**Introduction**

Understanding the evolution of regulatory networks is critical to understanding how cells work and how they successfully adapt to changing environments. Yeast are used to study regulatory networks because a lot of the metabolic pathways present in yeast are conserved in humans, and they are easily manipulated in a lab. Also, the genomes of all the Ascomycota yeast species are completely sequenced. This allows researchers to study disease such as diabetes and cancer which may result from mutations in genes that control metabolism.

Yeast central carbon metabolism is an interesting area of study because many different lifestyles evolved across the Ascomycota phylogeny (such as carbon source utilization and ability to deal with nutrient deprivation). For example, these yeast can be classified according to how they digest glucose. While most yeast are respiratory, some are respiro-fermentative. Respiro-fermentative yeast ferment even when there is sufficient oxygen in their environment. Moreover, this tendency to perform fermentation when oxygen is present is also a characteristic of cancer cells known as the Warburg effect.

Most species that are respiro-fermentative developed after a whole genome duplication that occurred about a hundred million years ago. This characteristic evolved two separate times in this whole genome duplication that occurred about a hundred million years ago. This characteristic evolved two separate times in this whole genome duplication that occurred about a hundred million years ago.

**Objective:** To determine the differential expression of metabolic genes in K.waltii (pre whole genome duplication yeast) and K.polysporus (post whole genome duplication yeast), two respiratory yeast species.

**Experimental Design:** Analyze differences in gene expression between samples of yeast grown in rich media with glucose and then shifted into minimal media with glycerol (a non-fermentable carbon source), either from plateau or exponential growth phase.

**Materials and Methods**

**Sample Taking**
- K.waltii and K.polysporus
  - BMG-rich media with 2% glucose
  - Mgly-minimal media with 2% glycerol

**Extracting RNA**
- Using Qiagen RNA extraction Kit
- BioAnalyzer
- RNA Gel

**RT Reaction & Labeling**
- Obtain complementary DNA strands; Label reference and experimental DNA with different dyes

**Hybridization**
- Competitive binding to DNA probes on species-specific microarrays

**DNA Microarray Scanner**
- Feature extraction software: Log ratios between reference and experimental DNA

**Analysis**
1. 
   - Kmeans Clustering with 25 clusters for K.waltii, 10 clusters for K.polysporus vs. K.waltii
   - Distance is correlation; 100 runs
2. 
   - Hypergeometric test for functional gene set enrichment
   - Used Gene Ontology gene sets: process, function, component
   - Calculated p-value for each gene set for each cluster
   - Determined significance using the Bonferroni method for multiple hypothesis correction

**Key**
- **Microarray Analysis**
- **Results**
  - **Comparing Gene Expression Between Varying Conditions in K.waltii**
    - **C0**
      1. Cell biomass and ribosomal genes
      2. Respiration genes
      3. Glycolysis genes
    - **C6**
      1. Genes involved in carbohydrate metabolism
      2. Respiration genes
      3. Ribosomal genes
    - **C10**
      1. Genes involved in protein degradation
      2. Respiration genes
      3. Ribosomal genes
    - **C15**
      1. Genes involved in transcription & translation
      2. Respiration genes
      3. Ribosomal genes
    - **C16**
      1. Genes involved in transcription & translation
      2. Respiration genes
      3. Ribosomal genes
    - **C17**
      1. Genes involved in amino acid metabolism
      2. Ribosome genes
      3. Ribosomal genes

**Conclusions**

By comparing across species, we are able to find sets of co-expressed genes that have either conserved or diverged regulation between species.

**Future Research**

Our group will get expression profiles for all fifteen species in the phylogeny in multiple conditions such as different carbon sources, stresses, and nitrogen sources. Then we will find sets of co-regulated genes whose expression differs between species and perform analysis of upstream regulatory elements, such as binding motifs, to discern the evolution of regulatory networks.

**Literature Cited**

1. Fernando Rodrigues, Paula Ludovico, and Cecilia Leao. "Sugar Metabolism in Yeasts: an Overview of Aerobic and Anaerobic Glucose Catabolism"
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