Emergent Gene Expression Patterns in *S. cerevisiae* Under Anaerobic, Mixotrophic, and Aerobic Regimes

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Abstract

Organisms must adapt to environmental changes to survive by occupying niches. Niches are the habitat of the organism, where it typically lives to survive. Entering a new niche is known to change the organism's physiology, and more recently its genes. These genes are responsible for the phenotype and metabolism, and it even keeps a history of all of the changes it has made when switching niches. The ability to change one's metabolism in response to the environment is regarded as metabolic plasticity. The regulatory basis of metabolic plasticity is understood in prokaryotes; however, this mechanism is understudied in eukaryotes. Brewer's yeast, also known as S. cerevisiae, gives us an opportunity to test this experimentally by utilizing its metabolic ability to grow with and without oxygen in multiple cellular states. Respiratory modes such as anaerobic, aerobic, and oxygen limited states have been seen to displace metabolic pathways via global gene regulation. Gene induction varies depending on the pathway and respiratory mode. However, a consistent finding is that S. cerevisiae does experience metabolic plasticity, through a glycolytic flux. This review will further elucidate how yeast rewires its genetic responses to different oxygen environments and metabolic conditions with further implications in cancer cell proliferation.

Keywords: Adaptation, yeast metabolism regulation, multicellular evolution, differential gene expression, snowflake yeast

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 gene properties in S. cerevisiae under anaerobic, aerobic, and mixotrophic conditions to uncover the complex interplay of yeast metabolism and genetic regulation. Here, isolation of S. cerevisiae and subject them to RNA

purification to extract RNA for sequencing (RNA-seq). Further bioinformatic analysis was conducted using DESeq2 to show emergent genes in different metabolic conditions. 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.

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. 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 not only seen in yeast and is common in other microorganisms. This 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 sources like maltose and galactose since 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 another study that shows how respiration and nutrient availability influences microbial growth. They cite the Crabtree effect, which is the glucose-induced repression of respiration, highlighting the role of oxidative phosphorylation by showing the importance of complex III and IV [12]. If complex III and IV cannot form into the supercomplex capable of transporting electrons, then the electron transport chain cannot be completed, and respiration will not occur at its fullest capacity. These findings illustrate how HDB is controlled through the availability of oxygen rather than the presence or absence of previous exposure to an environment [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.

Respiratory modes impact genetic regulation of metabolic pathways

Oxygen levels do not only impact metabolism, but also the genes regulating the 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]. Another study 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].

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 its initial steady growth more rapidly than other genes, suggesting that these specific genes are more regulated by environmental factors rather than metabolic pathways [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, yeast that have lost their mitochondrial function, may be a way to investigate this further. It has already been found that petite yeast grows slowly due to the inability to synthesize amino acids

[15]. Perhaps this can be used to take this global mechanism one step further by exploring how environmental changes, specifically oxygen availability, alter metabolic pathways that relate to loss of mitochondrial function.

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 [16].

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].

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].

When an important environmental resource like oxygen fluctuates, organisms must adapt to fit a new niche [9, 12]. S. cerevisiae, a facultative aerobic yeast, exhibits multiple adaptations to oxygen available (aerobic), oxygen limited (mixotrophic), and oxygen depleted (anaerobic) environment [3, 11]. Recent studies have shown that in response to these changes, their metabolism must adapt as well. This is regulated on a genetic level that can be kept within their genetic history. Gene induction appears to vary depending on its regulatory pathway, however specific corepressors have been found to control those respiratory genes [6, 7]. Most studies have focused on aerobic influences; however, S. cerevisiae's limited oxygen and depleted state seem to have understudied implications on metabolism, genetics, and mitochondrial function. Petite yeast has been used to model a loss of mitochondrial function like anaerobic yeast, with crucial metabolic pathways like the citric acid cycle being altered [15]. However, little is known about genetic regulation and the impact of such a major event. In the future, further investigation of the genetic, metabolic, and mitochondrial regulation of the oxygen-limited and depleted state of yeast should be used. It can also apply to other events, such as cancer cell proliferation and purine metabolism that can reveal the effect of drugs on our genome [24, 25].

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