Cold Adaptation and Mitochondrial Function in *S. cerevisiae*

BY

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Abstract

A major theory concerning the origin of life proposes that the first cells arose in cold, rather than hot, conditions. Regardless of its origin, life today can be found throughout the entire globe, even at the poles. Consequently, for survival to be possible, organisms must be capable of energy production under extreme temperatures. The main focus of this study is to determine the genetic basis of the ability of cells to grow at low temperatures. Yeasts are capable of fermentation in cold environments, but the effect of extreme temperatures on respiration is still under investigation. The objective of the experiment was to determine if *S. cerevisiae* mitochondrial function is important for growth at low temperatures. To test this hypothesis, we screened cold-sensitive mutants for their ability to grow on media that can only be metabolized via respiration. If the mutant gene is important for mitochondrial biogenesis or function, then its deletion may also hinder oxidative phosphorylation. Yeast mutants were grown in conditions at permissive and cold temperatures to observe differences in growth patterns. Our preliminary results suggest that most genes required for growth at low temperature do not affect mitochondrial function. Thus, most cold-sensitive mutants have robust mitochondrial function indicating that their inability to grow at low temperatures does not reflect an underlying mitochondrial defect.

Introduction

Mitochondria are universal organelles found in all eukaryotic cells, including yeasts and humans. Considerable data support the origin of mitochondria via endosymbiosis of independent bacterial cells to form a symbiotic relationship (8). Supporting this origin, mitochondria have many structural similarities to prokaryotes and a genome that suggests its ancestry to be from Protobeobacteria (8). Despite their symbiotic lifestyle, mitochondria still retain their genome and ribosomes so are able to translate mRNA and synthesize their own proteins, the mitochondria proteome (4, 13). However, evolution has resulted in cellular-mitochondrial interdependency. Specifically, in addition to their own genome-encoded proteome, mitochondria also require proteins encoded by nuclear DNA and imported into mitochondria from the cytosol (2). In turn, the mitochondria are able to produce ATP for the cell, as well as perform other biochemical functions (2).

The evolution of mitochondria resulted in cellular capability for aerobic respiration, which produces energy more efficiently than anaerobic respiration (27). This development is one of the characteristics that provided the opportunity for the evolution of complex multicellular organisms including humans (12). In addition to their ability to produce ATP through oxidative phosrphorylation (28), mitochondria also have essential roles in the synthesis of hormones, lipids, and amino acids (5). These molecules are vital in maintaining life in multicellular organisms, including humans. Hormones are important signal molecules within an organism. Lipids are important for cell structural support, insulation, and energy. Amino acids are needed to produce proteins. Therefore, the mitochondria have evolved to become a vital part of multicellular organisms.

Yeast and humans share many molecular pathways and machinery. As a result, the two organisms share many othologous genes, which share similarities due to inheritance from a common ancestor. Processes associated with the mitochondria, such as respiration and lipid biosynthesis, may be similar between humans and yeast. Particularly, mitochondria are vital for phospholipid metabolism and lipid import and export processes in eukaryotic cells (11). Mitochondria are able to synthesize acidic phospholipids independently, with the yeast mitochondria able to generate 70% of its cellular phospholipids (22). On a molecular level, phospholipids form the basic structure of cellular membranes. Previous studies have shown that lipid synthesis abnormalities result from defective mitochondria (22). Given the importance of membrane fluidity for cell function, it is possible that biosynthesis of lipids by mitochondria plays a role in cold adaptation. Cold adaptation is the ability of yeast to survive in cold temperatures. In determining the mechanism of cold adaptation we can broaden our knowledge on eukaryotic pathways, particularly in how cells function in cold temperatures.

The purpose of the experiment was to determine if the central mitochondrial function, oxidative phosphorylation, contributes to the cold tolerance of yeast cells. Our hypothesis is that many of the putative cold-sensitive (CS) mutations identified by the Wright lab may be cold-sensitive because of the effect of the mutation on mitochondrial biogenesis or function. If so, these mutations will not grow on media that cannot be fermented. Thus, we grew cold-sensitive mutants on media lacking glucose, but with ethanol and glycerol as carbon sources (YPEG). This medium is non-fermentable, and thus forces the yeast to use its mitochondria to produce ATP rather than fermentation, its preferred mechanism of energy production. If a cold-sensitive mutant has reduced or no growth on YPEG, then the mutant possesses a mutation that affects oxidative phosphorylation. A total of 307 mutants, which had been identified as putative cold-

sensitive mutants, were screened for their ability to grow on YPEG at both permissive and cold temperatures. These mutants were identified in a previous experiment of the Wright Lab to be potentially cold sensitive due to a difference in growth when compared to the growth of wild-type at cold temperatures. Interestingly, only 38 were unable to grow on YPEG at one or both temperatures. These observations did not support the hypothesis that defective mitochondrial functions were the physiological basis of the cold-sensitivity of many cold-sensitive mutants.

Materials and Methods

Model Organism: Saccharomyces cerevisiae

Budding yeast, *Saccharomyces cerevisiae*, were this study's model organism because they are inexpensive to grow, easy to culture, and possess a short generation time. This enables experiments to be conducted in an efficient manner. In addition, deletion strains of every known open reading frame (ORF) are available to study (32). *S. cerevisiae* can be transformed by deletion of specific ORFs via homologous recombination. Also, yeast genetics contain many human orthologs, which allows for comparative studies.

Master Plates

A Master Plate, containing the cold-sensitive, deletion mutants of interest was created as the template for replica plating. The yeast strains examined include haploid MATα, homozygous diploid, and heterozygous diploid deletion mutants of *Saccharomyces cerevisiae*. The complete list of screened putative cold-sensitive mutants is listed in Table 1. These strains were obtained from Invitrogen Corporation (Carlsbad, California).

The yeast strains were plated on YPD+G418+Amp solid media to produce the Master Plates. YPD (2% yeast extract, 2% proteose peptone, 2% agar, 0.0075% Amp, 0.0225% G418, and 2% glucose) is the standard complete medium, meaning it contains all the molecules required for cell growth. The media additive Ampicillin was used to prevent bacterial contamination in the Master Plates.

Using sterile toothpicks, frozen samples of the yeast strains were transferred individually to their assigned location on the Master Plates. The plates were then incubated overnight at room temperature to allow yeast growth. The Master Plates were then transferred to a 16^{o}C cold

room for five days, to precondition the cells and avoid cold shock. After this period, colonies/patches were evident and sufficient for replica plating.

Experimental Design

After replica plating, the yeast strains were grown at different temperatures to test the affect of temperatures on yeast growth and viability. One replicate per temperature was grown on YPD plates at 26°C and 10°C, providing positive controls for growth of strains on YPEG media (2% yeast extract, 2% proteose peptone, 2% agar, 1% galactose, and 1% ethanol). YPD contains glucose, a fermentable carbon source. Therefore, all yeast strains were expected to grow optimally at 26°C and minimally at 10°C on YPD. Any deviation from that phenotype on YPEG plates could then be identified. Strains were also grown on YPEG, which contains the non-fermentable carbon sources, ethanol and glycerol. This condition forces the yeast to metabolize via oxidative phosphorylation via their mitochondria. For the YPEG portion of the experiment, two replicates per temperature were grown at 10°C, 26°C, 30°C, and 37°C.

Replica Plating

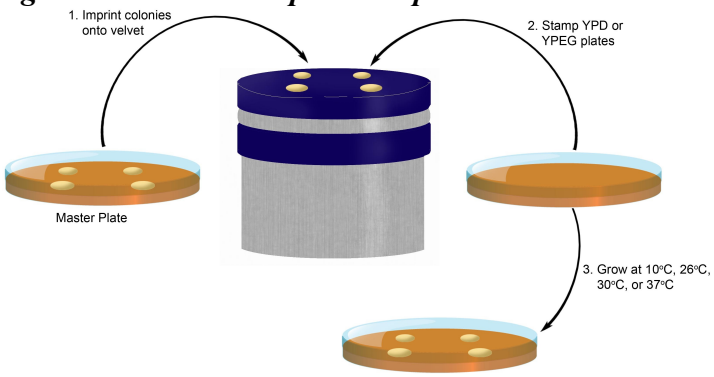
A total of 42 YPD and 168 YPEG plates were produced for the experiment. Once the plates were poured, they were stored at room temperature for seven days to dry. Dry plates ensure cells will stick onto the surface during velvet stamping.

Velvet Stamping Technique

The yeast strains were transferred from the Master Plates to the replica plates through velvet stamping. A visual representation of the technique can be viewed on Figure 1. The replica plates were then stamped by order of assigned temperature: 10°C, 26°C, 30°C, and 37°C. It is important to start stamping by lowest temperature first to ensure that plates assigned to the least favorable conditions contain the maximum number of cells. As plates are progressively

stamped, the yeast cells on the velvet reduce in number. When stamping, the grid of the replica plate must be aligned to the strains on the velvet to ensure identification is possible. The plate is laid on the velvet-covered mold and gently pressed to collect cells. Then the stamped plates were then transferred to their respective insulators and freezers.

Figure 1: Velvet Stamp Technique



Data Acquisition and Analysis

Each plate was photographed on two successive days once sufficient growth was observed. Plates growing at 37°C were photographed starting on Day 1, which was the day after they were stamped and incubated. Both the YPD and YPEG plates growing at 26°C and 30°C were photographed starting on Day 2. The plates grown at 10°C were photographed starting on Day 13.

Growth of a mutant strain on YPEG at low temperature indicated that the deletion did not impede cellular respiration or cold adaptation. The absence of growth on YPEG at any temperature indicated that the deleted gene coded for a vital component of the respiratory pathway in the mitochondria.

Yeast Genome Database Programs

The programs Biopixie (pixie.princeton.edu/pixie) and Biogrid (www.thebiogrid.org) were used to determine the relationship of individual genes to other yeast genes. Both work by

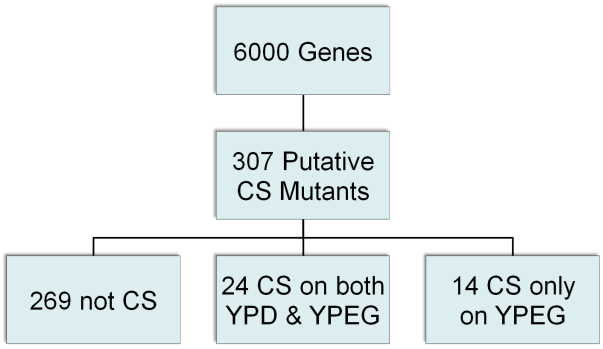
running the search query (gene of interest) through their genome databases to find associations of other genes to the gene of interest. Biopixie will then compile an interaction map of all genes related to the gene of interest. Biogrid will provide a list of interacting genes.

Results

Table 1 lists the temperature sensitivity of the 307 cold-sensitive mutants examined in this study. In most cases, haploid and homozygous diploid strains had similar cold-sensitive phenotypes. However, *MAC1* is only cold-sensitive as a homozygous diploid while *PEP3* and *BUD28* are cold-sensitive only as haploids (Table 2).

Of the 307 putative cold-sensitive mutants, only 38 were cold-sensitive in this experiment: 24 mutants were cold-sensitive on both YPD and YPEG and 14 mutants were cold-sensitive only on YPEG plates (Figure 2). These mutants are classified to be conditionally cold-sensitive or YPEG mutants for the purposes of this paper.

Figure 2: *Of the 307 mutants only 38 were confirmed to be cold-sensitive. 14 of the cold-sensitive genes were conditionally cold-sensitive on YPEG only.*



Gene Functions

I used the Saccharomyces Genome Database (www.yeastgenome.org) to examine currently known functions of the 14 conditional cold-sensitive genes (Table 2). Only *YME1* had a confirmed mitochondrial function, which is to degrade mitochondrial byproducts from oxidative stress (4). Instead, the majority of the genes encoded proteins involved in nuclear or protein transport or assembly and maintenance of the vacuole. Genes *BUD28* and *YKL118W* have unknown functions. Overall, the mutants affected proteins involved in intracellular transportation.

The two genes of unknown function are dubious open reading frames (ORF) that overlap with other genes. *BUD28* may not encode any protein and 98% of it overlaps with *RPL22A* (20). Therefore, *BUD28* may be part of the gene *RPL22A*. The ORF *YKL118W* overlaps with half of *VPH2* (6). It is highly possible *YKL118W* is the same gene as *VPH2*. *YKL118W* has an unknown but weak relationship with *NIP100* (Figure 3a), suggesting *VPH2* and *NIP100* should interact. However, this relationship remains inconclusive as *NIP100* and *VPH2* are not found to interact at all on *Biopixie* or *Biogrid*.

Table 2: YPEG Mutants

Only YME1 has a known mitochondrial function. The remaining 14 genes have nuclear, vacuolar, or cytosolic functions and have no known relationship to the mitochondria. Overall, the mutants identified have functions related to intracellular transportation. The left sub-columns of the Control and Experiment columns are haploid mutants. The right sub-columns are homozygous diploid mutants.

Gene	Control (YPD) *Haploid/Diploid*	Experiment (YPEG) *Haploid/Diploid*	Known Function
Nuclear Function			
SAC3			Pore-associated protein, forms complex with Thp1p, which is involved in transcription & mRNA export from nucleus (15, 24)
NUP188			Nuclear pore complex (NPC) subunit, involved in structural organization and permeability of envelope (19)
MAC1			Transcription factor, regulates genes required for high affinity Cu transport (23)
IES6			Protein that associates with INO80 chromatin remodeling complex under low-salt conditions; involved in DNA repair (11)
ARP1			Arp1-actin short filament related to dynactin complex via linking cytoplasmic dynein to its cargo through p150(glued); nuclear import (33)
NIP100			large dynactin complex subunit and ortholog of p150(glued); nuclear import(1, 18)
Vacuolar Function			
PEP3			Peripheral membrane protein that promotes vesicular docking/fusion reactions w/ SNARE; needed for vacuolar biogenesis (12)
VPH2			Vacuolar ATPase assembly protein that acts in ER (31)
Cytosolic Function			
SER1			3-phosphoserine aminotransferase, amino acid metabolism, needed for Ser & Gly biosynthesis (2)
RMD8			Cytosolic protein required for sporulation; needed for meiotic nuclear division (5)
RPL22A			Protein component of the large (60S) ribosomal subunit (26)
Mitochondrial Function			
YME1			Degrades improperly folded mitochondrial gene products in intermembrane space (4)
Unknown Function			
BUD28			Unlikely to encode protein, overlaps with RPL22A (20)
YKL118W			Overlaps with VPH2 (6)

Gene Relationships

Using the programs Biopixie and Biogrid, we were able to determine the relationship of individual genes to other yeast genes. None of the genes listed in Table 2 have any association with other known mitochondrial genes in *S. cerevisiae*. Furthermore, all the conditional mutants do not have any relationship with each other. However, a few small groups of genes are associated with each other. Among the nuclear genes that were identified, *SAC3, NUP188, ARP1*, and *NIP100* are known to be associated with each other (Figure 5). *NIP100* and *ARP1* are strongly associated with each other and are involved in the formation of the dynactin complex (Figure 3b). *SAC3* and *NUP188* are also associated with each other. Both encode nuclear pore-associated protein. *Nup188*'s relationship with *ARP1* and *NIP100* suggest a relationship between dynactin assembly and nuclear pore function.

Figure 3: *Diagrams of gene interactions and relationships according to information from Biopixie. (a) A weak connection between YKL118W and NIP100 was found. (b) A strong relationship between ARP1 and NIP100 was found.*

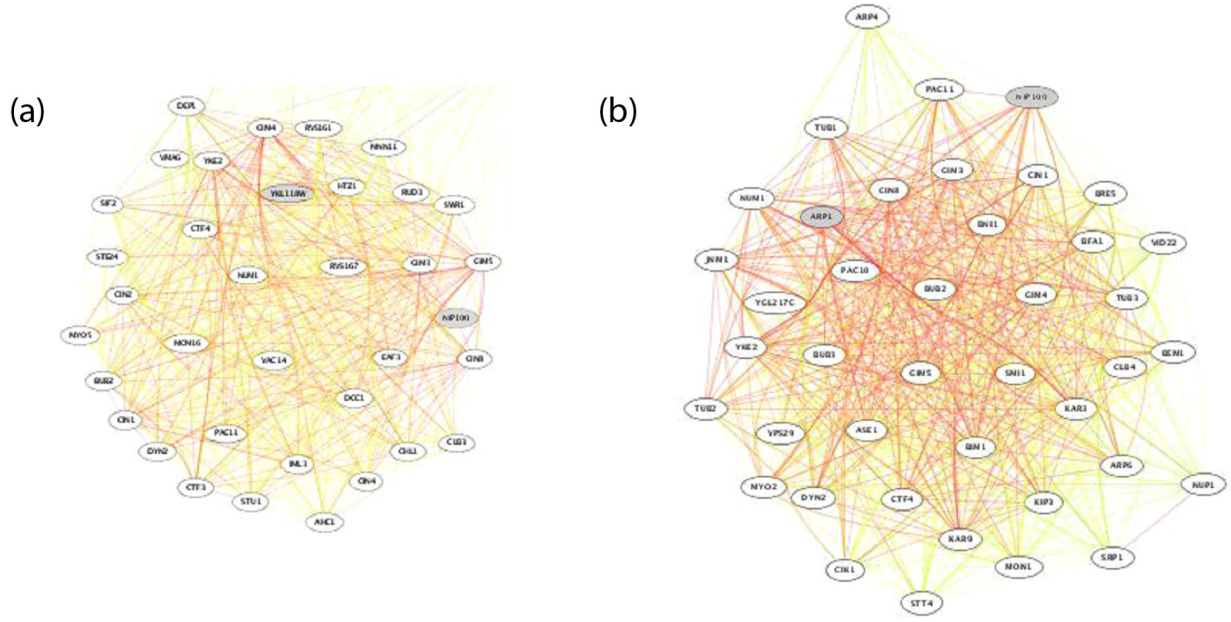
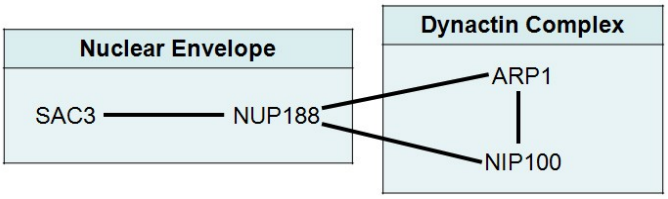


Figure 4: Relationship of Nuclear Genes

Diagram of interactions of four nuclear, YPEG-dependent CS genes according to information from Biogrid.



Discussion

Our overall results disproved the hypothesis that cold sensitivity resulted from dysfunctional mitochondria. In fact, only one of the YPEG mutants identified had an established role in mitochondrial function or morphology, although all mutants clearly grew poorly on a non-fermentable carbon source. The genes listed in Table 2 that have a known function are involved in intracellular biomolecular transportation, assembly, and regulation (Table 3). Nevertheless, the YPEG genes may impact mitochondrial function despite not coding for proteins that have a role in either mitochondrial biogenesis or function.

Though not essential for growth in all conditions, the YPEG mutants may be important for increased efficiency of cellular pathways involved in growth and reproduction. For most YPEG mutants, observable reduction in growth occurred only when the yeast experienced compound stresses (low temperature and lack of glucose) (7). Under permissive temperature YPEG mutants can respire, though less efficiently. Perhaps, maximum efficiency of the mitochondria is required for growth in cold temperatures in these mutants. Therefore, we hypothesize there are certain conditions, such as cold temperatures, when the yeast favors oxidative phosphorylation as its primary source of energy. Further research comparing the growth of known mitochondrial mutants at cold temperatures would be necessary to test this hypothesis.

Table 3: Molecular Function of YPEG-dependent CS genes

Transportation	Assembly	Regulation
SAC3	*SNU66*	*IES6*
NUP188	*VPH2*	*RMD8*
MAC1	*SER1*	*YME1*
ARP1	*RPL22A*	
NIP100		
PEP3		

Direct Impact on Mitochondria

YME1 was the only known mitochondrial gene among the YPEG mutants. It encodes a protein that degrades mitochondrial gene products modified by oxidative stress in the intermembrane space (4). Oxidative phosphorylation is known to cause oxidative stress, a condition that can lead to damage of all components of the cell (29). It is hypothesized that the deletion of *YME1* resulted in a lack of proteins necessary to dispose of the byproducts of oxidative stress and led to decreased survival rates at low temperatures.

There may be redundancy among genes encoding proteins that degrade byproducts of oxidative stress so the *yme1* mutants can still function normally on YPEG conditions at high temperatures. However, cold conditions may cause the inactivation of the redundant genes and leave Yme1p as the sole disposer of mitochondrial byproducts. According to this theory, *yme1* will accumulate byproducts from oxidative stress if the mitochondria are utilized at cold temperatures.

Nuclear Transportation

Half of the YPEG-dependent CS genes are known to be involved in nuclear transport (Table 2). *SAC3* and *NUP188* each code for nuclear pore complex (NPC) subunits and thus contribute to nuclear envelope entry and exit. Sac3p, located on cytoplasmic fibrils that extend from the nuclear pores, interact with mRNA export factors and nuclear pore associated proteins to facilitate mRNA export (10). Nup188p also facilitates nuclear export as well as well as nuclear port structure (12). The most vital function of Nup188p is to stabilize the pore as a core protein to allow for molecular entry and exit (15).

However, Nup188p is usually part of a three to five unit complex and pores that lack Nup188p still function, so its loss is not lethal to the cell (15). Perhaps at low temperatures and

in conditions of low ATP, the mutant nuclear pores no longer have sufficient function to support growth. Nup188p's interaction with other nuclear import and export proteins shows it has a central role in nuclear transport. For example, it is a major protein found in all nuclear pores and Sac3p interacts with it to open the pore for export (Figure 4). Deletion of either of these proteins could reduce the amount of mRNA that reaches the cytoplasm to be translated. Thus, the effects of nuclear transport on the cell's ability to grow at low temperatures may be indirect: vital proteins that are needed for cold resistance, growth, or proliferation may not be produced in adequate amounts needed by the cell at low temperature and therefore reduce yeast growth in the cold. Identification of proteins whose level increases under cold temperature conditions will be important to test this idea.

In addition to *NUP188* and *SAC3*, YPEG-dependent CS genes with nuclear function include two dynactin complex proteins, *ARP1* and *NIP100*. Dynactin is a dynein receptor that signals dynein to bind to cell organelles or molecular cargo that are to be transported along the microtubules (17). Then dynein transports its cargo toward the center of the cell (14). The genes *ARP1* and *NIP100* are two genes that encode for specific proteins in the dynactin complex. Arp1p is an actin short filament that links the cargo to dynein through p150(glued), which is encoded by *NIP100* (6, 3). Both *Biopixie* and *Biogrid* databases mapped a strong interaction between *ARP1* and *NIP100* (Figures 4, 5), further suggesting they may be part of the same complex.

Both dynactin complex genes *ARP1* and *NIP100* also interact with the NPC subunit gene *NUP188* to accomplish nuclear import (Figure 4). The import function of Arp1p and Nip100p suggest they are specific to the transport of vesicles formed by other organelles such as the endoplasmic reticulum (ER) rather than organelles. The molecular products are produced

outside the nucleus and packaged into vesicles that are marked as designated to the nucleus. Thus the vesicle is theorized to be transported to the nucleus by specific dynactin and its contents imported into the nucleus with the aid of dynein. In the absence of Arp1p and Nip100p, cell products like transcription factors will not be guided to the nucleus. The nucleus will then be unable to transcribe mRNA and thus the cell will not produce the proteins it needs for survival and proliferation.

Meiosis and Mitosis

The dynactin complex is also involved in chromosome alignment and spindle organization during mitosis (26). Nip100p, a dynactin subunit, is specifically known to play a role in mitotic spindle partitioning (1). Another YPEG-dependent CS gene is also involved in regulating cellular division. *RMD8* encodes a protein needed for meiotic nuclear division during sporulation (5). Mutations in either of the two proteins would affect the equal division of nucleus and chromosomes during meiosis and mitosis respectively. The observed reduction in growth at low temperatures can be the result of the yeast's inability to produce progeny with the correct set of chromosomes.

Redundancy is frequently found for proteins involved in cellular processes such as meiosis and mitosis. Hence, the *rmd8* and *nip100* mutants continue to grow at normal temperatures. However, changes in extracellular conditions such as temperature may cause the inactivation of multiple genes involved in nuclear division. In wild type cells, the genes *RMD8* and *NIP100* may be the only genes still activated in cold temperatures to facilitate even nuclear division during meiosis and mitosis respectively.

DNA repair, mRNA Transcription and Translation

IES6 is theorized to be involved in DNA repair (11). Thus, the fact that it is required for growth at low temperature, suggests that certain types of DNA repair may be particularly important at low temperature. Mac1p is transcriptional factor (23). The deletion of *MAC1* might inhibit or cause errors in the transcription of DNA and thus specific mRNAs would not be produced. The gene *RPL22A* codes for a component of the large ribosomal subunit (26). Its deletion might result in a dysfunctional ribosomal subunit and affect its ability to properly translate mRNA and thus result in a decrease in protein synthesis or the formation of incorrect polypeptides. A mutation in *SER1* can also pose problems for translation. Ser1p is a protein needed for Serine and Glycine biosythesis (2). Any protein requiring Serine or Glycine in its structure will not form properly and may compromise its function in the cell.

Vacuole Genes

Among the YPEG mutants are two vacuolar function genes, *PEP3* and *VPH2*, suggesting that vacuolar function is important for growth at low temperatures on YPEG (Table 2). Pep3p is needed for biogenesis and for function of the vacuole (12). Yeast vacuoles are involved in a multitude of processes, including maintaining the homeostasis of the cell and isolating toxic ions (33). Therefore, *PEP3* would contribute greatly to the survival of the cell in producing vacuoles. The vacuoles may play a role in detoxifying the cell of the byproducts from oxidative stress. As a result, the byproducts would damage the cell and decrease its survival rate at cold temperatures. As stated earlier, there may be multiple proteins involved in the degradation of oxidative stress byproducts but only a few can function in extreme conditions such as cold temperatures. Therefore, Pep3p may be involved in detoxifying the cell from oxidative stress at cold temperatures.

VPH2 encodes a vacuolar ATPase assembly protein found in the endoplasmic reticulum (ER) (31). The ATPase it produces is then sent to the vacuole, where it drives acidification of the vacuole (9). The acidic vacuole creates a proton motive force that drives cellular transportation of nutrients in or out of the vacuole and allows degradative enzymes to act (25). Thus, we theorize vacuoles may store components necessary to cold survival. Cold temperatures may trigger a signal that signals the ER to produce ATPase to export those components.

Indirect Impact on Mitochondria

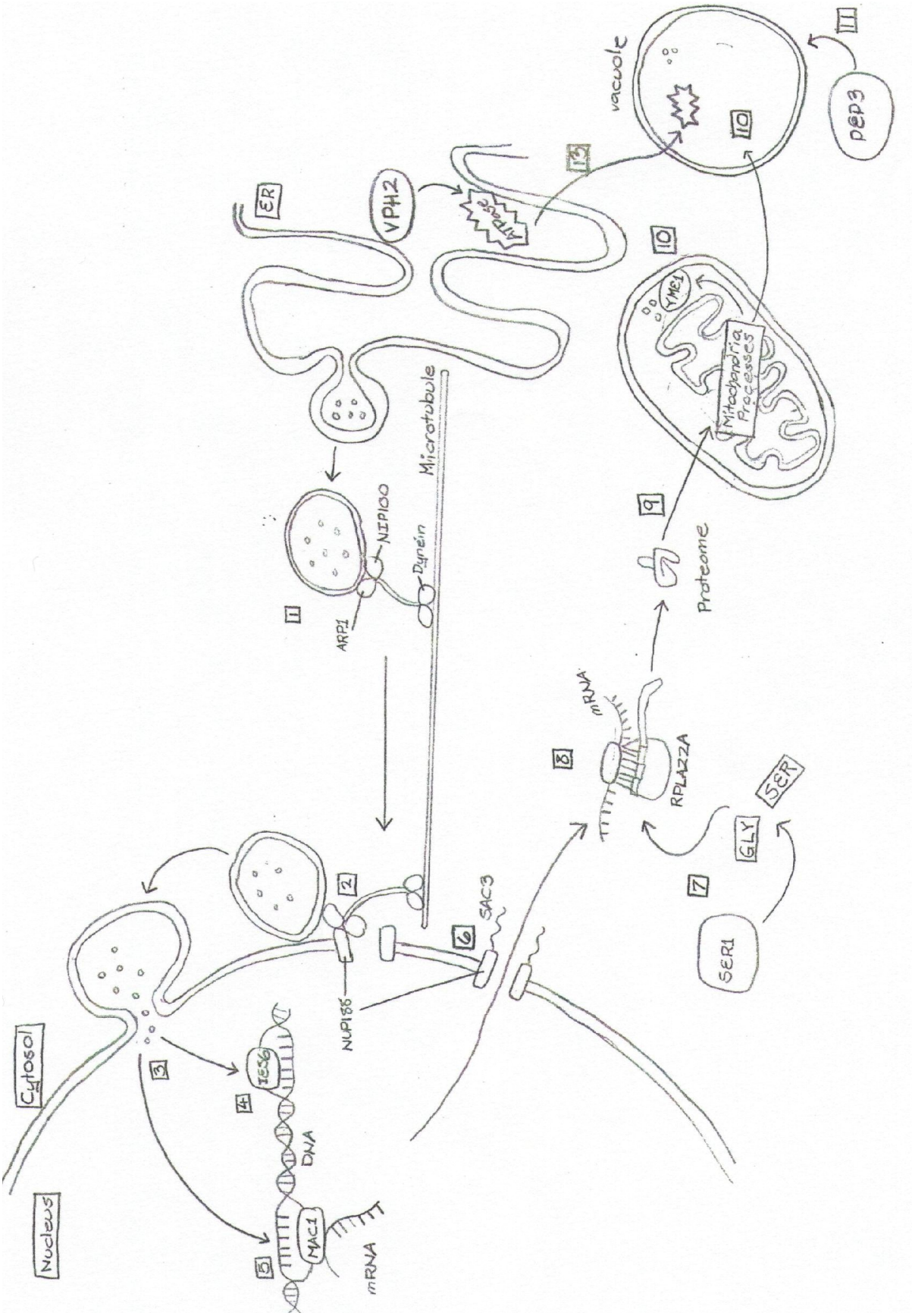
We theorize YPEG mutants indirectly affect mitochondrial function at low temperature. As observed in Table 2, the identified genes are involved in nuclear transportation, transcription, and translation—all general processes necessary to produce protein. Mitochondria require both imported proteins encoded by the nucleus and proteins synthesized inside the mitochondria (13). YPEG mutant cells may interfere with the production of nuclear-encoded proteins needed by the mitochondria. As a result, mitochondria may be unable to synthesize products such as lipids or amino acids that may, in turn, affect cell viability or proliferation. A diagram of the interactions of the YPEG mutant genes to produce protein for the mitochondria is provided in Figure 5.

Problems in nuclear transportation caused by the deletion of proteins from the NPC and dynactin complex will result in significant reduction in protein production, including mitochondrial proteomes. Similarly, problems in mRNA transcription and translation would also decrease mitochondrial proteome production.

Figure 5: *A simplified diagram of the cell interior to visualize the interactions of the YPEG mutants.*

1. *ARP1 and NIP100, found on the dynactin of nuclear bound vesicles, are detected by dynein. They facilitate the attaching of dynein to the dynctin. Dynein transports vesicle toward nucleus via microtubule.*
2. *Upon reaching the nucleus, ARP1 and NIP100 recognize NUP188 on NPC and deposit vesicle to nucleus.*

3. Vesicle cargo is imported into the nucleus. Cargo can include DNA repair proteins and transcription factors.
4. IES6 is protein involved in DNA repair.
5. MAC1 is a transcription factor.
6. SAC3 interacts with NUP188 to export mRNA into cytosol.
7. SER1 produces the amino acids Glycine and Serine, which are later attached to tRNA to contribute to polypeptides.
8. mRNA binds to ribosome. RPLA22A translates mRNA by binding to tRNA and polypeptides are produced. The proteins produced include mitochondrial proteomes
9. Proteomes are transported to the mitochondria. Mitochondria can then function, including performing oxidative phosphorylation.
10. *Toxic byproducts from oxidative stress are degraded by YME1 in the mitochondria and in the vacuole.*
11. PEP3 facilitates in vacuole biogenesis.
12. VPH2 facilitates ATPase assembly.
13. ATPase is sent to the vacuole acidify it.


Nucleus
Cytosol
DNA
mRNA
MAC1
IES6
NUP188
SAC3
ARP1
NIP100
Dynein
Microtubule
ER
VPH2
Golgi
vacuole
pep3
YME1
Mitochondria Processes
Proteome
mRNA
RPL22A
SER1
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Future Studies

The results found in this study raise questions for potential future research. *YME1* is theorized to be conditionally cold sensitive due to the inactivation of other similar functioning genes at cold temperatures. To test the theory, an experiment should be conducted to detect and quantify the amount of byproduct produced by oxidative stress in *yme1* mutants compared to wild-type strains when grown on YPEG medium. Other tests should confirm if inactivation of byproduct decomposition genes occurs at cold temperatures by growing their deletion mutants on YPEG while comparing growth between cold and permissive temperatures. The knockout mutants would be grown on YPEG with one replicate grown at cold temperatures and another at permissive temperature for comparative studies. The theory that inactivation of redundant genes occur in cold temperatures also applies to genes like *RMD8* and *NIP100*. Thus similar studies used to confirm the *YME1* theory can also be used to confirm the role of *RMD8* in meiosis and *NIP100* in mitosis. Known meiosis and mitosis mutants should be grown on YPEG to compare growth between cold and permissive temperatures.

Further research can also be conducted to determine the interaction of NPC proteins encoded by *SAC3* and *NUP188*. Quantitative studies should be performed to determine if there is a difference in protein production at cold and normal temperatures.

The new theories about dynactin structure and function should also be confirmed. Specific dynein subunits like Arp1p or Nip100p may have a recognition function when the dynactin has reached its destination. This theory can be tested by observing the growth of other deletion mutants of genes that encode nuclear pore subunits or dynein subunits. We also theorized that there are different types of dynactin that transport cargo to specific organelles. The absence of Arp1p and Nip100p only affected dynactin-aided transportation to the nucleus

while transportation of products between other organelles seemed unaffected. This second theory can be tested by growing known dynactin mutants on different media and conditions to observe changes in phenotype. Selective media can force yeast to use specific pathways to survive and grow. By growing different dynactin mutants on selective media, we can observe how the absence of various dynactin subunits affects cellular processes. The relationship between cold sensitivity and dynactin function should also be studied. Temperature can affect cell transportation by affecting the speed of the molecules in the system. Further research can determine if there is a lack of growth at cold temperature due to a physical change in dynactin or slowing of the molecules in the cell overall.

Explorative research will need to be conducted to further understand the mechanisms of cold adaptation. Other cellular processes should be studied to see how they function in cold temperatures. Knockout mutants of genes involved in a particular process can be grown in both cold and permissive temperatures for comparative studies.

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Appendix

Table 1

The table shows how each knockout strain of all the genes tested grew at cold temperatures on YPEG and YPD. The strain was considered cold sensitive (CS) if there was a significant lack of growth on the plate at cold temperatures in comparison to optimal temperatures.
The type of yeast knockout strains tested included haploid, heterozygous diploid, and homozygous diploid (duplicates of the homozygous diploid strain were placed in the HomoDiploid2 column). If certain strains of a gene were not tested, the column was marked not applicable (NA).

ORF	Gene	HetDiploid	HomoDiploid	HomoDiploid2	Haploid
YAL009W	SPO7	NA	not CS on either	NA	not CS on either
YAL011W	SWC3	NA	not CS on either	NA	not CS on either
YAL021C	CCR4	NA	not CS on either	NA	not CS on either
YAL026C	DRS2	NA	CS on both	NA	CS on both
YBL004W	UTP20	not CS on either	NA	NA	NA
YGL085W	YGL085W	not CS on either	NA	NA	NA
YBL020W	RFT1	not CS on either	NA	NA	NA
YBL023C	MCM2	not CS on either	NA	NA	NA
YBL067C	UBP13	NA	not CS on either	NA	not CS on either
YBL071C	YBL071C	NA	not CS on either	NA	not CS on either
YBL099W	ATP1	NA	CS on both	CS on both	CS on both
YBL100C	YBL100C	NA	CS on both	CS on both	CS on both
YBR068C	BAP2	NA	not CS on either	NA	not CS on either
YBR077C	SLM4	NA	not CS on either	NA	not CS on either
YBR081C	SPT7	not CS on either	not CS on either	NA	not CS on either
YBR082C	UBC4	not CS on either	NA	NA	NA
YBR106W	PHO88	NA	not CS on either	NA	not CS on either
YBR109C	CMD1	not CS on either	NA	NA	NA
YBR110W	ALG1	not CS on either	NA	NA	NA
YBR123C	TFC1	not CS on either	NA	NA	NA
YBR143C	SUP45	not CS on either	NA	NA	NA
YBR152W	SPP381	not CS on either	NA	NA	NA
YBR153W	RIB7	not CS on either	NA	NA	NA
YBR197C	YBR197C	NA	not CS on either	NA	not CS on either
YBR231C	SWC5	NA	not CS on either	NA	not CS on either
YBR255W	MTC4	NA	CS on both	NA	CS on both
YBR296C	PHO89	NA	not CS on either	NA	not CS on either
YCL016C	DCC1	NA	not CS on either	NA	not CS on either
YCL029C	BIK1	NA	not CS on either	NA	not CS on either
YCL041C	YCL041C	not CS on either	NA	NA	NA
YCR012W	PGK1	not CS on either	NA	NA	NA
YCR012W	PGK1	not CS on either	NA	NA	NA

YCR053W	THR4	NA	CS on both	NA	CS on both
YCR054C	CTR86	not CS on either	NA	NA	NA
YCR057C	PWP2	not CS on either	NA	NA	NA
YCR072C	RSA4	not CS on either	NA	NA	NA
YDL001W	RMD1	NA	CS on both	NA	not CS on either
YDL006W	PTC1	NA	not CS on either	NA	not CS on either
YDL015C	TSC13	not CS on either	NA	NA	NA
YDL016C	YDL016C	not CS on either	NA	NA	NA
YDL017W	CDC7	not CS on either	NA	NA	NA
YDL020C	RPN4	NA	not CS on either	NA	not CS on either
YDL028C	MPS1	not CS on either	NA	NA	NA
YDL043C	PRP11	not CS on either	NA	NA	NA
YDL084W	SUB2	not CS on either	NA	NA	NA
YDL087C	LUC7	not CS on either	NA	NA	NA
YDL092W	SRP14	not CS on either	NA	NA	NA
YDL100C	GET3	NA	not CS on either	NA	not CS on either
YDL143W	CCT4	not CS on either	NA	NA	NA
YDL172C	YDL172C	NA	not CS on either	NA	not CS on either
YDL173W	YDL173W	NA	not CS on either	NA	not CS on either
YDL190C	UFD2	NA	not CS on either	NA	not CS on either
YDL192W	ARF1	NA	not CS on either	NA	CS on both
YDR008C	YDR008C	NA	not CS on either	NA	not CS on either
YDR053W	YDR053W	not CS on either	NA	NA	NA
YDR054C	CDC34	not CS on either	NA	NA	NA
YDR064W	RPS13	not CS on either	NA	NA	NA
YDR083W	RRP8	NA	not CS on either	NA	not CS on either
YDR086C	SSS1	not CS on either	NA	NA	NA
YDR149C	YDR149C	NA	CS on both	NA	CS on both
YDR158W	HOM2	NA	not CS on either	NA	not CS on either
YDR159W	SAC3	NA	CS on YPEG	NA	CS on YPEG
YDR160W	SSY1	not CS on either	NA	NA	NA
YDR164C	SEC1	not CS on either	NA	NA	NA
YDR173C	ARG82	NA	not CS on either	NA	not CS on either
YDR177W	UBC1	not CS on either	NA	NA	NA
YDR187C	YDR187C	not CS on either	NA	NA	NA
YDR188W	CCT6	not CS on either	NA	NA	NA
YDR190C	RVB1	not CS on either	NA	NA	NA
YDR200C	VPS64	NA	not CS on either	NA	not CS on either
YDR212W	TCP1	not CS on either	NA	NA	NA
YDR226W	ADK1	NA	CS on both	NA	CS on both
YDR245W	MNN10	not CS on either	NA	NA	NA
YDR289C	RTT103	NA	CS on both	NA	CS on both
YDR323C	PEP7	NA	not CS on either	NA	not CS on either

YDR328C	SKP1	not CS on either	NA	NA	NA
YDR334W	SWR1	NA	not CS on either	NA	CS on YPEG
YDR355C	YDR355C	not CS on either	NA	NA	NA
YDR363W	ESC2	NA	not CS on either	NA	not CS on either
YDR389W	SAC7	NA	not CS on either	NA	not CS on either
YDR398W	UTP5	not CS on either	NA	NA	NA
YDR424C	DYN2	NA	not CS on either	NA	not CS on either
YDR431W	YDR431W	NA	not CS on either	NA	not CS on either
YDR472W	TRS31	not CS on either	NA	NA	NA
YDR473C	PRP3	not CS on either	NA	NA	NA
YDR478W	SNM1	not CS on either	NA	NA	NA
YDR485C	VPS72	NA	not CS on either	NA	not CS on either
YDR488C	PAC11	NA	not CS on either	NA	not CS on either
YDR527W	RBA50	not CS on either	NA	NA	NA
YEL027W	CUP5	NA	CS on both	NA	CS on both
YEL032W	MCM3	not CS on either	NA	NA	NA
YEL044W	IES6	NA	CS on YPEG	NA	CS on YPEG
YER008C	SEC3	not CS on either	NA	NA	NA
YER014CNAA	#N/A	NA	CS on both	not CS on Either	not CS on either
YER019W	ISC1	NA	not CS on either	NA	not CS on either
YER052C	HOM3	NA	CS on both	NA	CS on both
YER068W	MOT2	NA	not CS on either	CS on both	CS on both
YER084W	YER084W	NA	not CS on either	NA	not CS on either
YER111C	SWI4	NA	not CS on either	NA	not CS on either
YFL008W	SMC1	not CS on either	NA	NA	NA
YFL017C	GNA1	not CS on either	NA	NA	NA
YFL018WNAA	#N/A	not CS on either	NA	NA	NA
YFL023W	BUD27	NA	not CS on either	NA	not CS on either
YFR001W	LOC1	NA	not CS on either	NA	not CS on either
YFR001W	LOC1	NA	not CS on either	NA	not CS on either
YFR003C	YPI1	not CS on either	NA	NA	NA
YFR037C	RSC8	not CS on either	NA	NA	NA
YFR048W	RMD8	NA	CS on YPEG	NA	CS on YPEG
YGL012W	ERG4	NA	not CS on either	NA	not CS on either
YGL026C	TRP5	NA	not CS on either	NA	not CS on either
YGL054C	ERV14	NA	not CS on either	NA	not CS on either
YGL095C	VPS45	NA	CS on YPEG	NA	not CS on either
YGL113W	SLD3	not CS on either	NA	NA	NA
YGL120C	PRP43	not CS on either	NA	NA	NA
YGL122C	NAB2	not CS on either	NA	NA	NA
YGL148W	ARO2	NA	not CS on either	NA	CS on YPD
YGL150C	INO80	not CS on either	NA	NA	NA
YGL223C	COG1	NA	not CS on either	NA	CS on both

YGL244W	RTF1	NA	not CS on either	NA	CS on both
YGL247W	BRR6	not CS on either	NA	NA	NA
YGR020C	VMA7	NA	CS on both	NA	CS on both
YGR024C	THG1	not CS on either	NA	NA	NA
YGR056W	RSC1	NA	not CS on either	NA	not CS on either
YGR065C	VHT1	not CS on either	NA	NA	NA
YGR078C	PAC10	NA	not CS on either	NA	not CS on either
YGR083C	GCD2	not CS on either	NA	NA	NA
YGR092W	DBF2	NA	not CS on either	NA	not CS on either
YGR094W	VAS1	not CS on either	NA	NA	NA
YGR095C	RRP46	not CS on either	NA	NA	NA
YGR098C	ESP1	not CS on either	NA	NA	NA
YGR105W	VMA21	NA	CS on both	NA	CS on both
YGR132C	PHB1	NA	not CS on either	NA	not CS on either
YGR159C	NSR1	NA	not CS on either	NA	not CS on either
YGR163W	GTR2	NA	not CS on either	NA	not CS on either
YGR208W	SER2	NA	not CS on either	NA	not CS on either
YGR216C	GPI1	not CS on either	NA	NA	NA
YGR231C	PHB2	NA	not CS on either	NA	not CS on either
YGR240C	PFK1	NA	not CS on either	NA	not CS on either
YGR246C	BRF1	not CS on either	NA	NA	NA
YGR251W	YGR251W	not CS on either	NA	NA	NA
YGR262C	BUD32	NA	CS on both	NA	CS on both
YGR262C	BUD32	NA	CS on both	NA	NA
YGR285C	ZUO1	NA	not CS on either	NA	not CS on either
YGR285C	ZUO1	NA	not CS on either	NA	not CS on either
YHR004C	NEM1	NA	not CS on either	NA	not CS on either
YHR021C	RPS27B	NA	not CS on either	NA	not CS on either
YHR025W	THR1	NA	CS on both	NA	not CS on either
YHR026W	PPA1	NA	CS on YPEG	NA	CS on both
YHR036W	BRL1	not CS on either	NA	NA	NA
YHR042W	NCP1	not CS on either	NA	NA	NA
YHR060W	VMA22	NA	CS on both	NA	CS on both
YHR062C	RPP1	not CS on either	NA	NA	NA
YHR066W	SSF1	NA	not CS on either	NA	not CS on either
YHR068W	DYS1	not CS on either	NA	NA	NA
YHR069C	RRP4	not CS on either	NA	NA	NA
YHR129C	ARP1	NA	CS on YPEG	NA	CS on YPEG
YHR151C	MTC6	NA	CS on both	NA	CS on both
YHR154W	RTT107	NA	not CS on either	NA	not CS on either
YHR170W	NMD3	not CS on either	NA	NA	NA
YHR178W	STB5	NA	not CS on either	NA	not CS on either
YHR191C	CTF8	NA	not CS on either	NA	not CS on either

YHR196W	UTP9	not CS on either	NA	NA	NA
YHR200W	RPN10	NA	not CS on either	NA	not CS on either
YIL004C	BET1	not CS on either	NA	NA	NA
YIL046W	MET30	not CS on either	NA	NA	NA
YIL048W	NEO1	not CS on either	NA	NA	NA
YIL142W	CCT2	not CS on either	NA	NA	NA
YJL008C	CCT8	not CS on either	NA	NA	NA
YJL009W	YJL009W	not CS on either	NA	NA	NA
YJL014W	CCT3	not CS on either	NA	NA	NA
YJL054W	TIM54	not CS on either	NA	NA	NA
YJL057C	IKS1	NA	not CS on either	NA	not CS on either
YJL074C	SMC3	not CS on either	NA	NA	NA
YJL075C	APQ13	NA	CS on both	NA	not CS on either
YJL091C	GWT1	not CS on either	NA	NA	NA
YJL095W	BCK1	NA	not CS on either	NA	not CS on either
YJL111W	CCT7	not CS on either	NA	NA	NA
YJL115W	ASF1	NA	not CS on either	NA	not CS on either
YJL121C	RPE1	NA	not CS on either	NA	not CS on either
YJL124C	LSM1	NA	not CS on either	NA	CS on both
YJL180C	ATP12	NA	CS on both	NA	CS on both
YJL180C	ATP12	NA	CS on both	NA	NA
YJL204C	RCY1	NA	CS on both	NA	CS on both
YJR006W	POL31	not CS on either	NA	NA	NA
YJR059W	PTK2	NA	not CS on either	NA	not CS on either
YJR064W	CCT5	not CS on either	NA	NA	NA
YJR104C	SOD1	NA	not CS on either	NA	not CS on either
YJR105W	ADO1	NA	not CS on either	NA	not CS on either
YKL098W	MTC2	NA	not CS on either	NA	CS on both
YKL118W	YKL118W	NA	CS on YPEG	NA	CS on YPEG
YKL119C	VPH2	NA	CS on YPEG	NA	CS on YPEG
YKL139W	CTK1	not CS on either	CS on both	CS on both	CS on both
YKL180W	RPL17A	not CS on either	NA	NA	NA
YKL190W	CNB1	NA	not CS on either	NA	not CS on either
YKL196C	YKT6	not CS on either	NA	NA	NA
YKL212W	SAC1	NA	CS on both	not CS on Either	CS on both
YKR007W	MEH1	NA	CS on both	not CS on Either	not CS on either
YLL002W	RTT109	NA	not CS on either	NA	not CS on either
YLL043W	FPS1	NA	not CS on either	NA	not CS on either
YLL049W	LDB18	NA	not CS on either	NA	CS on YPEG
YLR061W	RPL22A	NA	not CS on either	CS on YPEG	CS on YPEG
YLR062C	BUD28	NA	CS on YPEG	not CS on Either	CS on YPEG
YLR065C	YLR065C	NA	not CS on either	NA	not CS on either
YLR079W	SIC1	NA	not CS on either	NA	not CS on either

YLR085C	ARP6	NA	not CS on either	NA	not CS on either
YLR087C	CSF1	NA	CS on both	NA	not CS on either
YLR089C	ALT1	NA	CS on both	NA	CS on both
YLR115W	CFT2	not CS on either	NA	NA	NA
YLR148W	PEP3	NA	CS on YPEG	not CS on Either	CS on YPEG
YLR182W	SWI6	NA	not CS on either	NA	not CS on either
YLR190W	MMR1	NA	not CS on either	NA	not CS on either
YLR268W	SEC22	NA	not CS on either	NA	not CS on either
YLR269C	YLR269C	NA	not CS on either	NA	not CS on either
YLR274W	MCM5	not CS on either	NA	NA	NA
YLR276C	DBP9	not CS on either	NA	NA	NA
YLR277C	YSH1	not CS on either	NA	NA	NA
YLR291C	GCD7	not CS on either	NA	NA	NA
YLR293C	GSP1	not CS on either	NA	NA	NA
YLR298C	YHC1	not CS on either	NA	NA	NA
YLR305C	STT4	not CS on either	NA	NA	NA
YLR310C	CDC25	not CS on either	NA	NA	NA
YLR317W	YLR317W	not CS on either	NA	NA	NA
YLR321C	SFH1	not CS on either	NA	NA	NA
YLR323C	CWC24	not CS on either	NA	NA	NA
YLR350W	ORM2	NA	not CS on either	NA	not CS on either
YLR357W	RSC2	NA	not CS on either	NA	not CS on either
YLR372W	SUR4	NA	not CS on either	NA	not CS on either
YLR397C	AFG2	not CS on either	NA	NA	NA
YLR409C	UTP21	not CS on either	NA	NA	NA
YLR436C	ECM30	NA	not CS on either	NA	not CS on either
YLR447C	VMA6	NA	CS on both	NA	CS on both
YML006C	GIS4	NA	not CS on either	NA	not CS on either
YML010WNAA	#N/A	NA	not CS on either	NA	not CS on either
YML013CNAA	#N/A	NA	not CS on either	NA	not CS on either
YML013W	UBX2	NA	not CS on either	NA	not CS on either
YML047C	PRM6	NA	not CS on either	NA	not CS on either
YML093W	UTP14	not CS on either	NA	NA	NA
YML098W	TAF13	not CS on either	NA	NA	NA
YML103C	NUP188	NA	CS on YPEG	NA	CS on YPEG
YML112W	CTK3	NA	CS on both	not CS on Either	CS on both
YML121W	GTR1	NA	not CS on either	NA	not CS on either
YMR005W	TAF4	not CS on either	NA	NA	NA
YMR016C	SOK2	NA	not CS on either	NA	not CS on either
YMR021C	MAC1	NA	CS on YPEG	CS on YPEG	CS on YPEG
YMR022W	UBC7	NA	not CS on either	NA	not CS on either
YMR038C	CCS1	NA	not CS on either	NA	not CS on either
YMR060C	SAM37	NA	not CS on either	NA	not CS on either

YMR073C	IRC21	NA	not CS on either	NA	not CS on either
YMR078C	CTF18	NA	not CS on either	NA	not CS on either
YMR125W	STO1	NA	not CS on either	NA	not CS on either
YMR186W	HSC82	NA	not CS on either	NA	not CS on either
YMR202W	ERG2	NA	CS on YPD	NA	CS on YPD
YMR216C	SKY1	NA	not CS on either	NA	not CS on either
YMR217W	GUA1	not CS on either	NA	NA	NA
YMR224C	MRE11	NA	not CS on either	NA	not CS on either
YMR229C	RRP5	not CS on either	NA	NA	NA
YMR264W	CUE1	NA	not CS on either	NA	not CS on either
YMR290C	HAS1	not CS on either	NA	NA	NA
YMR294W	JNM1	NA	CS on both	NA	CS on both
YMR296C	LCB1	not CS on either	NA	NA	NA
YMR299C	DYN3	NA	not CS on either	NA	not CS on either
YMR304W	UBP15	NA	not CS on either	NA	not CS on either
YMR309C	NIP1	not CS on either	NA	NA	NA
YNL059C	ARP5	NA	not CS on either	NA	not CS on either
YNL080C	EOS1	NA	not CS on either	NA	not CS on either
YNL118C	DCP2	not CS on either	NA	NA	NA
YNL147W	LSM7	NA	not CS on either	NA	not CS on either
YNL178W	RPS3	not CS on either	NA	NA	NA
YNL220W	ADE12	NA	not CS on either	NA	not CS on either
YNL225C	CNM67	NA	CS on both	CS on both	CS on both
YNL229C	URE2	NA	not CS on either	NA	not CS on either
YNL241C	ZWF1	NA	not CS on either	NA	not CS on either
YNL248C	RPA49	NA	CS on both	NA	not CS on either
YNL250W	RAD50	NA	not CS on either	NA	not CS on either
YNL258C	DSL1	not CS on either	NA	NA	NA
YNL307C	MCK1	NA	not CS on either	NA	CS on both
YNR010W	CSE2	NA	not CS on either	NA	not CS on either
YNR050C	LYS9	NA	not CS on either	NA	not CS on either
YOL012C	HTZ1	NA	CS on both	NA	not CS on either
YOL040C	RPS15	not CS on either	NA	NA	NA
YOL041C	NOP12	NA	CS on both	NA	CS on both
YOL097C	WRS1	not CS on either	NA	NA	NA
YOL111C	MDY2	NA	not CS on either	NA	not CS on either
YOL127W	RPL25	not CS on either	NA	NA	NA
YOL146W	PSF3	not CS on either	NA	NA	NA
YOL148C	SPT20	NA	CS on both	NA	CS on both
YOR007C	SGT2	NA	not CS on either	NA	not CS on either
YOR027W	STI1	NA	not CS on either	NA	not CS on either
YOR102W	YOR102W	not CS on either	NA	NA	NA
YOR116C	RPO31	not CS on either	NA	NA	NA

YOR164C	YOR164C	NA	not CS on either	NA	not CS on either
YOR184W	SER1	NA	CS on YPEG	NA	CS on YPEG
YOR225W	YOR225W	NA	not CS on either	NA	not CS on either
YOR272W	YTM1	not CS on either	NA	NA	NA
YOR308C	SNU66	NA	CS on YPEG	NA	CS on YPEG
YOR322C	LDB19	NA	not CS on either	NA	not CS on either
YOR323C	PRO2	NA	not CS on either	NA	not CS on either
YOR349W	CIN1	NA	not CS on either	NA	not CS on either
YPL069C	BTS1	NA	not CS on either	NA	not CS on either
YPL077C	YPL077C	NA	not CS on either	NA	not CS on either
YPL142C	YPL142C	not CS on either	NA	NA	NA
YPL143W	RPL33A	not CS on either	NA	NA	NA
YPL146C	NOP53	not CS on either	NA	NA	NA
YPL174C	NIP100	NA	CS on YPEG	NA	CS on YPEG
YPL178W	CBC2	NA	not CS on either	NA	not CS on either
YPL205C	YPL205C	NA	not CS on either	NA	not CS on either
YPL213W	LEA1	NA	not CS on either	NA	not CS on either
YPR024W	YME1	NA	CS on YPEG	NA	CS on YPEG
YPR082C	DIB1	not CS on either	NA	NA	NA
YPR085C	ASA1	not CS on either	NA	NA	NA
YPR094W	RDS3	not CS on either	NA	NA	NA
YPR187W	RPO26	not CS on either	NA	NA	NA