The Significance of the Nuclear Gene *KU80* in Mitochondrial Genome Stability of *Saccharomyces cerevisiae*

A Senior Honors Thesis

Submitted in Partial Fulfillment of the Requirements for Graduation in the Honors College

By

Brooke Scott

Biology Major

The College at Brockport

May 9, 2019

Thesis Director: Dr. Rey Sia, Assistant Dean School of Arts and Sciences

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Abstract

Mitochondria are essential organelles in eukaryotes. They are often referred to as the powerhouse of the cell because mitochondria manufacture ATP, which is required for the successful completion of many cellular processes. Mitochondria have individual genomes, separate from the nuclear DNA, which encode proteins required for respiration. In humans, mutations in the mitochondrial DNA (mtDNA) result in the loss of mitochondrial function which leads to neuromuscular and neurodegenerative disorders. The focus of this study is to determine the role of the nuclear gene *KU80* in maintaining mtDNA stability in the budding yeast, *Saccharomyces cerevisiae*. The product of the *KU80* gene is the protein, Ku80p. Ku80p, in humans, is encoded by the *XRCC4* gene. Ku80p along with Ku70p forms a heterodimeric protein complex, which binds to DNA double-strand break ends and is required for the non-homologous end joining (NHEJ) pathway of DNA repair. The goal of this research is to determine whether loss of the *KU80* gene plays a role in mitochondrial genome stability. Mitochondrial genome instability can arise via spontaneous point mutations or deletion events. Assays were performed to measure the spontaneous respiration loss rate between wild type and *ku80*-Δ mutant strains. The respiration loss assay showed a 1.90-fold increase ($p=0.001256$) in spontaneous respiration loss compared to the wild type strain. Strains were constructed to determine the role of *KU80* in spontaneous direct-repeat mediated deletion (DRMD) events within the mitochondrial genome as well as the nuclear genome. The rate of DRMD events in the mitochondrial and nuclear genomes showed a 1.94-fold decrease ($p=0.08711$) in spontaneous mutation rates in mitochondrial DNA and a 5.87-fold decrease ($p=0.000786$) in nuclear DNA compared to the wild type. These results suggests that Ku80p plays a role in maintaining the integrity of the mitochondrial genome in budding yeast.

Introduction

Saccharomyces cerevisiae as a Model Organism

In order to study the effects of *KU80,* it is essential that a superior model organism is used for research. *S. cerevisiae,* yeast, are unicellular fungi that are naturally found in terrestrial, aerial, and aquatic environments (Rodrigues 2006). Yeast is also known as a facultative anaerobe, which means it can grow in either the presence or absence of oxygen, so it will alter its metabolic physiology in response to the sugar composition of the media and the availability of oxygen (Rodrigues 2006).

The ubiquitous nature of yeast is largely due to their ability to metabolize a number of carbon sources in a respiratory or fermentative way. Yeast are single-celled organisms, so they grow in easily observed colonies. Usually these colonies are identical phenotypically; however, if the mitochondrial DNA (mtDNA) is mutated, yeast are unable to respire and must undergo fermentation. When this happens, the colonies appear to be smaller and are known as "petites" due to their smaller size. Petite colonies develop through various means. For example the entire mitochondrial genome could be lost or a deletion mutation in the mtDNA could cause these non-respiring colonies to grow (Dujon 1981).

Yeast and humans share similar genomes, with many genes and proteins functioning the same way in both organisms. Due to this, any significant findings in yeast with the *KU80* gene and protein will be comparable to the human versions of the gene and protein.

Mitochondrial Genome

Mitochondria are membrane-bound organelles within most eukaryotic cells that house the sites for cellular respiration. Cellular respiration is the process of breaking down glucose and

producing ATP. In addition to their ability to carry out cellular respiration, they also have their own genome known as mitochondrial DNA (mtDNA).

The size and composition of the mitochondrial genome varies between species. The size of human mtDNA is 16.6 kb long (Taanman 1999), and the size of *S. cerevisiae* mtDNA is 85.8 kb (Foury 1998). Even though the genomic lengths are so different, as shown in Figure 1, both organisms contain a similar number of genes in their mtDNA. Human mtDNA contains 37 genes (Zhang 2017) and yeast mtDNA contains 35 genes (Langkjaer et al. 2003).

Researchers use the large mitochondrial genome of *S. cerevisiae* to their advantage when researching mitochondrial functions and disorders. The abundance of non-coding sequences allows genes to easily be knocked-in or knocked-out (Rea et al. 2010). Genes can be added or removed without interfering with the function or expression of existing mitochondrial genes.

Mitochondrial DNA is referred to as dispensable if an organism can survive without it. As a facultative anaerobe, *S. cerevisiae* can utilize fermentable carbon sources as a means of ATP production even if their mtDNA is massively damaged or lost altogether. This characteristic of *S. cerevisiae* allows researchers to investigate genomic changes in the mitochondria that are normally incompatible with life (McAtee 2016).

In humans, mutations in the mitochondrial DNA (mtDNA) result in the loss of mitochondrial function which leads to neuromuscular and neurodegenerative disorders. It is essential to understand the processes involved in the repair of damaged mtDNA. These processes can be understood using *S. cerevisiae* as a model organism and then applied to human mtDNA.

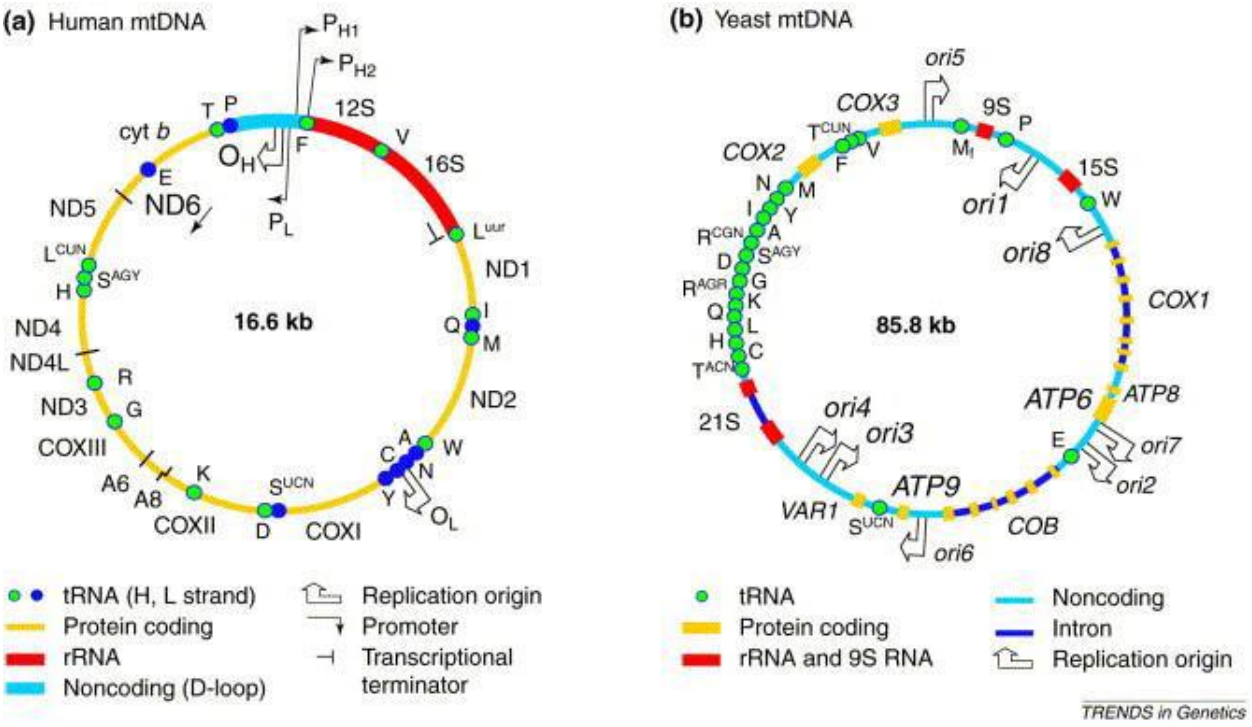

Figure 1. A comparison of the mitochondrial genomes of humans and yeast. The human mtDNA has a size of 16.6 kb, and the yeast mtDNA has a size of 85.8 kb (Jacobs 2001).

Background of *KU80*

In the experiments completed for this thesis, the gene *KU80*, involved in non-homologous end joining (NHEJ), was observed in genetic assays performed on the model organism *Saccharomyces cerevisiae*. This gene expresses a protein that is a subunit of the Ku heterodimer of polypeptides, which works in conjunction with the Ku70p protein expressed from the gene *KU70*. This Ku70-80p heterodimer is capable to bind free ends of DNA. As Figure 2 shows, the two subunits surround DNA and function together. The arrangement of proteins is such that the complex has an open ring-like shape with the DNA threaded through the aperture (Doherty et al. 2001).

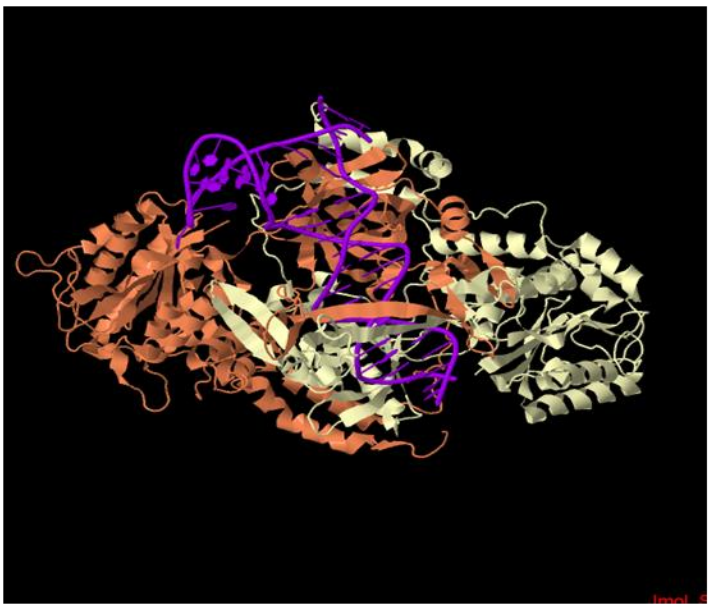

Figure 2. This is a 3D view of the crystal structure of the Ku heterodimer attached to the ends of DNA. The beige structure shows the Ku70p subunit while the orange structure is the Ku80p subunit. DNA is the purple helix centered between the two subunits. This figure was obtained from the Protein Data Bank (Walker et al. 2001).

The Ku complex is appealing for research because it is conserved throughout eukaryotes and can be seen in humans and many other species including yeast, mice, rats, birds and many others. Ku80p is important for normal functioning of the cell because it is involved in genomic stability by repairing double-strand breaks, one of the most lethal forms of DNA damage, by means of non-homologous end joining. Double-strand breaks are dangerous because, if left unrepaired, they cause the cell to undergo programmed cell death, or apoptosis, and cell aging, known as senescence (Davis and Chen 2013).

In eukaryotes, the NHEJ pathway is utilized when the Ku heterodimer forms a complex with DNA-dependent protein kinase, an enzyme required for NHEJ. In yeast, however, these DNA-Pkcs are absent, and the NHEJ depends on the heterodimer and DNA ligase IV (Palmbos et al. 2008). Though the necessary proteins are present in yeast to perform NHEJ, more often than not, yeast will undergo homologous recombination to repair DNA breaks (Doherty et al.

2001). Homologous recombination is favored by yeast because it is a more accurate mechanism to repair DSB and less prone to DNA sequencing errors that frequently occur after NHEJ. Figure 3 shows the repair processes of homologous recombination and non-homologous end joining.

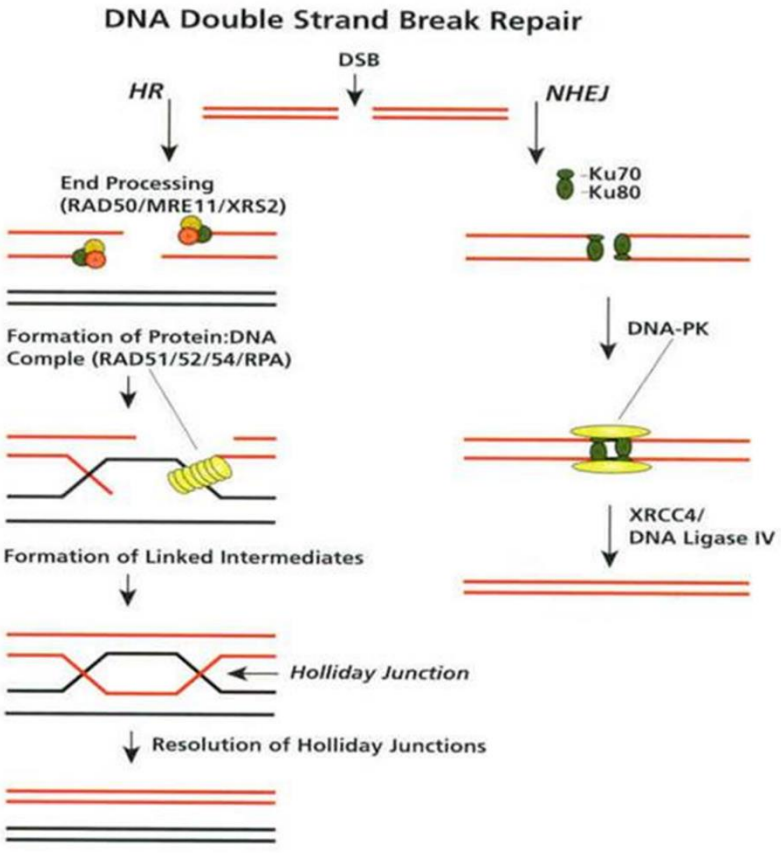

Figure 3. This figure shows the repair processes of homologous recombination and non-homologous end joining. Ku80 and Ku70 are involved in NHEJ (Chapman 2012).

Non-Homologous End Joining

The process of non-homologous end joining (NHEJ) includes the Ku complex, DNA-PKcs, ligase IV, and x-ray cross complementing protein 4 (XRCC4). Ku forms a ring that encircles duplex DNA, cradling two full turns of the DNA molecule. By forming a bridge between the broken DNA ends, Ku acts to structurally support and align the DNA ends, to protect them from degradation, and to prevent promiscuous binding to unbroken DNA. Ku

effectively aligns the DNA, while still allowing access of polymerases, nucleases and ligases to the broken DNA ends to promote end joining (NHEJ, How It Works).

Ku70/80 directly recruits DNA-PKcs to the DNA ends to form the active DNA-PK complex and the interaction between the two requires the presence of DNA. Binding of DNA-PKcs to the DNA-Ku complex results in translocation of the Ku heterodimer inward on the dsDNA strand and ultimately results in activation of the DNA-PKcs kinase activity (Davis and Chen 2013). The XRCC4/DNA ligase IV ligation complex is recruited to join the DNA ends together. DNA ligase IV carries out the ligation step, but it requires the binding of XRCC4 to do so. XRCC4 functions as a regulatory element to stabilize DNA ligase IV, to stimulate ligase activity, and to direct the ligase to the site of DNA breaks via its recognition helix and DNA-binding capacity (NHEJ, How It Works).

Importance of DNA Repair

DNA damage can be induced by several chemically reactive species and physical agents or may occur spontaneously through intrinsic instability of chemical bonds in DNA. Even under normal physiologic conditions, DNA is being damaged continuously. This is why DNA repair mechanisms are necessary for the proper functioning of cells. A number of disorders or syndromes are linked to an inherited or acquired defect in one of the DNA-repair pathways, and some DNA repair–linked disorders show a mixed phenotype, including neurologic symptoms, cancer predisposition, and accelerated aging (Altieri et al. 2008).

There is evidence for base excision repair (BER), direct damage reversal, mismatch repair, and recombinational repair mechanisms in mitochondria, while nucleotide excision repair

(NER), as we know it from nuclear repair, is not present (Croteau 1999). There is no definitive evidence for NHEJ working in the mitochondria.

Research Focus

The focus of this study was to determine the functions of Ku80p in the yeast mitochondria. By performing a knockout of this gene and creating a *ku80*-Δ strain, the effects of this were observed via genetic assays of respiration loss and direct-repeat mediated deletion (DRMD). The respiration loss assay determines the rate of spontaneous mutations that impact mitochondrial function. The DRMD assay determines if a particular gene functions in stabilizing the mitochondrial genome from mutations caused by recombination events. The results from these experiments were analyzed.

Materials and Methods

Yeast Strains and Growth Media

The yeast strains used in this study are shown in Table 1. Growth media included YPG, YPD, YPG + 0.2% dextrose, SD-Trp, and SD-Ura-Arg. YPG, or yeast peptone glycerol, included the following ingredients: 10 grams of yeast extract, 20 grams of bacto peptone, 25 grams of agar, 900 milliliters of deionized (DI) water, and 100 milliliters of 20% glycerol solution. YPD, or yeast peptone dextrose, included the following ingredients: 10 grams of yeast extract, 20 grams of bacto peptone, 25 grams of agar, 1000 milliliters of DI water, and 20 grams of dextrose. YPG + 0.2% dextrose plates were made with the same ingredients as the YPG plates, except 2 grams of dextrose was also added. Synthetic dextrose (SD) was another medium that was used. SD minus tryptophan (SD-Trp) contained 0.74 grams of CSM-Trp, 1.7 grams of yeast nitrogen base, 5 grams of ammonium sulfate, 20 grams of dextrose, 25 grams of agar, and

1000 milliliters of DI water. SD minus uracil and arginine (SD-Ura-Arg) contained 0.74 grams of CSM-Ura-Arg, 1.7 grams of yeast nitrogen base, 5 grams of ammonium sulfate, 20 grams of dextrose, 25 grams of agar, and 1000 milliliters of DI water.

Table 1.

Strain	Genotype
DFS188	*MATa ura3-52 leu2-3, 112 ly2 his3 arg8::hisG*
NRY169	DFS188 *ku80*Δ*::*kanMX
LKY196	DFS188 Rep96::*ARG8m::cox2* Rep96::*URA3::trp1*
RCY382	LKY196 *ku80*Δ*::*kanMX

Respiration Loss Assay

Assays were performed with wild type (DFS188) and *ku80*-Δ (NRY169) yeast strains. Each strain was suspended in 250 μL of a 20% glycerol solution and frozen at -72 °C. As illustrated in Figure 4, the strains were patched and grown on a YPG plate in a 30 °C warm room. After two days, the cells were streaked for singles onto a YPD plate. The colonies grew for three days. Then fifteen individual yeast colonies from streaked YPD plates were transferred to fifteen different microcentrifuge tubes such that each microcentrifuge tube contained one yeast colony. Each yeast colony was diluted in sterile dH_2O to a final dilution factor of 10^{-5}. 50 μL of the final dilution solution and 100 μL of sterile dH_2O were plated onto YPG + 0.2% dextrose. Plated cells were incubated for three days at 30 °C.

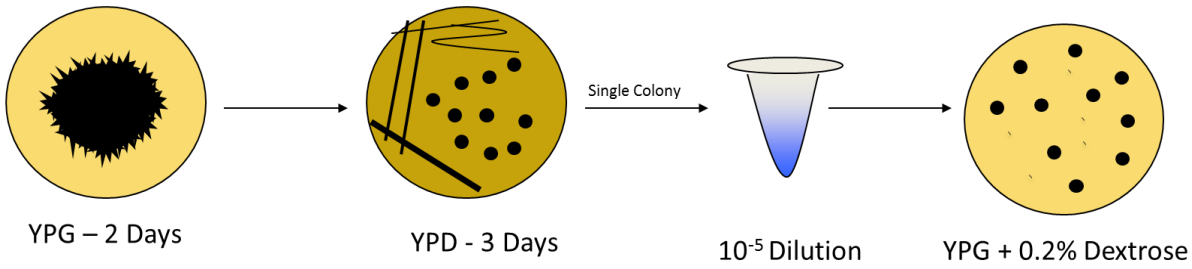

Figure 4. This figure shows the necessary steps to complete a respiration loss assay. First, the strain is patched on YPG and incubated for two days. Next, the patched cells were streaked for singles on YPD and incubated for 3 days. Individual yeast colonies were transferred to microcentrifuge tubes, diluted by a factor of 10^{-5}, plated on YPG + 0.2% dextrose plates and incubated for three days.

Colonies were counted based on phenotype. Non-respiring (rho^-) colonies appeared petite while respiring (rho^+) colonies appeared large, as shown in Figure 5.

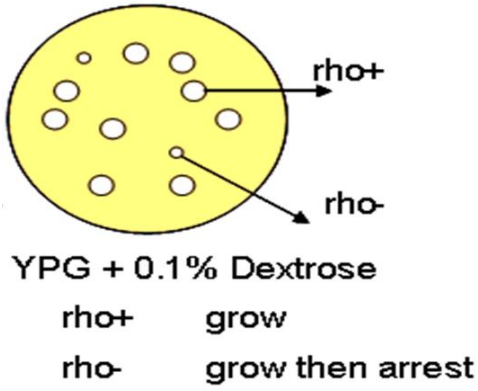

Figure 5. Growth from a respiration loss assay. Rho^- (petite) are non-respiring colonies while rho^+ are respiring colonies. Petite colonies and the total number of colonies were counted.

The percent respiration loss was calculated by dividing the number of petite colonies by the total number of colonies on each plate. The fraction of petite colonies was multiplied by 100 to obtain the percent petite for each plate. Once the percent petite for each plate was determined, the median percent petite was calculated from all of the plates in the assay. At least three assay repetitions were performed for mutant and wild type strains. The average of the median percent

petites was calculated to give the overall frequency of respiration loss for each strain. Standard deviations and p-values were also calculated.

Direct-Repeat Mediated Deletion Assay

Wild type (LKY196) and *ku80*-Δ (RCY382) strains were used to measure the rates of recombination in nuclear and mitochondrial DRMD events. Each strain was suspended in 250 μL of a 20% glycerol solution and frozen at -72 °C. As illustrated in Figure 6, each strain was patched onto an SD-Ura-Arg plate and grown in the warm room at 30 °C for two days and then streaked for singles on a YPD plate. Streaked yeast cells were incubated for three days at 30 °C.

Fifteen individual yeast colonies from the streaked YPD plates were transferred to fifteen microcentrifuge tubes, labeled 1 through 15, so that each microcentrifuge tube contained one yeast colony. Each yeast colony was suspended in 100 μL of sterile dH_2O. Five microliters of yeast cell suspension, from tubes 1 through 15, was vortexed and transferred to fifteen corresponding microcentrifuge tubes containing 500 μL sterile dH_2O (a dilution factor of 100) labeled 1^{-2} through 15^{-2}. Five microliters of cell suspension from microfuge tubes labeled 1^{-2} through 15^{-2} was transferred to to corresponding microfuge tubes labeled 1^{-4} through 15^{-4} containing 500 μL sterile dH_2O (a dilution factor of 20,000).

As shown in Figure 6, 95 μL of yeast cell suspension from tubes 1 through 15 was plated on SD-Trp plates to determine the nuclear recombination rate. 100 μL of yeast cell suspension from tubes 1^{-2} through 15^{-2} was plated on YPG plates to determine the mitochondrial recombination rate. 50 μL of yeast cell suspension from tubes 1^{-4} through 15^{-4} was plated on YPD plates to determine the total cell count.

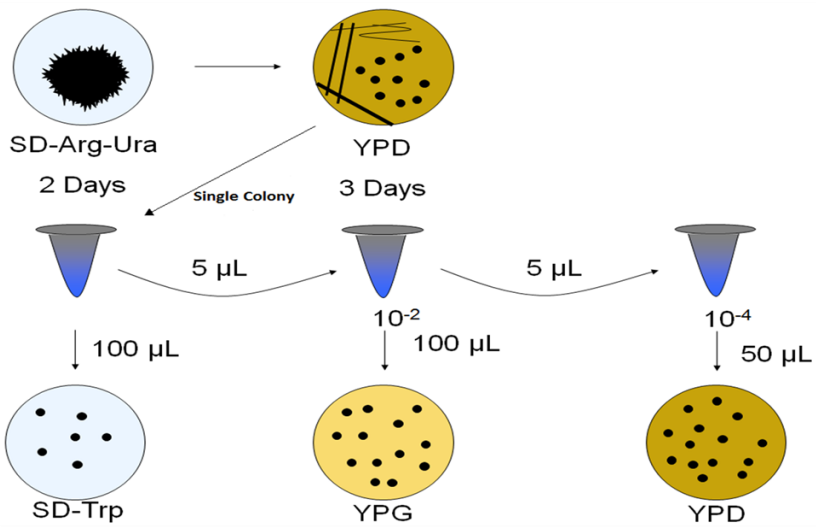

Figure 6. This figure shows the necessary steps to complete a DRMD assay. First the strain is patched on SD-Trp and incubated for two days. Next, the patched cells were streaked for singles on YPD plates and incubated for three days. Individual yeast colonies were picked and transferred to microcentrifuge tubes, diluted, and plated onto SD-Trp, YPG and YPD plates.

After the plates were stored in the warm room for three days, the number of colonies were counted and recorded. All colonies on the SD-Trp and YPD plates were recorded. Only larger yeast colonies were counted on YPG plates. YPG plates contained a significant amount of background that was not counted.

Lea and Coulson Method Calculations

The Lea and Coulson method was used to calculate the nuclear and mitochondrial recombination rates for both the wild type and mutant strains. First the total number of colonies was obtained from the YPD plates by counting the colonies that grew on each plate for a single assay. The average was then determined for all fifteen plates within a single assay trial. The average was then multiplied by two and divided by two to determine the maximum and minimum number of colonies in order to take out any outliers that may have skewed the average. If the number of colonies that grew on a YPD plate did not fall within the range of the maximum

and minimum, the data associated with those plates was disregarded. After this was done, a new average was calculated without the outliers. Then, the median numbers of colonies grown on the SD-Trp and YPG plates were found to determine nuclear and mitochondrial recombination rates respectively. These medians were then used in a series of calculations using the Lea and Coulson table to determine the number of mutations per cell (Lea and Coulson 1949).

Results

Respiration Loss Assay

Respiration loss assays are used as indicators to determine whether the mitochondrion is functioning correctly by determining the rate of spontaneous mutations. Yeast cells that are mitochondria deficient have lost the ability to respire, but can still implement fermentation as a means of energy production. Utilizing the knowledge that glycerol is a non-fermentable carbon source and dextrose is a fermentable carbon source, the rate of spontaneous mutation can be determined. As shown in Figure 7, yeast cells patched on plates containing 20 g/L of dextrose (YPD) allows respiring (rho^+) and non-respiring (rho^-) cells to grow. Yeast cells patched on plates containing 2% glycerol (YPG) as the sole source of carbon ensures that only respiring cells grow. Yeast cells patched on plates containing 2% glycerol and 0.2% dextrose (YPG + 0.2% dextrose) allows respiring and non-respiring cells to grow, but non-respiring cells arrest growth after the limited supply of dextrose is fermented. The colonies who arrest appear petite. Comparing the number of petite colonies to the total number of colonies provides a method to quantitatively determine differences in spontaneous mutation rates between wild type and mutant strains of *S. cerevisiae.*

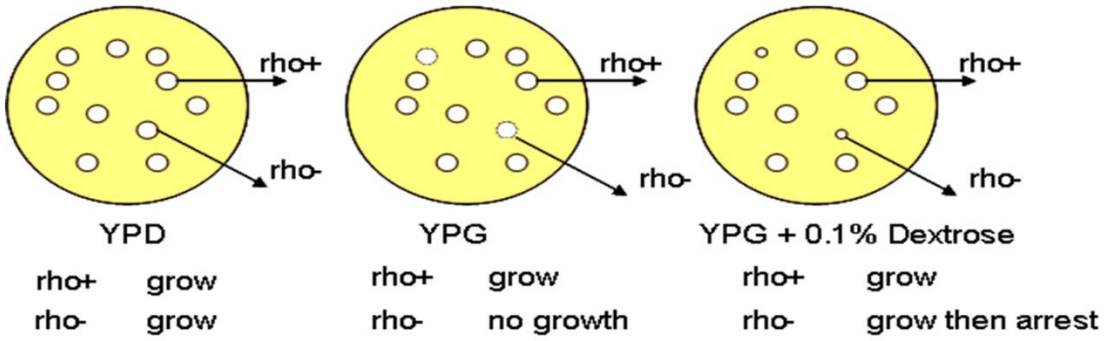

Figure 7. This figure shows respiring (rho^+) and non-respiring (rho^-) colonies ability to grow on YPD, YPG, and YPG + 0.2% dextrose plates. Both respiring and non-respiring colonies grow on YPD plates. Only respiring colonies grow on YPG plates. Respiring and non-respiring colonies grow on YPG + 0.2% dextrose, but non-respiring colonies arrest growth and remain petite in size.

The results of the respiration loss assays are shown in Figure 8. The average frequency of respiration loss in the wild type strain was 2.16%. The loss of *KU80* showed a 1.90-fold increase in the mutant strain frequency to 4.10% compared to the wild type. The average frequency of respiration loss for DFS188 and NRY169 were compared and a T-test analysis produced a p-value of 0.001256. Therefore, a statistically significant difference was found in the average frequency of respiration loss between the wild type and *ku80*-Δ strains of *S. cerevisiae.*

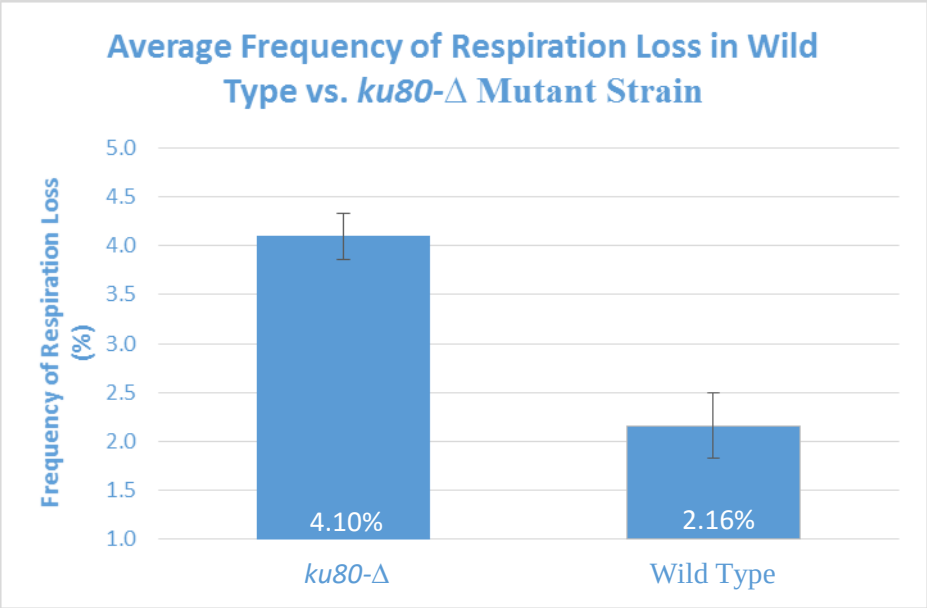

Figure 8. This figure shows the average percent respiration loss for the wild type (DFS188) and *ku80*-Δ (NRY169) strains. The average frequency of respiration loss for DFS188 was 2.16%. The average frequency of respiration loss for NRY169 was 4.10%. There is a statistically significant increase (p=0.001256) in the frequency of respiration loss between the DFS188 and NRY169 strains.

Direct-Repeat Mediated Deletion Assay

In the yeast mitochondrial genome, there are many regions where sequences of DNA repeat themselves. These regions are referred to as direct repeat sequences and are known for high frequencies of recombination events because of the redundancy in their repetitiveness. Within mtDNA, many deletions and point mutations occur, and these deletions are commonly flanked by direct repeat sequences. This suggests that DRMD events occur within the direct repeat sequences. Several mechanisms allow for DRMD events to occur, including DNA polymerase slippage, HR, and NHEJ (Phadnis *et al.* 2005).

To measure the rate at which these events occur in the nucleus and mitochondria, two genetically engineered reporters were used. As shown in Figures 9 and 10, the construct is very similar for each reporter. For the nuclear reporter, the gene *URA3* interrupts *TRP1*. *URA3* is

important because it is the nuclear gene that encodes for a protein required in the uracil biosynthetic pathway, while *TRP1* encodes for a protein required in the tryptophan biosynthetic pathway. The *URA3* gene is inserted between two 96 base pair repeat sequences of *TRP1*. If a recombination event occurs between these repeat sequences, the *URA3* gene will be removed and *TRP1* will regain function. This can be selected for on media that does not contain tryptophan.

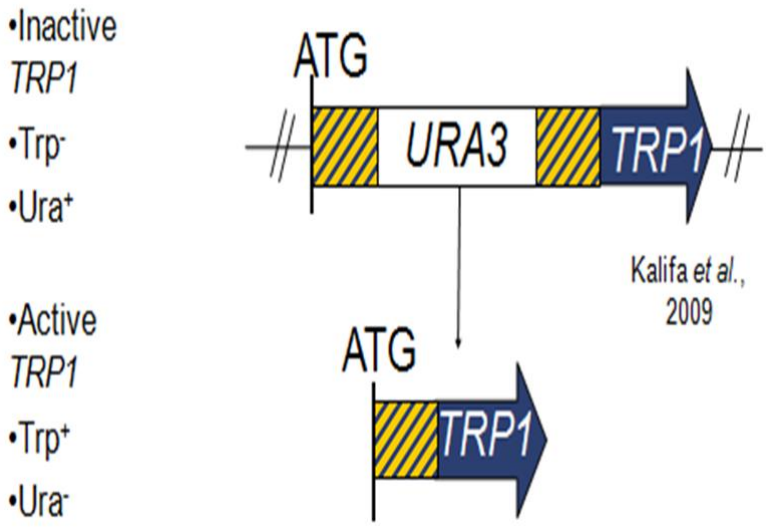

Figure 9. This figure shows the DNA construct created to determine whether a direct-repeat mediated deletion (DRMD) event occurred in the nucleus. The *TRP1* gene is inactive when it is interrupted by the *URA3* gene. When recombination occurs, *URA3* is deleted and the cells' ability to synthesize tryptophan is restored. To determine if this occurred, plating was done on SD-Trp plates.

The average rate of nuclear recombination events was 3.39×10^{-6} for the wild type strain (LKY196) and 5.78×10^{-7} for the *ku80*-Δ (RCY382), which was a 5.87 fold decrease in homologous recombination events. The results from this assay were statistically significant with a p-value of 0.000786.

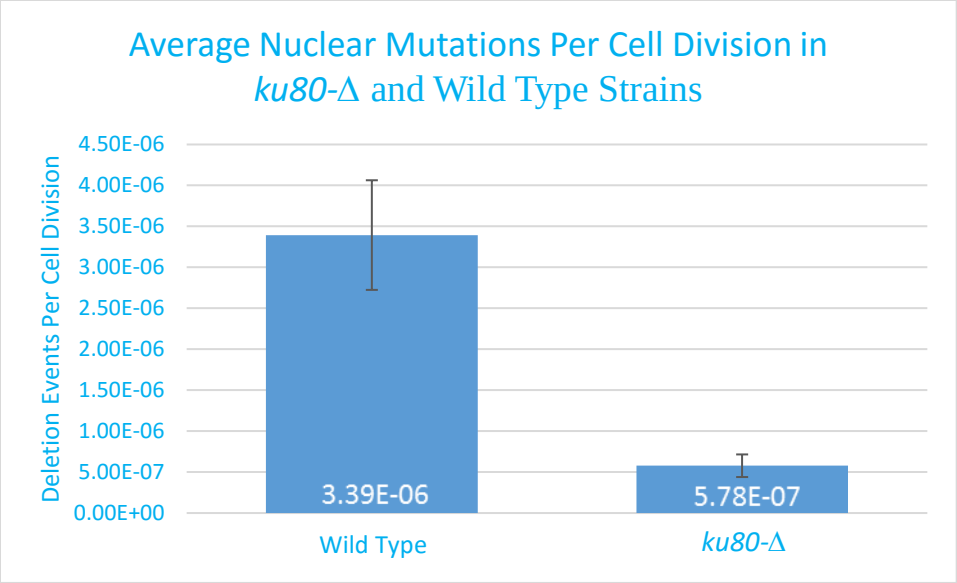

Figure 10. This figure shows the average nuclear mutation rate per cell division in the wild type and *ku80*-Δ strains. The average nuclear DRMD rate for wild type (LKY169) was 3.39 x 10^{-6} deletion events per cell division. The average nuclear DRMD rate for *ku80*-Δ (RCY382) was 5.78 x 10^{-7} deletion events per cell division. The 5.87 fold decrease between LKY169 and RCY382 was determined to be statistically significant (p=0.000786).

The other reporter was used to determine the rate of direct-repeat mediated deletions in the mitochondria. The mitochondrial reporter, shown in Figure 11, consisted of the essential mitochondrial respiratory gene *COX2* interrupted by a gene producing arginine, $ARG8^m$. $ARG8^m$ which is flanked by direct repeats, is prone to direct-repeat mediated deletions. When these deletions occur, the activity of *COX2* is restored and cells regain their ability to respire. The restoration of respiration is measured by plating yeast colonies on media containing an exclusively non-fermentable carbon source such as glycerol.

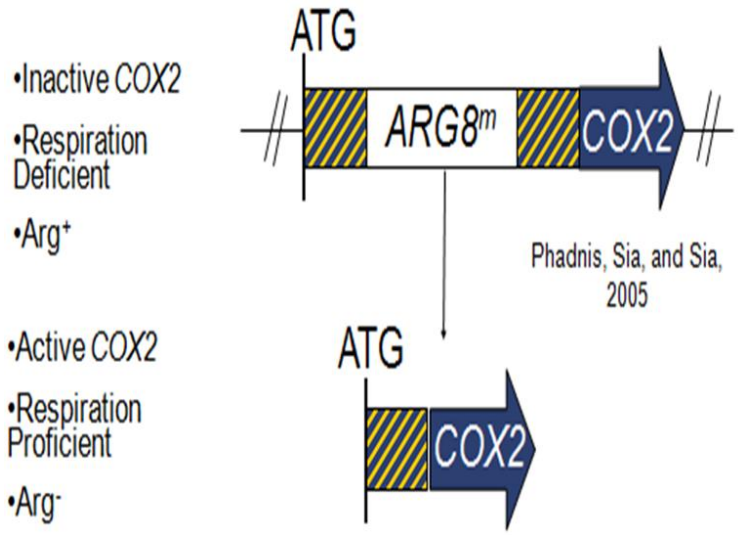

Figure 11. This figure shows the DNA construct created to determine whether a direct-repeat mediated deletion (DRMD) event occurred in the mitochondria. The *COX2* gene is inactive when it is interrupted by the *ARG8*^{m} gene. When recombination occurs, *ARG8*^{m} is deleted and the cell is able to undergo cellular respiration. To determine if this occurred, plating was done on YPG plates.

The average rate of mitochondrial recombination events was 1.61×10^{-4} for the wild type strain (LKY196) and 8.29×10^{-5} for the *ku80*-Δ (RCY382), which was a 1.94-fold decrease in homologous recombination events. The results from this assay were statistically insignificant with a p-value of 0.08711.

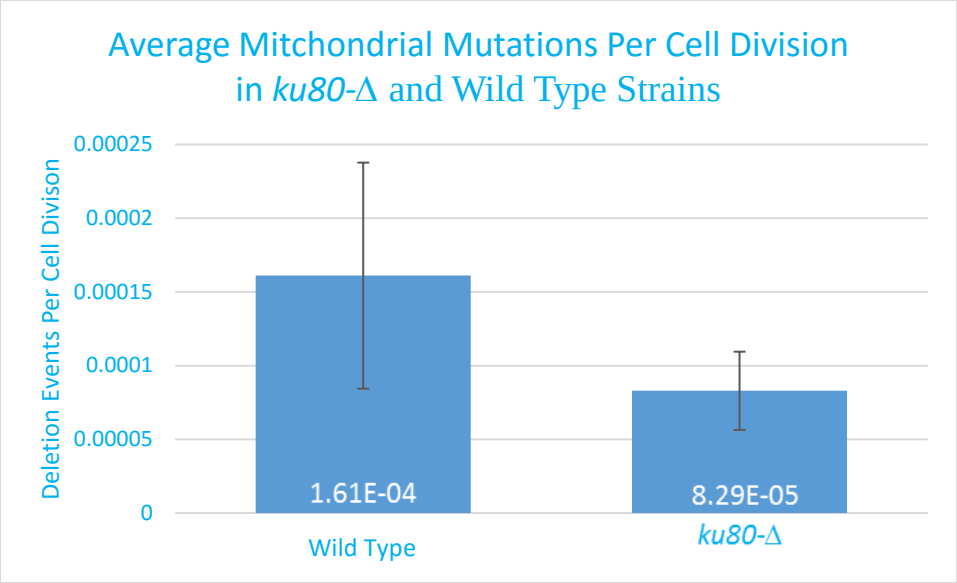

Figure 12. This figure shows the average mitochondrial mutation rate per cell division in the wild type and *ku80*-Δ strains. The average mitochondrial DRMD rate for wild type (LKY196) was 1.61 x 10^{-4} deletion events per cell division. The average mitochondrial DRMD rate for *ku80*-Δ (RCY382) was 8.29 x 10^{-5} deletion events per cell division. The 1.94-fold decrease between LKY196 and RCY382 was determined to be statistically insignificant (p=0.08711).

Discussion

The purpose of this project was to determine the role of the nuclear gene *KU80* on mitochondrial genome stability in the budding yeast, *Saccharomyces cerevisiae.* The study of this project is important because mutations in the mtDNA result in the loss of mitochondrial function which leads to neuromuscular and neurodegenerative disorders. By studying the effects on the mitochondria when the *KU80* gene is knocked out, we can better understand the genome stability of this organelle.

ku80-Δ Strains Show a Significant Difference in Respiration Loss

The purpose of the respiration loss assay was to phenotypically determine the rate of spontaneous mutations in mtDNA. The frequency of spontaneous mutations for the wild type and *ku80*-Δ strains were 2.16% and 4.10% respectively. These frequencies produced a significant p-

value of 0.001256. Because the rate of spontaneous mutations significantly increased with the loss of *KU80,* it was concluded that *KU80* does impact the rate of spontaneous mutations within mtDNA.

ku80-Δ Strains in Nuclear and Mitochondrial DRMD Events

The DRMD assay was used to quantify the rate of homologous recombination events in *S. cerevisiae.* The nuclear rate of deletion events per cell division for wild type and *ku80*-Δ strains were 3.39×10^{-6} and 5.78×10^{-7} respectively. The 5.87-fold decrease between the wild type and *ku80*-Δ strains produced a significant p-value of 0.000786.

The mitochondrial rate of deletion events per cell division for wild type and *ku80*-Δ strains were 1.61×10^{-4} and 8.29×10^{-5} respectively. The 1.94 fold decrease between the wild type and *ku80*-Δ strains produced an insignificant p-value of 0.08711.

The results from this assay showed that both the nuclear and mitochondrial DRMD rates decreased in the *ku80*-Δ strain compared to the wild type strain. This data suggests that *KU80* plays a greater role in the nucleus than the mitochondria. The mitochondrial data could be explained by the presence of the mitochondrial endonuclease Ccel, or by the notion that homologous recombination is not used as a specific repair pathway for double stranded mtDNA breaks (Larsen, Rasmussen & Rasmussen, 2005).

The small and statistically insignificant decrease in the mitochondrial DRMD assay could be the result of a process known as Ku-independent end joining. Studies have shown that yeast are still able to undergo NHEJ when the Ku complex is absent. It has been proposed that when the Ku complex is absent, terminal microhomologies promote the rejoining of DNA strands at a DSB. XRCC4 and DNA ligase are able to repair the damage (Gu *et al.* 2007). For this research,

it is hypothesized that in *ku80*-Δ strains, NHEJ becomes more efficient by utilizing these terminal microhomologies, thus, causing the rates of respiration loss and DRMD events to decrease compared to the wild type. When *KU80* is absent, Ku-independent end joining may be the repair pathway taking over, promoting the stability of mtDNA by repairing DSB in the cell.

Future Work

In conclusion, *KU80* did show statistically significant results in the respiration loss, and nuclear DRMD assays. The standard DRMD assay does not recognize between different methods of DNA repair such as NHEJ or homologous recombination. The future of this research is to perform induced DRMD assays, which is a more specific assay that only measures the rate of homologous recombination, and to perform assays using *KU70* to further investigate the role of the Ku heterodimer on the stability of the mitochondria in yeast.

Acknowledgements

I would like to thank Dr. Rey Sia for allowing me to be a part of his research group for the past two years. His guidance and support has helped to make me a better scientist.

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