The diauxic shift at the single cell level

Student project – Molecular Systems Biology

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Introduction

Micro-organisms are masters in adapting to novel circumstances. One of the best known examples hereof is the ability of the yeast *Saccharomyces cerevisiae* to shift between fermentative and respiratory growth (Figure 1). In an environment rich in glucose, it rapidly metabolizes glucose to ethanol in a process called fermentation. Upon exhaustion of this glucose, it however needs to turn to respiratory growth to utilize its waste product ethanol. This process is commonly known as the diauxic shift and it is accompanied by widespread changes in gene expression, protein localization and cellular morphology (DeRisi *et al.* 1997; Galdieri *et al.* 2010; Guidi *et al.* 2010).

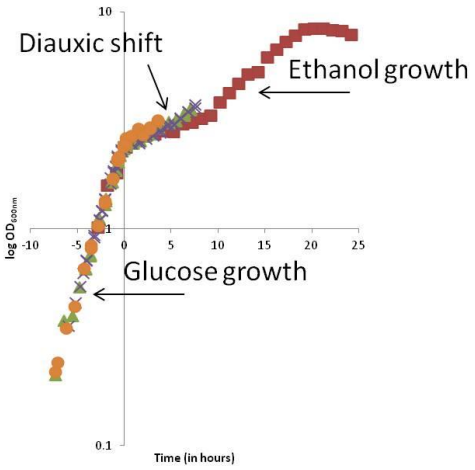

Figure 1: Example of a growth curve of yeast on glucose. Growth on each of the different carbon sources is indicated.

Goal of this project

In this project you will study the ability of single cells to adapt to a shift from glucose to ethanol. We will use advanced techniques, such as fluorescence microscopy and flow cytometry to compare mother and daughter cells. Features we will compare are growth rate, cell size, and protein expression and localization patterns.

What to expect

→ Basic lab techniques, such as culturing yeast and . simple genetic work, such as GFP tagging of proteins

→ Learn how to make your own microfluidics chips and run them (Figure 2)

- We will look at the growth rate and cell size of cells prior, during and after the shift from glucose to ethanol. In addition we will use strains containing fluorescently tagged proteins, such as Hxk2 and Msn2/4 to study changes in their localization. These proteins are markers for changes in glucose availability and stress responses.

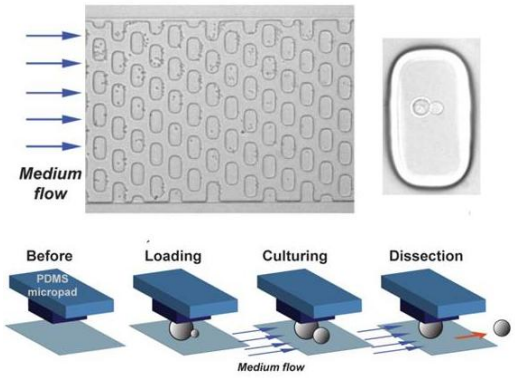

Figure 2: Schematic illustration of our microfluidics chip. Upon loading, cells are retained in the chip. Fresh media is supplied through the chip providing the cells with fresh nutrients and washing out the newly born smaller daughter cells.

→ Analysis of microscopy data using programs such as ImageJ/CellProfiler

→ Magnetic cell sorting in combination with flow cytometry (Figure 3)

- To study a large number of cells at the single cell level, we will shift yeast strains from glucose to ethanol medium and compare GFP expression between mother cells and daughter cells. We will in particular be interested in proteins involved in respiration and fermentation, such as Pdc1, Tpi1 and Cit1.

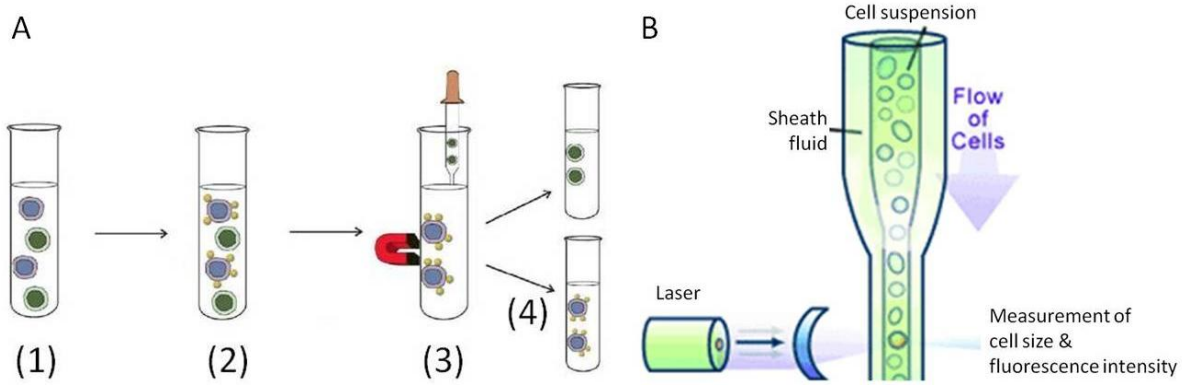

Figure 1: (A) Overview of the magnetic cell sorting procedure. Mother cells are labeled (1) and as a result bind to small iron beads (2). These beads are collected using a magnet (3). The mother cells clinging to the beads are then fished out of the general cell population (4). (B) In flow cytometry, cells are passed one by one past a detection point at high speed. Cells are counted and features, such as cell size and fluorescence intensity, are measured.

References

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