will explore the use of this technology in other applications such as single-molecule studies of protein-DNA interactions.

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Annette Granéli has several years of experience working at the interface of physics and biology on problems ranging from cell-membrane mimicry using surface-sensitive techniques, to single-molecule biophysics studying protein-DNA interactions, to single-cell behavior using optical tweezers.

References