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Growth of Yeast, Saccharomyces cerevisiae, under Hypergravity Conditions

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Introduction

The yeast, *Saccharomyces cerevisiae,* is a eukaryote that can exist in a haploid or diploid form. The haploid state consists of 16 chromosomes while the diploid form consists of 32 chromosomes. This unicellular organism reproduces by budding (Bond et al. 2001). *Saccharomyces cerevisiae* is classified under the division of Ascomycota and the kingdom of Fungi (Bond et al. 2001). The genome of the yeast *Saccharomyces cerevisiae* was the first eukaryote sequenced, and a deletion set is available to help better understand this organism as a model eukaryote.

A species tested with the expectation that what is discovered will provide insight on other organisms is considered a model organism. The yeast, *Saccharomyces cerevisiae*, was first introduced as a model organism in the 1930s to further understand the life cycle as well as the transmission of genetic material. (Forsburg 2005). By understanding cellular processes of *S. cerevisiae*, this model can be applied to study other organisms.

Simple eukaryotes share many cellular processes with metazoan systems and provide simple methods of understanding higher eukaryotes (Forsburg 2005). As a eukaryotic organism, *S. cerevisiae* shares cell processes that can be applied to other eukaryotes such as cytoskeletal organization, metabolic regulation, receptor and second messenger signaling, and chromosomal structure (Botstein and Fink 1988). *Saccharomyce cerevisiae*

has been used as a model to study cellular processes such as oxidative stress through studying the peroxisome (Forsburg 2005).

Saccharomyce cerevisiae was the first eukaryote to be sequenced (Goffeau et al. 1996) and was developed further to understand genomic patterns. Over 600 scientists from all over the world contributed to sequencing the genome of *S. cerevisiae*, allowing for its 6,000 genes to be further screened by genetic analysis (Goffeau et al. 1996). The genes can be efficiently screened to understand protein functions, genomic patterns, and cellular processes. Previous research suggests the yeast *Saccharomyces cerevisiae* is a good model for genome evolution since the genome has undergone evolutionary duplications and gene deviations in expression or function (Forsburg 2005).

The fast growth rate of this organism contributes to the development of *S. cerevisiae* as a model organism (Botstein and Fink 1988) and allows a large number of cells to be screened to better understand more about the genome (Forsburg and Nurse 1991). *Saccharomyces cerevisiae* cells can be kept frozen and stored at -70^{o}C for decades, and the haploid cells can generally regenerate in 2-4 hours (Forsburg 2005). The fast generation time of *S. cerevisiae* allows for mutants to be studied easily.

The ability to easily isolate phenotypes of *S. cerevisiae* makes it a good model organism. Mutants can either be classified as lethal or viable. The

mutation is considered lethal if there is no growth, and the mutation is considered viable if the mutant strain can be isolated and studied (Botstein and Fink 1988). Recessive mutations of the yeast *Saccharomyces cerevisiae* allow for easy identification since the cells can regenerate as haploids or diploids (Forsburg and Nurse 1991).

The genome of the yeast *S. cerevisiae* has been useful in understanding diseases, specifically human diseases such as inherited colon cancer and Werner's syndrome. Mutated yeast genes were isolated to study the cellular phenotypes of these two diseases. The isolated genes are used to find a common function through homology to the human genes that have been mapped by linkage, cloned, and sequenced (Botstein et al. 1997).

The entire genome of *S. cerevisiae* has been scanned for proteins by looking for open reading frames (ORFs). The open reading frames are structured with a start codon, at least 100 codons that specify amino acids, and a stop codon. ORFs are designated in a manner that denotes their location. The first letter Y denotes "Yeast," the second letter (A to P) denotes the chromosome, the third letter (L or R) determines whether the gene lies to the left or right of the chromomere (Bond et al. 2001). The three digit number that follows identifies to the order of the open reading frame from the centromere. The last letter (W or C) denotes if the open reading frame is within the Watson or Crick strand (Bond et al. 2001).

There are about 5,885 open reading frames that can be used to further understand protein functions (Goffeau et al. 1996). The sequenced genome of *S. cerevisiae* allows for proteins to be compared to other proteins on the basis of amino acid sequence similarity. The description of the proteins synthesized is described by the term "proteome" (Goffeau et al. 1996) Homologs can be found on the basis of the amino acid sequence comparison of proteins, which can then be classified by function. The proteome of the yeast cell contributes 11% to metabolism, 3% to energy production, 3% to DNA replication, repair, and recombination, 7% to transcription, and 6% to translation (Goffeau et al. 1996). Identification of these proteins has results in classifying 430 proteins in intracellular trafficking and 250 proteins maintaining structural functions (Goffeau et al. 1996).

The life cycle of the yeast *S. cerevisiae* is divided into four phases: G1, S, G2, and mitosis and allows for a short generation time with yeast cells doubling about every 100 minutes (Herskowitz 1988, Forsburg and Nurse 1991). The cell starts with 17 haploid chromosomes, which eventually undergo mitosis (Herskowitz 1988, Forsburg and Nurse 1991). During the cell cycle, the G1/S phase is the checkpoint used to regulate the cell by repairing DNA damage and choosing the correct pathways (Forsburg 2005).

Yeast cells grow by the process of budding, and the cells can grow as a haploid or diploid. The mother gives rise to a smaller daughter cell that grows independently, which becomes the daughter cell once cytokinesis

occurs to produce two separate cells (Herskowitz 1988, Chant and Pringle 1991). The daughter cell will eventually pinch off the mother cell depending on the present conditions of the cell cycle.

Similar to the G1/S checkpoint, *S. cerevisiae* undergo a damage checkpoint at the G2 phase that controls the metaphase transition (Forsburg 2005). Once the cell continues past the G2 phase, mitosis will occur. During mitosis, the cell will be divided into two identical daughter nuclei. Cells have several pathways to choose from when they reach the G1 phase. The first pathway involves continuing in the life cycle and eventually dividing (Herskowitz 1988, Forsburg and Nurse 1991). Second, environmental factors and lack of nutrients cause the cell to become arrested and enter the stationary phase, where they remain dormant and undergo a lag phase before they can re-enter the cycle (Herskowitz 1988, Forsburg and Nurse 1991). Third, the cells can mate if they are haploid to form a diploid and undergo mitotic divisions. Diploids can also choose to sporulate meiotically forming four stress resistant haploid spores or diploids can choose to grow invasively. Invasive growth involves the development of long filaments that can burrow into the neighboring environment for nutrients (Forsburg and Nurse 1991).

Saccharomyces cerevisiae can be used as a model organism to study how eukaryotic cells respond to hypergravity. High gravity simulation can be used to study the role of the deleted genes in this process. Previous research has

exposed other species to hypergravitational studies such as *C. elegans*, fruit flies, turtles, rodents, and chickens to examine similarities in their responses (Wade 2005). The study by Wade found a relationship between the size of rodents and their ability to survive in an ambient gravity level. The study compared rats and mice and found that due to larger size, rats were more sensitive to gravitational changes (Wade 2005).

Artificial gravity is achieved by accelerating or decelerating an object, and centrifugation can be ideal in order to achieve these changes. Using a centrifuge, artificial gravity is reached through angular acceleration. Rotations per minute, the radial distance from the center of the centrifuge, and angular velocity determine the actual artificial gravitational force (Clement and Pavy-Le Traon 2004, Yang et al. 2007). Hypergravity is reached with centrifugal accelerations, and studies have been conducted to determine physiological responses in space by using a centrifuge (Hader 1999).

The importance of testing exposure to hypergravity conditions can be related to space flight studies. Upon returning from space flight, astronauts were found to develop cardiovascular problems such as lowered blood volume, central venous pressure and stroke volume, cardiac atrophy, attenuated baroreflex capacity, and as much as 25% reduction in exercise capacity(Clement and Pavy-Le Traon 2004). Centrifugation studies to simulate hypergravity environments have been used to obtain more research on cardiovascular deconditioning in humans post space flight.

Another previous study found similar cardiovascular effects from space flight in addition to musculoskeletal and vestibular systems. The loss of muscle in space flight after less than three weeks has been shown to be reduced by 5-15% and as much as 40% loss after 6 months of space flight (Yang et al. 2007). The physical deconditioning effects also include significant loss of orthostatic tolerance as well as bone mass loss, which has been found to range from 1.5-3% per month (Yang et al. 2007). In order to study the negative effects of microgravity, artificial gravity has been used to replace the 1g gravity on earth as well as simulate high gravity conditions.

S. cerevisiae has become a model organism to study how eukaryotic cells response to stress and oxidative regulation. A study on the relationship between yeast stress and efficient ethanol production was conducted to study the environmental and stress conditions that cause cells to adjust their metabolic abilities (Zhao and Bai 2009). The study found a relationship for high expression of several genes involved in cell wall organization and the resulting defense mechanisms against stress conditions by adjusting cell wall structure and arrangement (Zhao and Bai 2009). Before these studies commenced we hypothesized that many of the genes we identify that involved in resistance to hypergravity may be involved in stress response and cell wall organization.

Materials, Methods, and Results

Four standard wild type haploid strains and two diploid strains were plated on Petri dishes after incubation. These Petri plates were centrifuged for 2-3 days at a time and then examined for growth. These six wild-type strains were incubated under simulated hypergravity conditions through the process of centrifugation to test the limits of growth.

Table 1 Wild-Type *S. cerevisiae* Strains

Genotype	Strain	Haploid/ Diploid
MATa his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1	DBY2006	Haploid
MATa lys2 ade2-1 his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1	DBY2007	Haploid
2006/2007	Mes101	Diploid
MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	BY4741	Haploid
MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	BY4742	Haploid
4741/4742	BY4743	Diploid

(Schmitt and Clayton 1992)

Standard concentrations of agar were used to streak the wild-type strains on Petri plates, but these plates were found to be not durable enough during centrifugation.

Initially, the plates worked under a low centrifugation speed, but the plates eventually experienced cracking once higher simulated gravity was

reached. Petri plates were used in the experiment until forces exceeded the strength of the Petri plates.

Fig. 1 Standard 4% YPD plate with 6 wild-type strains streaked onto Petri dish and incubated for 48 hours at 2,600 rotations per minute.

The Petri plates were replaced with TH4 microcarrier plates, which we found were able to withstand higher forces. The concentration of agar was increased from the standard 2% to 5% to prevent disintegration of the media during centrifugation. For our experiment, we used a Beckman TJ-6 centrifuge with a swinging bucket rotor and used inserts that could carry either standard Petri plates or the microcarrier dishes.

This experiment was used to initially test a maximum centrifuge rate the yeast can withstand. The purpose of this is to determine maximal artificial gravity that will allow for a yeast colony to form at a rate comparable to cells at 1g.

Table 2 Different concentrations of YPD media

2% YPD (1 Liter)	5%YPD (1 Liter)
20g agar	50g agar
20g peptone	20g peptone
20g dextrose	20g dextrose
10g yeast extract	10g yeast extract

The final limit was 2,114 g where the yeast continued to grow but a little slower than usual. Technical barriers prevented testing at higher speeds and hence higher levels of hypergravity.

A trial and error process was used to select for a medium that will allow for colony formation. The centrifugation speed limit is read as rotations per minute on a centrifuge and is then converted to gravity using the equation:

$$\mathbf{RCF=.00001118 \times r \times (RPM)^2}$$

where r is the radius measure from the center of the centrifuge to the surface of the agar plate where the yeast is growing. The rotations per minute (RPM) in the table were determined by allowing the centrifuge to run without any plates inside. These values were recorded and then converted into gravity.

The medium was initially centrifuged at a low RPM in order to identify maximum speed the yeast can grow. With continued growth, the speed was increased over time.

Table 3 Centrifugal Speed and Relative Gravity

Centrifuge Speed	Rotations/minute (RPM)	Relative Gravity (g)
8	3,200	2,114
7	3,000	1,666
6	2,600	1,247
5	2,100	813
4	1,800	597

A collection of 4800 different yeast strains, each with a viable gene deletion was screened for their ability to grow at high gravity. The deletion set of *Saccharomyces cerevisiae* is important to screen in order to learn more about the genes involved in adaption to higher gravitational limits.

Microcarrier plates were used with a 5% agar concentration. A Virtek Robot was used to efficiently plate all of the strains with deleted genes onto these plates for incubation as well as centrifugation. A total of nineteen microcarrier plates were used for the entire deletion collection. Two 5% YPD plates were centrifuged while two 2% YPD plates were incubated for the

same 24 hour time period. This method allowed for the screening of 768 separate gene deletions in one 24 hour period.

Using a scanner, the plates were scanned and compared for differences in the ability to grow for the screened genes. The location of the genes were denoted by assigning letters A through P to each row while the numbers one through twenty four were assigned to each cell in a row. The data were recorded and compared for further analysis.

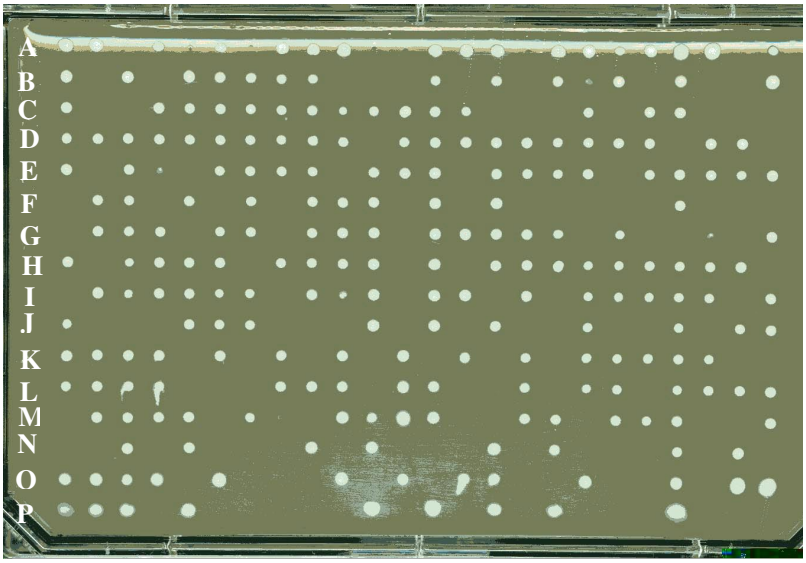


Fig. 2 Microcarrier plate used for plating the deletion set. Strains containing the deleted genes were denoted by location with rows 1 through 24 and columns A through P.

The plates that were subjected to simulated high hypergravity conditions were compared to the plates incubated at 30^{o}C and 1g. To test the effects of high speed centrifugation, we used a 24 hour time period. This

allowed for avoiding cracking of the microcarrier plates and efficiently screening the deletion set. The plates were scanned, allowing for comparison between the two plates grown under different conditions.

Gene Ontology term	Cluster frequency	Background frequency	P-value	Genes annotated to the term
mitochondrion organization | AmiGO	8 out of 14 genes, 57.1%	232 out of 4751 background genes, 4.9%	4.67e-06	IMG2/YCR071C, UGO1/YDR470C, MRPL8/YJL063C, MRPL49/YJL096W, ATP12/YJL180C, MEF1/YLR069C, GEP5/YLR091W, MSS51/YLR203C
cellular component organization at cellular level | AmiGO	12 out of 14 genes, 85.7%	937 out of 4751 background genes, 19.7%	1.40e-05	SPO7/YAL009W, CYC3/YAL039C, IMG2/YCR071C, UGO1/YDR470C, PRP18/YGR006W, MRPL8/YJL063C, MRPL49/YJL096W, ATP12/YJL180C, MEF1/YLR069C, GEP5/YLR091W, PEP3/YLR148W, MSS51/YLR203C
cellular component organization or biogenesis at cellular level | AmiGO	12 out of 14 genes, 85.7%	1136 out of 4751 background genes, 23.9%	0.00013	SPO7/YAL009W, CYC3/YAL039C, IMG2/YCR071C, UGO1/YDR470C, PRP18/YGR006W, MRPL8/YJL063C, MRPL49/YJL096W, ATP12/YJL180C, MEF1/YLR069C, GEP5/YLR091W, PEP3/YLR148W, MSS51/YLR203C
cellular component organization | AmiGO	12 out of 14 genes, 85.7%	1184 out of 4751 background genes, 24.9%	0.00021	SPO7/YAL009W, CYC3/YAL039C, IMG2/YCR071C, UGO1/YDR470C, PRP18/YGR006W, MRPL8/YJL063C, MRPL49/YJL096W, ATP12/YJL180C, MEF1/YLR069C, GEP5/YLR091W, PEP3/YLR148W, MSS51/YLR203C
mitochondrial translation | AmiGO	5 out of 14 genes, 35.7%	94 out of 4751 background genes, 2.0%	0.00033	IMG2/YCR071C, MRPL8/YJL063C, MRPL49/YJL096W, MEF1/YLR069C, MSS51/YLR203C
cellular component organization or biogenesis | AmiGO	12 out of 14 genes, 85.7%	1285 out of 4751 background genes, 27.0%	0.00053	SPO7/YAL009W, CYC3/YAL039C, IMG2/YCR071C, UGO1/YDR470C, PRP18/YGR006W, MRPL8/YJL063C, MRPL49/YJL096W, ATP12/YJL180C, MEF1/YLR069C, GEP5/YLR091W, PEP3/YLR148W, MSS51/YLR203C
organelle organization | AmiGO	10 out of 14 genes, 71.4%	807 out of 4751 background genes, 17.0%	0.00069	SPO7/YAL009W, IMG2/YCR071C, UGO1/YDR470C, MRPL8/YJL063C, MRPL49/YJL096W, ATP12/YJL180C, MEF1/YLR069C, GEP5/YLR091W, PEP3/YLR148W, MSS51/YLR203C

Fig. 3 GO term results of yeast strains that did not grow under hypergravity conditions. Fourteen genes were found to be related to cellular mitochondrial function and organization (Hodges et al. 1998).

The deletion strains that did show expected phenotypes at high gravitational limits were entered into a GO term finder to observe for any pathway similarities. The results indicated these genes were related to mitochondrial expression and cellular organization. The smaller p-values indicate a higher significance for the genes involved in mitochondrial organization. Although different terms were generated in this search, the

fourteen genes are all related to mitochondrial function in different ways. The difference in gene location in the mitochondria can be studied to better understand how these genes contribute to mitochondrial pathways and intracellular organization.

Table 4 Gene Observation Results by Gravity

Grow better high g	Grow better 1 g	No growth high g
YEL029C	YJL188C	YAL009W
YEL036C	YJL140W	YJL063C
YEL050C	YOR035C	YJL209W
YPL129W	YLR047C	YAL039C
YER050C	YLR202C	YJL096W
YPL144W	YGL143C	YJL180C
YPL031C	YDR462W	YLR148W
YPL029W	YHR147C	YLR091W
YBR163W	YCR020W-B	YLR069C
YBL058W	YFR036W	YLR201C
YDL167C		YLR203C
YDL062W		YDR470C
YDL135C		YGR006W
YDL044C		YCR071C
YDL069C		
YJR090C		
YGL218W		
YGR219W		
YHR039C-B		
YLR242C		
YKL170W		
YLR380W		
YPR163C		
YPR124W		
YBR132C		

The system of presence or absence was used to denote if the plate of all the strains containing the deleted genes grew better or worse at high simulated gravity. Yeast cells that grew better in either of the conditions

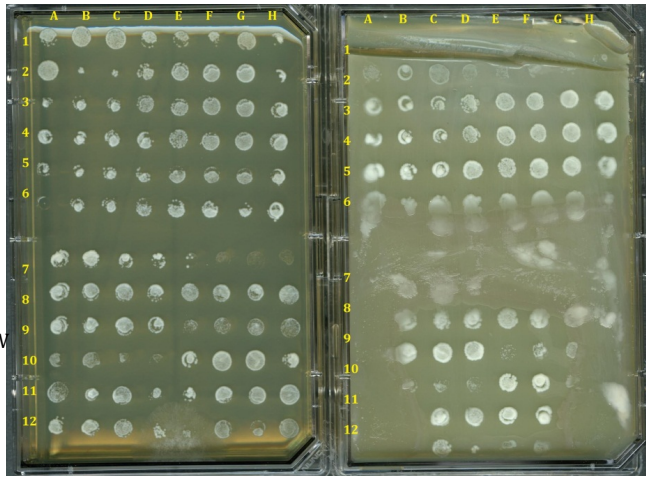
were recorded as well as knockout lines for genes that showed no growth under centrifugation conditions. The different gene strains were rescreened to confirm the results found through the frogger method. Rescreening focused on the genes that did not survive under simulated hypergravity conditions.

The frogger method replicates experimental results to confirm strains that showed no growth in high speed centrifugation. Two microtiter plates were used to rescreen 48 genes with the frogger method. The frogger dimensions were 6 by 8 spokes so each plate required two separate transfers by placing the frogger into the well. Ethanol flame sterilization was used between each frogger transfer.

The results indicated forty seven genes needed to be rescreened for differences in growth. The strains containing the deleted genes were streaked on to Petri plates, incubated for forty eight hours, and kept in a refrigerator. This allowed us to isolate these knockout strains on separate Petri plates and store for further rescreening. A series of dilutions was created for each strain recorded using ninety six well microtiter plates.

The plate dimensions were twelve wells by eight wells, and each plate held twenty four strains. Columns A through D and columns E through H were used for the dilutions of each strain separately. The twelve rows were each assigned two strains, one for columns A through D and the other for

columns E through H. The dilutions were created using an initial one hundred microliters (µl) of sterile water in each well. Sterile toothpicks were used to pick colonies into an individual well in the microtiter plate, and each strain was designated a row of one through 12 in columns A and H. Since a total of 47 different deletion gene-lines were found and two microcarrier plates hold 48 lines, the line with gene YLR047C was repeated. The dilution was created by transferring 20 µl from column A to B, 20 µl from B to C, and 20 µl from column C to D. Serial dilutions were used to compare two different concentrations of the yeast line.



SHE4
HOF1
SPO7
MRPL49
YLR074C
ATP12

MRPL28
MRF1
YBR163W
SHP1
AGP2
CTR1

TIF3
MSS51
YLR202C
YLR201C
YLR074C
MRPL6

PRP18
GRR1
YGL218W
YPL144W
YER050C
ANC1

Fig. 4 Frogger serial dilution results on microcarrier plate with the first dilution in rows A1 through A12 and H1 through H12. (Left) First plate incubated under standard 1g conditions for 48 hours and second plate (right) incubated for 48 hours under hyper gravitational conditions.

A second method was used to rescreen the genes by streaking individual strains onto microcarrier plates. The plates were marked off using a grid system with each grid square designated for a separate strain. A total of four plates were used for this method of rescreening with two 5% YPD plates and two 2% YPD plates.

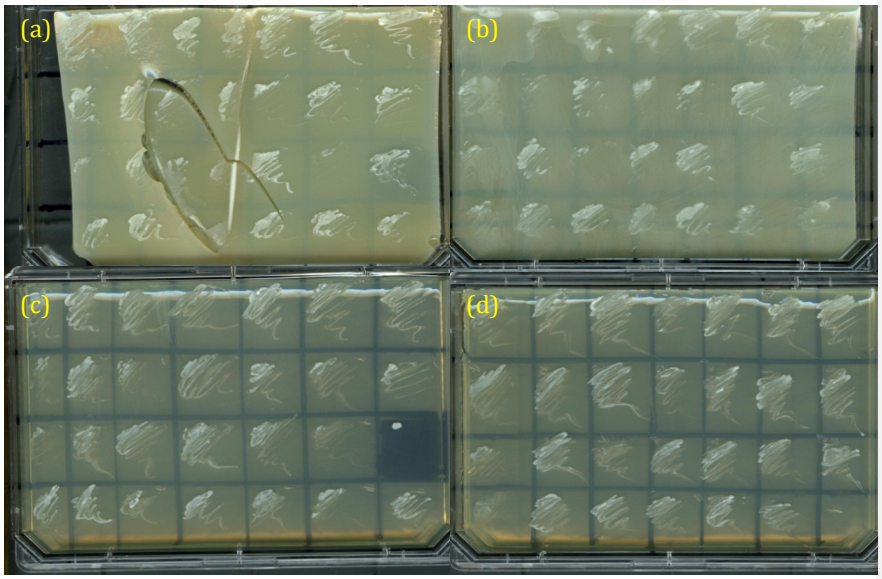


Fig. 5 Second method of rescreening by individually streaking gene deletion-lines. (a) 5% concentration YPD microcarrier plate 1 with hypergravity effects on media (b) 5% concentration YPD microcarrier plate 2 (c) 2% concentration YPD plate 1 (d) 2% concentration YPD microcarrier plate 2. Inconclusive results for this rescreening method.

Discussion

Yeasts have been exposed to various conditions such as heat shock, osmotic stress, and oxidative stress. The responses of yeast genes to these effects have been studied to better understand the reactions and mechanisms used by yeasts upon exposure to these conditions. In a previous study,

osmotic stress was studied after yeast cells were exposed to hyperosmotic shock by counteracting cell dehydration and protecting the cell structure (Estruch 2000). Although yeasts in this study were tested under osmotic stress conditions, it is useful to understand different mechanism of cell maintenance as well as MAP kinase pathways to increase osmolarity (Estruch 2000).

In a different study, the effect of vacuum pressure on ethanol fermentation was observed using baker's yeast. The study found that baker's yeast was able to ferment under high vacuum pressure. The yeasts converted glucose to ethanol with an increasing rate of fermentation as the pressure was increased, and vacuum pressure was run continuously without running the pump through the effect of liquid nitrogen trapping carbon dioxide (Nguyen 2009). Although the experiment reported here focuses on hypergravity effects, the changes in osmotic pressure can also greatly affect cell activity and response. Baker's yeast under high vacuum conditions was able to survive and continue the process of fermentation. This is similar to this study by the yeast, *Saccharomyces cerevisiae*, surviving under hypergravity conditions and continuing to express the phenotype of the knockout strains.

The mitochondrion is an organelle that serves as the powerhouse of the cell by providing a large supply of energy. Mitochondria are complex since these large organelles have their own DNA, and mitochondria perform

a number of processes such as fatty acid oxidation, the citric acid cycle, respiration, metabolite biosynthesis, ion homeostasis, and oxidative phosphorylation (Kanki et al. 2009, Solieri 2010). Larger numbers of proteins also perform functions such as carbohydrate metabolism, iron homeostasis, and are responsible for organelle morphology. Mitochondria contain two lipid bilayers and will experience effects from high gravity even if this means pushing them all to one side of the cell. Based on the results from this experiment, it is difficult to predict what other effects these mitochondria are experiencing.

The yeast, *Saccharomyces cerevisiae*, is a facultative anaerobe with a genome that encodes around 500 to no more than 800 proteins localized in the mitochondrion (Lipinski et al. 2010). Furthermore, there are over 600 genes that contribute to mitochondrial function. A large number of proteins can be found in the nucleus or cytoplasm while less than half, or 44% of proteins, is found in other subcellular regions (Huh et al. 2003).

The mitochondrial DNA, or mtDNA, of *S. cerevisiae* encode polypeptides that are essential to respiratory metabolism (Berger and Yaffe 2000, Lipinski et al. 2010). Mitochondrial DNA molecules are synthesized throughout the cell cycle, but the exact process of mitochondrial DNA replication remains unknown (Berger and Yaffe 2000, Lipinski et al. 2010). Large noncoding sequences in the yeast, *Saccharomyces cerevisiae*, allow for

diversity in the yeast genome that is further studied by extensive mitogenomic research to better understand cellular activity (Solieri 2010).

Compared to mammalian mitochondrial genes, yeast mitochondrial genes are about four times larger in size and are intron-rich (Dieckmann and Staples 1994, Solieri 2010). Yeast genes are transcribed from several different promoters that are adjacent to transcription initiation sites while mammalian mitochondrial genes are transcribed from a pair of promoters (Dieckmann and Staples 1994).

During the process of budding, the transfer of mitochondrial DNA from a mother to daughter cell is complex since mitochondria have their own DNA. Mitochondrial cells are synthesized in the cell cycle and passed onto daughter cells, where each cell has 20-25 copies of mitochondrial DNA molecules (Berger and Yaffe 2000).

The results of this experiment showed that high gravity sensitive genes are involved in mitochondrial development. Before rescreening, the fourteen genes that did not survive growth under simulated hypergravity were entered into a GO term finder, and the results indicated a relation to mitochondrial development. The nuclear genes identified have protein products that are imported into the mitochondria. Of the 400 different nuclear encoded gene products imported into the mitochondria, a small subset were found in this experiment that was involved in mitochondrial

translation. The products of the genes were located within different parts of the mitochondrion and further rescreening was needed to test for development of mitochondria.

The forty seven genes found to have differences in growth were rescreened through the frogger method and the streaking method. The results of the streaking method cannot be used to confirm the mutants for differences in growth since the amount streaked onto the plates may differ for each strain. As a consequence of this difficulty with the method, retesting is required.

The GO term finder results show similarities for gene functions such as mitochondria cellular organization. Although the results are organized by different gene ontology terms, the genes are all related to mitochondrial function but each function may be localized in different parts of the mitochondria rather than the same location. The fourteen genes were found to be related by mitochondrial function

Mitochondria are required for essential function in the cell such as mitochondrial fusion. Mitochondrial fusion is not necessary in *Saccharomyces cerevisiae*, but the absence results in respiratory defects. Mitochondria are also essential in cell functions such as metabolic processing, ATP generation and calcium buffering (Coonrod et al. 2007). Two proteins involved in mitochondrial fusion include GTPases Fzo1p and Mgm1p. The function of the

outer membrane protein Ugo1 is unknown, but previous work has shown the Ugo1 protein interacts with the two mitochondrial fusion proteins (Coonrod et al. 2007).

The PEP3 gene (YLR148W) is a vacuolar peripheral membrane protein that promotes vesicular docking and fusion reactions (Srivastava et al. 2000). Unlike other fusion events, mitochondrial fusion is not mediated by SNAREs and works by the action of dynamin-related proteins (Hoppins et al. 2009). The transport vesicles involved in intracellular protein dislocation use a SNARE complex to ensure the fusion of the membrane protein after docking occurs, and PEP3 serves as a necessary trafficking protein to the vacuole (Srivastava et al. 2000).

Vacuolar trafficking in the yeast, *Saccharomyces cerevisiae*, can be used to study intracellular transport. The vacuole is an acidic organelle containing hydrolases, which include carboxypeptidase Y (CpY), proteinase A (PrA), proteinase B (PrB) aminopeptidase I (ApI), and alkaline phosphatase (ALP) (Srivastava). The process of trafficking occurs by receiving proteinaceous cargo through a variety of pathways (Srivastava et al. 2000).

SUV3 is a nuclear coded gene that functions as an ATP-dependent helicase and is required for mitochondrial gene expression in yeast (Golik et al. 2004). SUV3 functions at the post-transcriptional level, and its primary function involves exonucleolytic trimming of the 3’ ends of the mRNA and

mtRNA processing (Golik et al. 2004). While the SUV3 gene is associated with the mitochondrial ribosome, multiple proteins are used to successfully achieve docking through different vacuole pathways.

The results of this experiment indicate a correlation to involvement of these genes in the mitochondrial pathway and development. The appearance of the large subunit mutants can be attributed to possible protein density differences. Protein density plays an important role in ultracentrifugation experiments and can be studied to further understand the difference in the products of the mutants observed. The average density of proteins is directly related to the volume of a macromolecule and is equal to 1.35 g/cm^3 (Fischer et al. 2004).

Very few proteins have a greater density than 1.5 g/cm^3 while the yeast mitochondrial ribosome has a density of 1.64 g/cm^3 (Fischer et al. 2004). A possible explanation for the differences in the size of the mutants observed is if there is a loss of enough of the large subunits, only the small subunits will remain and acts as bullets. This hypothesis can be explained in terms of thinking of cell membranes, or in this case the mitochondrial membrane, like a thin piece of glass.

Setting a tennis ball or placing an empty plastic bucket on the thin piece of glass will have little or no effect. However, if the weight of the plastic bucket was manipulated by increasing the gravity or artificially putting in

water, the thin piece of glass will shatter at some point as a result. The glass shatter is analogous to the rupture of the mitochondrial membrane and killing the cells. A possible solution to test this hypothesis would be to get rid of all mitochondrial ribosomes. Eliminating the presence of mitochondrial DNA would allow for testing the resistance of the ribosomes. Protein translation is not disrupted by gravity but rather because of the mutants identified that did not survive.

Although growth was not quantitatively measured in this study, the differences in protein density can be used to try to understand the differences in the physical appearance of the mutants. An alternative approach to testing the bullet hypothesis would be to take one of the large subunit knockouts and delete a small subunit gene to observe if the phenotype becomes suppressed.

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Growth of Yeast, Saccharomyces cerevisiae, under Hypergravity Conditions

Saccharomyces cerevisiae is a eukaryote that was used to study the effects of hypergravity on a life. This organism was chosen as a model organism, which is a species tested with the expectation that what is discovered will provide insight on other organisms. Characteristics of the yeast S. cerevisiae that enable this organism to be used to study higher organisms are its ability to duplicate quickly, isolate phenotypes, and the availability of a deletion set. The yeast can exist in a haploid form, containing 16 chromosomes, or a diploid form, containing 32 chromosomes, and the haploid cells can usually regenerate in 2-4 hours. This allows for a large number of cells to be screened to understand more about the genome. Isolated phenotypes of the cell are useful in identifying observable traits of the organism as well as mutants.

The genome of the yeast Saccharomyces cerevisiae has been sequenced to allow for further screening of the genes, and the deletion set is available to help better understand this genome as a model organism. Genes can be efficiently screened to understand protein functions, genomic patterns, and cellular processes. High gravity simulation was used to study how these eukaryotic cells respond to hypergravity. Six standard wild-type strains were used to determine a maximum gravitational force the yeast can withstand and still grow. Wild-type strains refer to the naturally found phenotype, or observable traits, of the strain. Artificial gravity was generated using a centrifuge, and the deletion set was then screened to identify genes that could not

withstand simulated hypergravity compared to the growth of genes under normal gravity (1g) conditions.

Artificial gravity was achieved by using a centrifuge, which is a machine with a swinging bucket rotor that works by spinning at high speeds and centripetal acceleration. A trial and error system was used to find the maximum artificial gravity the yeast can withstand and continue to grow. Petri plates were used with standard concentrations of agar media to streak the wild-type strains onto the plate. The agar concentrations ranged from 2-5%, and the medium was initially centrifuged at a low speed of rotations per minute, and the speed was increased over time with continued growth.

These plates were then simulated under hypergravity conditions, and the plates initially worked under low centrifugation speed. The centrifugation speed limit is read as rotations per minute and then converted to gravitational force, and the final limit achieved in this experiment was 2,114g. Technical barriers prevented testing at higher speeds and hence higher levels of hypergravity.

Once a maximum centrifugal rate was determined, a collection of 4800 different yeast strains were screened for their ability to grow and adapt to higher gravitational limits. Since Petri plates were not durable enough to withstand higher gravitational limits, microcarrier plates were used instead. These rectangular plates are thicker than standard Petri plates and can spin at higher gravity without cracking. The 4800 different yeast strains were plated onto the microcarrier plates to create a library of the strains using a Virtek robot, which allowed for the screening of 768 separate genes in one 24

hour period. A total of 4 plates (plates A, B, C, and D) were plated using the robot at a time. Plates A and B had lower agar concentrations and were placed in the incubator for a 24 hour time period, and plates C and D had higher agar concentrations and were in simulated hypergravity conditions for a 24 hour time period. An incubator is a device used to regulate growth conditions such as temperature and humidity, and in this experiment the plates were incubated at 30°C and 1g (normal gravity). A scanner was used to compare a plate from the incubator, plate A, and a matching plate of deletion strains from the centrifuge, plate C, to compare the differences in ability to grow.

The plates were observed using a presence/absence system to denote if the plate of all the strains containing the deleted genes grew better or worse at high simulated gravity. Yeast cells that grew better in either the incubator or in the centrifuge were recorded, but the focus of the experiment was rescreening the genes that did not survive under simulated hypergravity conditions. Rescreening is a process of duplicating experimental results to confirm the strains that showed no growth at high speed.

The frogger method uses a frogger with spokes of dimensions 6 by 8 to transfer genes from microtiter plate wells onto microcarrier plates that undergo centrifugation. Microtiter plates, which are rectangular plates with the dimensions of 12 X 8 wells, are used to create a series of dilutions for each of the strains. The microcarrier plate dimensions were twelve wells by eight wells, and each microtiter plate held 24 strains. Columns A through D and columns E through H were used for the dilutions of each strain separately. Two twelve rows were each assigned two strains, one for columns A through D and the other for columns E through H. The dilutions were created using an

initial one hundred microliters (µl) of sterile water in each well of the microtiter plates. Sterile toothpicks were used to pick colonies into an individual well in the microtiter plate, and each strain was designated a row of one through 12 in columns A and H. The dilution was created by transferring 20 µl from column A to B, 20 µl from column B to C, and 20 µl from column C to D. Serial dilutions were used to compare two different concentrations of the yeast line.

Yeasts have been exposed to a variety of conditions such as osmotic stress, heat shock, and oxidative stress. The responses of yeast can be used to better understand oxidative reactions and mechanisms of survival upon exposure to these conditions. This study focused on the growth of the yeast S. cerevisiae under hypergravity conditions. Many of the strains that failed to grow at high gravity were found to be related to mitochondrial gene expression. The mitochondrion is an organelle that provides a large supply of energy to the cell and is involved in processes such as the citric acid cycle and respiration of the cell. The genes that did not survive growth under simulated hypergravity were entered into a Gene Ontology (GO) term finder, which generates similarities in the biological process, molecular function, and cellular component of a gene product. The GO term finder was used to determine products of the genes were located within different parts of the mitochondrion. The results would need to be rescreened further to test for the development of the mitochondria.

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