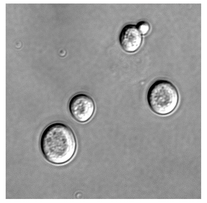
Differentially Regulated Genes in *K. waltii* and *K. polysporus* Offer Insight Into How Gene Networks Evolve



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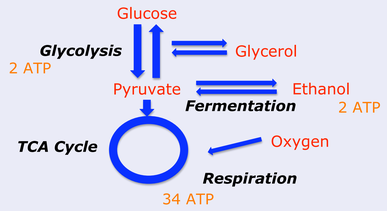
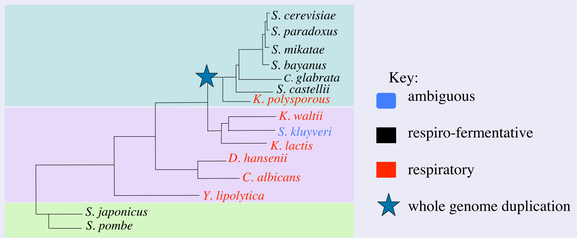
Introduction

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 diseases 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 phylogeny, suggesting that it may have an adaptive quality.



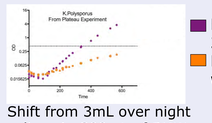
Objective: To determine the differential expression of metabolic genes in *K.waltii* (pre whole genome duplication yeast) and *K.polysporus* (post whole genome duplication yeast), two respiratory yeast species.

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

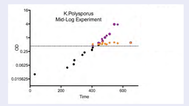
Sample Taking

K.waltii and *K.polysporus*



Shift from 3mL over night culture to 300mL of BMW or Mgly

- BMW-rich media with 2% glucose
- Mgly-minimal media with 2% glycerol



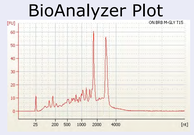
Shift from 600mL of BMW at mid log to 300mL of BMW or Mgly

Extracting RNA

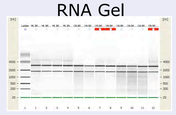
Using Qiagen RNA Extraction Kit

Check RNA Integrity

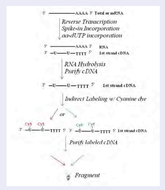
BioAnalyzer Plot



RNA Gel

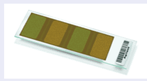


RT Reaction & Labeling



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

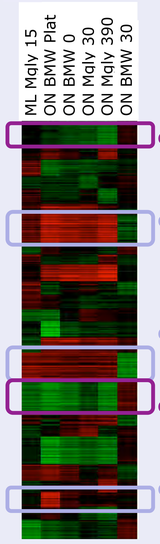
Results

KEY

Genes are: induced repressed

ML: switch from mid log culture (high glucose)
ON: switch from 3mL over night culture (low glucose)
Mgly: minimal media with glycerol
BMW: rich media with glucose
C: Cluster
PI: Plateau (glucose has been used up)
**Reference is exponential growth in BMW

Low glucose



Comparing Gene Expression Between Varying Conditions in *K.waltii*

C0: Cell biogenesis and ribosomal genes
Ribosome genes, p<2.45e-12
Cellular biosynthesis genes, p<7.62e-12

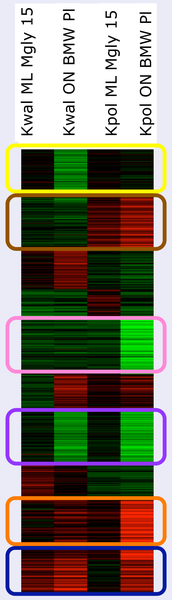
C6: Genes involved in carbohydrate metabolism
Electron transport genes, p<1.53e-9
Aerobic respiration genes, p<2.78e-7

C15: Genes involved in protein degradation
Protein targeting to vacuole genes, p<4.41e-6
Ubiquitin dep. protein catabolism, p<7.45e-6

C16: Genes involved in transcription & translation
RNA polymerase complex genes, p<4.47e-12
Ribosome biogenesis genes, p<1.34e-12

C22: Genes involved in amino acid metabolism
Methionine metabolism genes, p<2.81e-7
Lysine metabolism genes, p<5.78e-6

Low glucose



Comparing Gene Expression Between *K.polysporus* and *K.waltii*

C0: Glycolysis genes
PGI1, FBA1, TDH1, ENO1, ENO2

C1: Mating, reproductive and respiration genes
Reproduction-genes, p<2.72e-8
IDH2, LSC1, KGD1, FUM1

C4: Ribosomal genes
Ribosomal large subunit biogenesis genes, p<5.46e-6

C6: Ribosomal genes; glycolysis genes
Ribosome-genes, p<2.15e-12
PFK1, FBA1, GPM1, CDC19

C8: Translation genes; respiration genes
Ribonucleotide metabolism genes, p<6.67e-7
PYC1, ACO1, MLS1, KGD2, LSC2, MDH3

C9: Respiration genes
Mitochondral etc genes, p<1.12e-4
Acetyl-coA metabolism genes, p<1.10e-5

Conclusions

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

Comparing Gene Expression Between Varying Conditions in *K.waltii*

- **C0 & C16** genes involved in transcription and translation and cell growth
  repressed in low glucose environment

Comparing Gene Expression Between *K.polysporus* and *K.waltii*

Convergent Expression:
- **C6** ribosome and glycolysis genes
  repressed in low glucose environments in both species
- **C8** genes involved in respiration
  induced in low glucose environments in both species

Divergent Expression:
- **C0** glycolysis genes
  more down regulated in *K. waltii*
- **C1** genes involved in TCA cycle and respiration
  up regulated in *K. polysporus*
  slightly down regulated in *K. waltii*
- **C4** ribosomal genes
  more down regulated in *K. polysporus*
- **C9** genes involved in respiration
  up regulated in plateau time points in both species
  more up regulated in *K. waltii* in minimal media than in *K. polysporus*

Our minimal media with glycerol condition gave us more examples of 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"
2. Wapinski, Ilan. "Natural History and Evolutionary Principles of Gene Duplication in Fungi."

Acknowledgements

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