EMERGENT GENE EXPRESSION PATTERNS IN *S. CEREVISIAE* UNDER ANAEROBIC, MIXOTROPHIC, AND AEROBIC REGIMES

A Thesis Proposal

by

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TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION

Organisms must adapt to survive, sometimes occupying new niches. Each niche has extensive diversity, regarded as niche breadth. The variation contributing to this biodiversity has been influenced by extrinsic and intrinsic elements like environmental and genetic factors [1]. However, further details that include metabolism changes in response to these factors were not incorporated. A new conceptual niche has been proposed that overcomes the previous shortcomings which includes the metabolic plasticity of organisms [2, 3]. The organism's metabolism has plasticity (the ability to adapt to different environments) to switch substrates and generate energy in the form of ATP depending on its physiology, nutrient availability, and environment, permitting it to fit into unique niches over time [4]. This has commonly been seen in prokaryotes and haloarchaea in extreme geothermal environments [5, 6]. However, this mechanism has been understudied in eukaryotes. Oxygen availability is thought to play a role in the evolution of large organisms, which provides a change of niches for organisms like S. cerevisiae [7]. S. cerevisiae has shown to be able to express multicellularity and unicellularity, allowing greater exploration into the evolution of multicellularity [8]. Using S. cerevisiae (snowflake yeast) genes linked to their metabolic regulation can be located when they are introduced to different environments, as well as further comparison to their multicellular and unicellular forms.

The aim of this study is to examine the emergent regulatory changes in S. cerevisiae under anaerobic, aerobic, and mixotrophic conditions to uncover the complex interplay of yeast metabolism and genetic regulation. To study the different gene expression profiles, we extracted RNA from yeast growing in these anaerobic (PA), mixotrophic (PM), and aerobic conditions (PO). Using Illumina sequencing, we obtained RNA-Seq. Next, we generated expression levels of each gene using DeSeq2,

and obtained relative change in gene expression, by comparing PO vs PA, PO vs PM, PM vs PA, and PM vs Y55, the unicellular yeast. Through this, understanding of emergent gene patterns of complex lifestyles can be used to contribute to the knowledge of evolutionary mechanisms like genetic plasticity and metabolic regulation.

Microbes can adapt to different environments with varying oxygen availabilities, using strategies such as modification of their physiology, metabolic pathways, and genetic regulation [1, 2]. Specifically, microbes have the ability to alter their metabolism in response to changes in environmental factors, regarded as metabolic plasticity [3-6]. How metabolic plasticity is regulated in different oxygen availabilities is understudied, particularly in Eukaryotic lineages. S. cerevisiae can grow in anaerobic, aerobic, and mixotrophic (oxygen limited) conditions. Additionally, it has a short generation time and is one the most studied eukaryotic model organism, presenting itself to be an ideal model system to investigate [7,8]. Currently, other studies have included specific genes involved in metabolic regulation, how aerobic or anaerobic regimes have impacted mitochondrial complexes, and the ability to store a previous adaptation within genes in a combination of two oxygen availabilities [9-14, 19]. To our knowledge, no work has been conducted where yeast has been subjected to these three conditions, and long-term adaptation under such conditions.

Here, we will grow three ancestral yeast strains and their descendants in aerobic, anaerobic, and mixotrophic conditions, extract their RNA using RNA-Seq, and analyze emergent differences in metabolic gene regulation before and after their evolution. We expect to find if yeast can rewire their transcriptome profile immediately and if long-term adaptation makes strict-regulatory changes that are harder to retract.

CHAPTER 2. LITERATURE REVIEW

2.1 Respiration as a key factor to overcome lag phases in HDB

Recent studies show that *S. cerevisiae* not only responds physiologically to environmental changes, but alters their genotype and retains its history in their genes. Typically, cells adapt to changes in their environment by activating or repressing genes, however this causes significant depletion of time and resources which can potentially reduce the functional optimization of the cell, known as the lag phase. When the same stimulus re-occurs, the lag time can shorten, a phenomenon known as history dependent behavior (HDB). This can also occur metabolically where HDB (history dependent behavior) was seen in glucose to maltose and glucose to galactose shifts, as well as many others that suggested this is a common phenomenon [9]. HDB is thought to be an epigenetic behavior that relates to genetic heterogeneity and has been shown when yeast switch from glucose to maltose [10]. Glucose is a primary food source to produce carbon and is preferred over secondary carbon sources such as maltose and galactose as it provides a greater energy yield [11]. However, HDB does not depend on previous exposure to maltose or galactose. Moreover, *MAL* gene induction is required for a cell to resume fast exponential growth and does not seem to be a primary factor in determining lag phase duration. Respiratory activity appears to be the primary factor, with reduced respiratory activity resulting in longer lag phases. Specifically, cells that initially escape the lag phase exhibit slow growth around the time respiratory proteins are induced, which is hours before *MAL* gene induction [9]. This is further supported by Peres-Samper et al. (2018), which shows how

respiration and nutrient availability influence microbial growth. They cite the Crabtree effect, which is the glucose-induced repression of respiration. They showed the importance of complex III and IV in determining microbial fitness in fluctuating carbon environments [12]. Complex III and IV are essential in the electron transport chain and for the adaptation to galactose. More specifically, *S. cerevisiae* strains in glucose with higher respiration were thought to have a quicker lag phase when glucose is removed. Therefore, the proper formation of the supercomplex may provide a key role in the length of lag phases in yeast. These findings illustrate how HDB is controlled through the availability of oxygen rather than the presence or absence of previous exposure to an environment as well as the impact of respiration in lag phase duration [9, 12]. This also raises the question of whether the induction of other genes like *MAL* occurs in a similar fashion by first the availability of oxygen as an environmental cue, then repression or activation of a metabolic pathway.

2.2 Respiratory modes impact genetic regulation of metabolic pathways

Oxygen levels do not only impact metabolism, but also the genes regulating metabolic cycles. To induce or repress specific pathways, a cue must be given, which is found to most likely be aerobic, anaerobic, or mixotrophic conditions. S. cerevisiae was used to determine the effects of oxygen on 17 metabolites, and 69 genes related to carbon metabolism. In anaerobic conditions, the metabolic levels increased in the TCA (citric acid cycle) and upper glycolysis, however gene induction varied [13]. *COX5b* and *CYC7* have been found to be anaerobic genes that are only expressed when oxygen solubility is low. *COX5b* was detected at low levels in anaerobic cultures and *CYC7* had higher expression in aerobic cultures, which could be due to batch variability or strain variation. Other genes such as *CYC1* and *COX5a* were more highly expressed in aerobic conditions, as well as *ACS2* which is a part of the acetyl coenzyme synthetase gene. For genes involved in glucose dependent regulation, Dbp2 is responsible for proper gene expression in metabolic pathways [14]. Wang et al. (2017) found

that *CYC8* and *TUP1* form a complex and inhibit transcription of genes and corepress common genes with Dbp2. The loss of either Dbp2 or *CYC8* increases respiration, and co-repressed genes are typically found with lncRNA which can be upregulated in the absence of Dbp2 to decrease the binding of *CYC8* to promoters [14]. This further supports the notion of metabolic adaptation in response to a changing environment and nutrient availability [9, 12, 13, 14].

2.3 Facultative and anaerobic yeast gene regulation

S. cerevisiae is a facultative aerobe, which can survive in a wide range of oxygen availability [3]. The studies place *S. cerevisiae* in an oxygen available or unavailable environment, however an oxygen-limited environment is also worth exploring. When oxygen is depleted from fully aerobic and oxygen-limited cultures there is downregulation of genes related to growth and proliferation. The oxygen-limited culture responded more rapidly. Interestingly, the transcription of ribosomal genes returned to their initial steady growth more rapidly than other genes, suggesting that these specific genes are directly regulated by environmental factors [3]. These processes of respiration take place in the mitochondria. Genes encoding mitochondrial membrane proteins and genes related to mitochondrial function were downregulated in both oxygen-limited and aerobic cultures when anoxia was induced. The lowest expression of these genes was observed in fully aerobic cultures, which indicates an unknown function of mitochondria under oxygen limited and aerobic conditions. Petite yeast, which have lost their mitochondrial respiratory functions, may be ideal to investigate this further. It has already been found that petite yeast grows slowly due to the inability to synthesize amino acids [15]. Petite yeasts also experience reduced efficiency of amino acid production, which we will explore further by focusing on the expression levels of genes in those pathways.

Commented [GM1]: Name?

The genetic regulation of intermediate oxygen and low oxygen levels are understudied. Recent studies show that under the conditions of intermediate oxygen, significant post-transcriptional changes to the S. cerevisiae genome occur. Additionally, under anaerobic and limited oxygen, mitochondrial translation genes were also upregulated. This alludes to a regulatory mechanism that controls the need for increased energy production under different conditions that can be further explored in future experiments [16].

2.4 Yeast metabolism and regulators

The basis of yeast metabolism involves the uptake of simple sugars, like monohexose. Monohexose is regulated by *HXT* (hexose transporters, while Snf3p and Rgt2p sense extracellular glucose levels and is controlled by facilitated diffusion [17]. The regulation of such machinery is at the genetic level, with transcription factors and chromatin remodelers affecting the nucleosome backbone to either express or repress such genes [18].

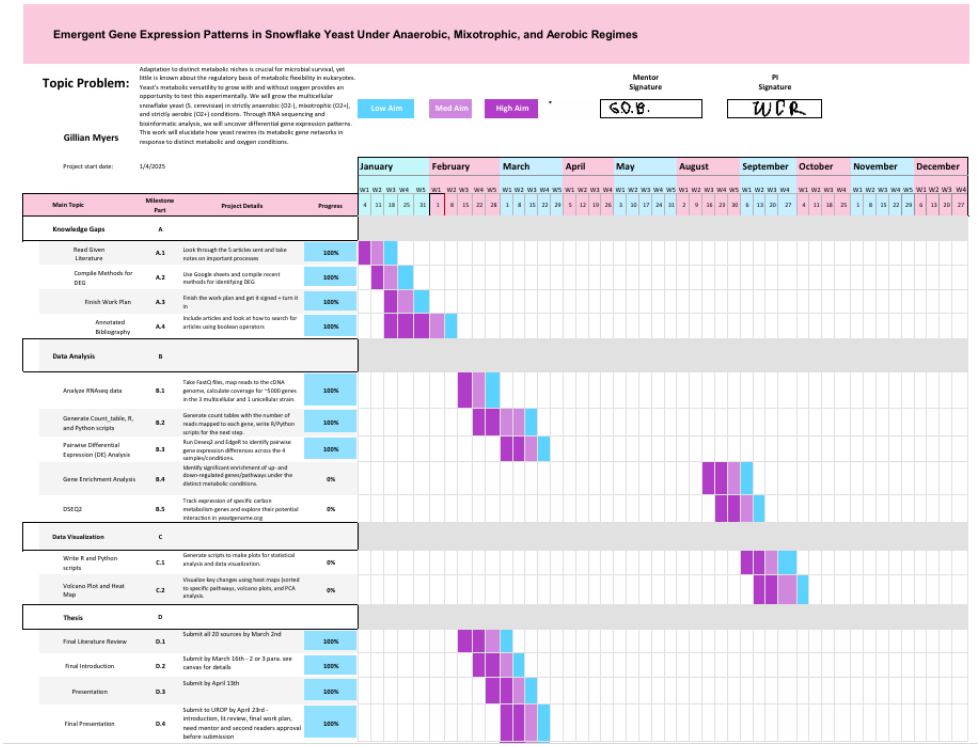
Respiration takes place in two parts: The Krebs cycle and fatty acid beta-oxidation, and through the respiratory chain and ATP synthase. In the presence of oxygen, the pyruvate generated during glycolysis will be transported from the cytoplasm to the mitochondria. It is then decarboxylated and oxidized by pyruvate dehydrogenase via fatty acid beta-oxidation. The products are typically acetyl CoA, NADH, and CO2. However, S. cerevisiae can also use fatty acid as a replacement carbon source. Acetyl CoA is then made through the removal of two carbons at a time [19]. The Krebs cycle (TCA) is crucial for de novo biosynthesis of amino acids and accounts for the majority of total oxidation of carbon. Here, Acetyl CoA reacts with oxaloacetate catalyzed by citrate synthesis to produce citrate. Three genes have been labeled responsible for encoding citrate synthases: CIT1 and CIT3 encode mitochondrial enzymes and CIT2 encodes the peroxisomal isoenzyme

responsible for exportation. When the compound is exported, citrate synthetase Cit2p is used to allow citrate to leave through the peroxisome compartment. The transportation of citrate across the mitochondrial membrane is through Ctp1 and Yhm2. Little is known about these transporters, except for the transportation of metabolic intermediates which allow the Krebs and glyoxylate cycles to regulate cell survival in the stationary phase [20].

2.5 Metabolic flexibility of *S. cerevisiae*

The natural mechanism of metabolic plasticity in yeast is the crab tree effect, which describes when, in the presence of high glucose, yeast will prefer fermentation to generate ATP. This is shown by an increase in glycolytic flux and repression of genes involved in mitochondrial respiration, glyoxylate cycle, and more [20]. The Crabtree effect is an evolved adaptation to fit *S. cerevisiae's* ecological niche, however its metabolism can be rewired in a laboratory setting to present Crabtree negative [21]. This can also be shown experimentally through diauxic growth conditions and nutrient availability. Diauxic growth is the utilization of the best source of carbon before consuming the second-best source. The best source is typically glucose and in any studies galactose is used as the second-best source. When S. cerevisiae was grown under glucose limitation in anaerobic conditions, galactose metabolism was induced in the absence of oxygen. However, slow diauxic growth has been shown in a sudden switch from glucose to galactose in anaerobic conditions. This was due to the low availability of ATP in anaerobic cultures [22]. This highlights the capacity for metabolic flexibility under oxygen availability. S. cerevisiae also experiences glycolytic fluxes. When switched from respiratory to fermentative conditions, glycolysis is upregulated. There were found to be two levels to this change, through the regulation of metabolic levels and metabolic concentrations [23].

CHAPTER 3. WORK PLAN



Emergent Gene Expression Patterns in Snowflake Yeast Under Anaerobic, Mixotrophic, and Aerobic Regimes

Topic Problem: Adaptation to distinct metabolic niches is crucial for microbial survival, yet little is known about the regulatory basis of metabolic flexibility in eukaryotes. Yeast's metabolic versatility to grow with and without oxygen provides an opportunity to test this experimentally. We will grow the multicellular snowflake yeast (S. cerevisiae) in strictly anaerobic (O2-), mixotrophic (O2+), and strictly aerobic (O2+) conditions. Through RNA sequencing and bioinformatic analysis, we will uncover differential gene expression patterns. This work will elucidate how yeast rewires its metabolic gene networks in response to distinct metabolic and oxygen conditions.

Low Aim | Med Aim | High Aim

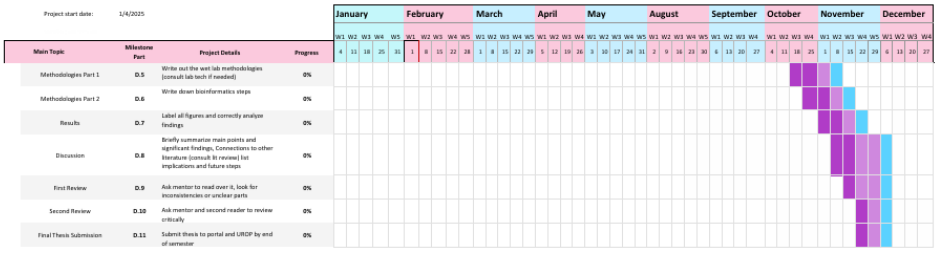
Mentor Signature: S.O.B.

PI Signature: WCR

Gillian Myers

Project start date: 1/4/2025

Main Topic	Milestone Part	Project Details	Progress
Knowledge Gaps	A		
Read Given Literature	A.1	Look through the 5 articles sent and take notes on important processes	100%
Compile Methods for DEG	A.2	Use Google sheets and compile recent methods for identifying DEG	100%
Finish Work Plan	A.3	Finish the work plan and get it signed + turn it in	100%
Annotated Bibliography	A.4	Include articles and look at how to search for articles using boolean operators	100%
Data Analysis	B		
Analyze RNAseq data	B.1	Take FastQ files, map reads to the cDNA genome, calculate coverage for ~5100 genes in the 3 multicellular and 1 unicellular strain.	100%
Generate Count_table, R, and Python scripts	B.2	Generate count tables with the number of reads mapped to each gene, write R/Python scripts for the next step.	100%
Pairwise Differential Expression (DE) Analysis	B.3	Run Deseq2 and EdgeR to identify pairwise gene expression differences across the 4 samples/conditions.	100%
Gene Enrichment Analysis	B.4	Identify significant enrichment of up- and down-regulated genes/pathways under the distinct metabolic conditions.	0%
DSEQ2	B.5	Track expression of specific carbon metabolism genes and explore their potential interaction in yeastgenome.org	0%
Data Visualization	C		
Write R and Python scripts	C.1	Generate scripts to make plots for statistical analysis and data visualization.	0%
Volcano Plot and Heat Map	C.2	Visualize key changes using heat maps (sorted to specific pathways, volcano plots, and PCA analysis.	0%
Thesis	D		
Final Literature Review	D.1	Submit all 20 sources by March 2nd	100%
Final Introduction	D.2	Submit by March 16th - 2 or 3 para. see canvas for details	100%
Presentation	D.3	Submit by April 13th	100%
Final Presentation	D.4	Submit to UROP by April 23rd - introduction, lit review, final work plan, need mentor and second readers approval before submission	100%



Project start date: 1/4/2025

Main Topic	Milestone Part	Project Details	Progress
Methodologies Part 1	D.5	Write out the wet lab methodologies (consult lab tech if needed)	0%
Methodologies Part 2	D.6	Write down bioinformatics steps	0%
Results	D.7	Label all figures and correctly analyze findings	0%
Discussion	D.8	Briefly summarize main points and significant findings, Connections to other literature (consult lit review) list implications and future steps	0%
First Review	D.9	Ask mentor to read over it, look for inconsistencies or unclear parts	0%
Second Review	D.10	Ask mentor and second reader to review critically	0%
Final Thesis Submission	D.11	Submit thesis to portal and UROP by end of semester	0%

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