A Study of Gene Expression in Respiring *Saccharomyces cerevisiae* during Cold Acclimation using Microarray Analysis

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**Introduction**

*S. cerevisiae* is a complex unicellular eukaryote organism that contains 6,400 genes. *S. cerevisiae* is an excellent model organism because its genome has been sequenced and yeast microarrays are available for studying gene expression. A yeast microarray is a glass slide with about 6,400 DNA genes printed on it. To use a microarray, RNA is isolated from the cells and made into complementary DNA (cDNA), which binds to homologous sequences on the array. The control sample is labeled with a green fluorescent dye and the experimental sample is labeled with a red fluorescent dye. The microarray is then scanned to determine the ratio of red-to-green signal.

The first study performed using microarrays examined gene expression during the diauxic shift from anaerobic to aerobic metabolism (*DeRisi*, 1997). We based our experimental methods on this landmark study.

**Materials & Methods**

- **Grow Yeast** at 28°C & 10°C in YPGAL:
  - Harvest warm samples and harvest the cold samples at 30 minutes, 2 hours, and 8 hours and flash freeze at -80°C
  - Check quality of RNA using Nano-drop and quality using agarose electrophoresis
  - Isolate RNA from the cells using Ambion RiboPure kit
  - Synthesize cDNA adding capture sequence to warm samples that will bind green dye and capture sequence to cold samples that recognize red dye using Genisphere kit
  - Hybridize the array with the cDNA at 50°C overnight
  - Wash and hybridize the array with red and green dyes and incubate at 4 hours at 50°C
  - Wash microarray and send to Davidson College for scanning
  - Data analysis of the red & green intensities using Magic Tool

**Results**

- The respiration rate of yeast grown in YPGAL was 4.005 umol/min/g as compared to YPD which was 0.32 umol/min/g

**Discussion**

Our studies show that in response to cold temperature, the expression of genes involved in protein biosynthesis and the ubiquitin pathway are up regulated at 30 minutes in yeast. This suggests that low temperature has a denaturing effect on proteins. Ubiquitin is a small protein that gets attached to damaged proteins and targets them for degradation. Genes involved in amino acid biosynthesis may be up regulated to replace damaged proteins. In addition, the expression of chaperones was induced. Chaperones assist in protein folding.

After 8 hours at 10°C, many of the stress response genes and genes involved in protein biosynthesis were no longer up regulated and some were repressed. This suggests that the cell has adapted to the cold.

In this study, we were surprised that cells grown in YPGAL did not appear to experience more oxidative stress than cells grown in YPD. We expected that because cells grown in YPGAL have more mitochondria for oxidative phosphorylation, they would experience more oxidative stress in the cold. Instead, the genes that were induced or repressed in response to cold temperature were actually quite similar between cells grown in YPD and YPGAL. One exception was that cells grown in YPGAL had more mitochondrial genes up regulated at 30 minutes.

**Literature Cited**


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