Analysis of PCK1 Expression in *Saccharomyces cerevisiae* Strain S288C During the Diauxic Shift

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Introduction

Microarrays are a very efficient lab technique in that they are quite cost-efficient as they enable scientists to study the expression of thousands of genes at a time. The technique is now very commonly used in molecular biology and medical labs, since it allows entire genomes to be explored at once (Hedge *et al.,* 2000). This is a much more sophisticated technique compared to that of Northern Blotting, which only allows one gene or DNA expression product to be explored at a time (Weaver, 2008).

Microarrays can be used for various comparative analyses of anything from different gene expression levels in tumors used to design more personalized treatments for cancer patients, to looking at the changes in gene expression levels under specific and controlled growth conditions. These types of analyses are better known as gene expression profiling. They can also be employed to determine the relatedness of different species (Hedge *et al.,* 2000; *Schena, et al.,* 1995).

Yeast, *Saccharomyces cerevisiae*, is a model organism whose genome has been previously sequenced. It is fascinating that under aerobic and anaerobic growth conditions, the genes of this organism are expressed at different levels. It is the induction and repression of certain genes that allow scientists to investigate what is ocuring during specific times and conditions of growth. It is intriguing to look at well known genes such as phosphoenolpyruvate carboxykinase (PCK1), an enzyme that is key to gluconeogenesis. PCK1 is an enzyme in yeast that catalyzes early gluconeogenic reactions while its transcription is repressed by glucose (Haurie, *et al.,* 2001).

The simplicity of using a microarray is something that can be done even in the undergraduate laboratory. In this experiment, *Saccharomyces cerevisiae,* gene expression was explored during the diauxic shift. When the yeast switch from anaerobic metabolism to aerobic metabolism, a shift in gene expression is observed and various genes are induced or repressed. Information gained from such observations can provide valuable insight for scientists seeking to determine certain genes' biological roles in the organism (DeRisi, *et al.,* 1997).

Materials & Methods

Culturing Yeast

Yeast, strain S288C, was used in this experiment and two samples were grown by Dr. Lee in a shaking incubator in yeast peptone dextrose (YPD) medium at 30 °C. The yeast employing anaerobic metabolism were grown for 12.75 hrs at 250 rpm at 30 °C, while the yeast employing aerobic metabolism were grown for 17 hrs at 250 rpm at 30 °C. The cells were removed, the absorbance was checked, and the yeast cultures were pelletted in a centrifuge. The experimental sample (aerobic conditions) required a repeat of the centrifugation step and absorbance was once again observed. 2.0×10^8 cells were isolated from the log phase of each pellet.

Preparing the Total Yeast RNA

The total yeast RNA was then isolated using an Ambion RiboPure Yeast Kit (Ambion, Austin, Texas). The yeast pellets were resuspended, transferred into a tube containing cold Zirconia beads, and subsequently vortexed to mix the yeast solution throughout the bead chamber. Binding Buffer was then used to bind the RNA to the beads. Several subsequent wash solutions were added and the samples were centrifuged to remove unwanted RNA. Elution Buffer was finally added to remove the desired RNA from the beads.

Checking Quality and Quantity of RNA, Labeling Microarrays

In order to check for the presence of RNA, an agarose gel was run via electrophoresis. Once the presence of RNA at a sufficient level was detected, cDNA preparations for both the reference and experimental conditions were synthesized and the RNA templates were degraded. The cDNA was concentrated using the QIAquick PCR Purification Kit (QIAgen, Valencia, CA). Figure 1 demonstrates the experimental procedure concerning Microarray labeling with the Genisphere Array 350 Kit (Genisphere, Hatfield, PA).

Analysis

Analysis was performed on previously obtained data from Drs. DeBerry and Lee using MagicTool.

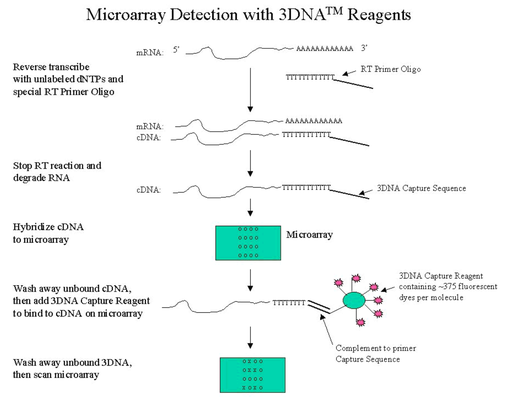

Figure 1. Protocol based on the Genisphere Array 350 kit.

Results

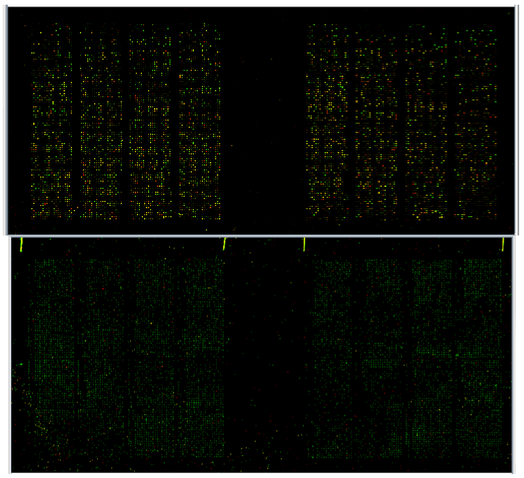

Figure 2. Yeast Genome Microarrays. 6233 genes from the *Saccharomyces cerevisiae* genome were hybridized in duplicate to a microarray slide with gene probes already present. Probes of cDNA were prepared from mRNA from yeast cells grown in 2 different conditions. The yeast from which the reference (anaerobic metabolism) mRNA was obtained was grown 12.75 hrs at 250 rpm at 30 °C, while the yeast from which experimental (aerobic metabolism) mRNA was obtained was grown for 17 hrs at 250 rpm at 30 °C. Each sample of isolated mRNA was subsequently reverse transcribed in the presence of unlabeled dNTPs using an RT primer. RNA was degraded and the cDNA preparations were hybridized to the microarray. Different capture tags were bound to each cDNA preparation, each of which bound a different fluorescent dendrimer tag. This allowed visualization of the amount of red (Cy5 dendrimer tag) and green (Cy3 dendrimer tag) fluorescence of the two cDNA preparations in the two different conditions. The image on the bottom is of a microarray (shown at contras =7000%) prepared according to the protocol previously described but did not provide sufficient data for exploration. The image on the top is of a microarray (shown at contrast=1500%) prepared with the same protocol by Dr Lee and was utilized for gene expression analysis. In this second image, mRNA expression before the diauxic shift is represented as green (Cy3), and mRNA expression after the diauxic shift is represented as red (Cy5). Genes expressed at approximately equal levels correspond to yellow spots, and no expression corresponds to black spots.

Table 1. Yeast gene expression change by a factor of 8 or more during the diauxic shift[1]

Gene	Description	Fold change
ACH1	long-chain fatty acid transporter activity	4.48
CYB2	L-lactate dehydrogenase (cytochrome) activity	4.49
FBP1	fructose-bisphosphatase activity	5.86
HXT5	glucose transporter activity	5.22
ICL1	isocitrate lyase activity	4.46
IDP2	isocitrate dehydrogenase (NADP+) activity	5.31
MLS1	malate synthase activity	5.84
PCK1	phosophoenolpyruvate carboxykinase (ATP) activity	*6.02*
PDH1	molecular function unknown	4.10
PYC1	pyruvate carboxylase activity	4.35
SFC1	succinate/fumarate antiporter activity	5.51
SIP18	phospholipid binding activity	6.21
SPG1	molecular function unknown	4.40
YDR070C	molecular function unknown	4.00

[1] 14 of the 6233 genes analyzed from *Saccharomyces cerevisiae* were found to have been induced or repressed by a factor of 8 or more during the diauxic shift (from anaerobic metabolism to aerobic metabolism). Those genes that were induced in the experimental conditions (aerobic) are indicated by the red fold increase values, while those repressed are indicated by the green values. PCK1 was induced by a factor of 6.02 in the experimental conditions.

Conclusions

Troubleshooting

- The absence of usable data from the microarray prepared by us may be due to a 10 minute pause in the protocol at too cool of a temperature during the second cDNA hybridization.

- Another possible reason is that too much of the cDNA was removed from the microarray slide during either of the washes.

- These possibilities could be further explored by repeating the experiment, not allowing any non-specified breaks in the protocol to occur and centrifuging the slide at a slower speed when washing.

Discussion

- Yeast cells anaerobically ferment glucose to ethanol to make ATP in the presence of sufficient glucose. As they deplete available glucose, they begin to metabolize the stored ethanol aerobically. Metabolizing ethanol involves converting it to pyruvate and subsequently acetyl-CoA to enter the Citric Acid Cycle.

- High-energy electrons are harvested and transported to the Electron Transport Chain (ETC). The ETC uses these to pump protons out of the mitochondrial matrix creating an electro-chemical gradient utilized by ATP synthase to convert ADP to ATP.

- Oxaloacetate (OAA) formed in the CAC is converted to phosphoenolpyruvate (PEP) by phosphoenolpyruvate carboxykinase (PCK1). PEP can enter gluconeogenesis to ultimately make more glucose.

- As expected, we found multiple genes active in gluconeogenesis and the Citric Acid Cycle that were substantially upregulated in *Saccharomyces cerevisiae* after the diauxic shift. This is logical because these processes become the main source of ATP during yeast aerobic metabolism. Stimulating PCK1 to convert OAA to PEP which can be entered into gluconeogenesis is efficient for yeast cells as they need to make more glucose in response to depleting levels that signaled the diauxic shift.

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