The Significance of the MICOS Protein Complex on Maintaining the Function of Cellular Respiration is *Saccharomyces cerevisiae*

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Abstract

The mitochondria are essential organelles to the survival of cells due to their important role in cellular respiration. Mitochondria have their own set of DNA (mtDNA), which encodes proteins needed for the execution of successful oxidative phosphorylation. One of these gene complexes, known as the MICOS complex, contains six genes and is responsible for encoding proteins needed for the maintenance of the inner architecture of the organelle (1). Oxidative phosphorylation is only possible due to the proton gradient that is produced across the inner mitochondrial membrane. The MICOS gene complex encodes proteins that facilitate the building of the inner membrane of the mitochondria, as well as the cristae junctions, which are required for a sufficient rate of cellular respiration. While it has been shown that other mitochondrial genes contribute to the integrity of the mtDNA, there is little research about the contribution of the MICOS complex to the integrity of the mtDNA.

The lab has developed a set of mutant strains that each represent a single gene knockout from the MICOS complex. Specifically, the *mic19Δ* mutant strain will be compared against the wild type strain, *MIC19,* in a respiration loss assay to develop an understanding of the significance of the MICOS complex on cellular respiration. Rich growth media containing dextrose and raffinose as the carbon sources were used to monitor spontaneous respiration loss in both the *MIC19* and *mic19Δ* strains. When plated using dextrose as the sole carbon source after growth on glycerol media, the *mic19Δ* strain demonstrated an increase in cellular respiration loss compared to that of the wild type. When plated using raffinose as the sole carbon source after growth on glycerol media, the *mic19Δ* strain again demonstrated an increase in cellular respiration loss compared to that of the wild type. This shows that Mic19p plays a significant role in maintaining a functional mitochondrion.

Introduction

Saccharomyces cerevisiae as a Model Organism

Budding yeast are a great model organism when performing repetitive assays, such as a respiration loss assay, due to their short growth time and visible colonies. They have been used in many experiments involving mitochondrial mutations and diseases. This organism is a useful comparison to human mtDNA due to the presence of homologs within their mitochondrial genomes; knowledge about the effects of certain mtDNA mutations in yeast can be directly applied to mtDNA mutations and diseases in humans (2).

Yeast are also able to respire both aerobically through oxidative phosphorylation and anaerobically through glycolysis, or fermentation. The presence of certain carbon sources that allow for either fermentation or only aerobic respiration as well as the integrity of the mtDNA determine the growth of the yeast. This is useful in a respiration loss assay as the colonies will still grow on a fermentable carbon source even if they have spontaneous mutations in their mitochondrial DNA. This allows for a quantitative comparison between colonies that have undergone mutations and colonies that have not.

Architecture of the Mitochondrion

The mitochondria is often referred to as the powerhouse of the cell due to its function to provide the cell with energy through cellular respiration. It contains two membranes, the inner and outer membranes, which make up the architecture of the organelle. The inner membrane is the location of cellular respiration and contains folds called cristae junctions, which increase the surface area of the membrane to allow for an adequate rate of cellular respiration (3). Without these cristae junctions, the cell wouldn't have enough surface area in the inner membrane to

create a proton gradient across and wouldn't be able to fit all the protein complexes required for ATP production. Ultimately, the formation of cristae junctions within the organelle is essential to the survival of the cell.

Cellular Respiration

Cellular respiration can either occur through aerobic or anaerobic processes. Anaerobic respiration only involves the breakdown of carbon sources through glycolysis to produce ATP; however, this process produces very little ATP compared to that of aerobic respiration. Aerobic respiration involves glycolysis, the citric acid cycle (which produces electron carriers, ATP, CO_2, and H_2O), and the production of many ATP through oxidative phosphorylation. While glycolysis occurs in the cytosol of the cell, the citric acid cycle occurs within the matrix of the mitochondria and oxidative phosphorylation occurs across the inner membrane of the mitochondria. Therefore, a functional mitochondria is required for successful completion of aerobic respiration, though it is not required for anaerobic respiration (3).

Significance of Different Carbon Sources

Saccharomyces cerevisiae prefer anaerobic respiration through fermentation, which does not require a functioning mitochondria (4). However, plating yeast on certain carbon sources can affect which path the cells take to respire. For example, plating yeast on dextrose allows for fermentation to occur because it is a 6-carbon molecule that can be turned into a 3-carbon molecule through glycolysis. This allows for spontaneous mutations to occur in the mtDNA because the mitochondria is not required for the survival of the cells when they are allowed to respire anaerobically. Plating yeast on glycerol allows only for aerobic respiration through oxidative phosphorylation because glycerol is already a 3-carbon molecule and therefore cannot

be broken down through just glycolysis. Thus, plating the yeast on glycerol ensures that all cells that grow have a functioning mitochondria. When plating the yeast on media with mostly glycerol and 0.2% dextrose, the cells that have undergone spontaneous mutations in their mtDNA will only be able to grow into petit colonies before their growth arrests while cells that have functioning mitochondria will grow to complete size. This creates a size difference between colonies on the plate, which allows for quantitative analysis of respiration loss (5).

Raffinose can also be used as a carbon source for the growth of yeast. Raffinose is similar to dextrose in that it's made up of 6-carbon sugars; it is a trisaccharide made of glucose, galactose, and fructose. Like dextrose, this carbon source allows for fermentation to occur and therefore allows spontaneous mutations to occur in the mtDNA.

Mitochondrial Genome

The mitochondrial genome is different from the nuclear genome in its size, shape, and function. The mitochondrial genome is much smaller than the nuclear genome; it only encodes about thirteen polypeptides. It's also circular rather than linear in shape and functions to produce proteins that are essential to the functionality of the mitochondria. The proteins that mtDNA encodes are all essential to the process of cellular respiration and therefore the survival of the cell (6). Mutations in the mitochondrial genome are often lethal in nature, which is why research about the physiological effects of mutations is so important.

The mitochondrial genome is very similar in both humans and yeast, which can be seen in *Figure 1.* As stated before, this makes yeast a great model organism when researching the effects of mutations within the mitochondrial genome.

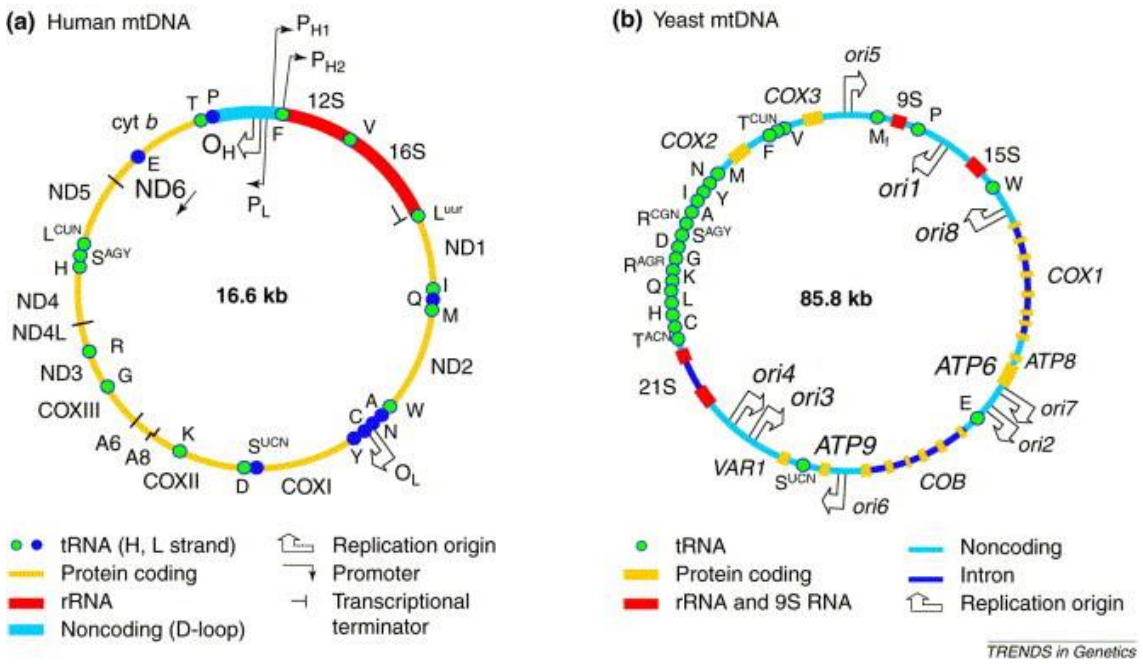

Figure 1. Mitochondrial genome of humans vs yeast. The above image shows the similarities and differences between the mitochondrial genomes of humans and yeast. Both genomes are circular, relatively small in size, and function to encode proteins required for the successful function of the mitochondria (Adapted from Jacobs, 2001).

MICOS

The mitochondrial genome contains a gene complex that encodes a protein complex called MICOS. MICOS stands for the Mitochondrial Inner Cristae Organization System and encodes proteins that contribute to the formation of the inner architecture of the mitochondria. Specifically, the MICOS protein complex is located within the inner mitochondrial membrane and helps to form the cristae junctions, which are essential for the successful function of cellular respiration (7). It is made up of six different proteins which form two main subcomplexes, Mic60p/Mic19p complex and Mic27p/Mic12p/Mic10p complex (8).

Previous research suggests that the MICOS complex has a functional relationship with the phospholipids within the inner mitochondrial membrane. For example, proteins in the

MICOS complex recruit phospholipids and other proteins that induce fusion and curvature of the membrane during formation (9). Specifically, the MICOS complex aids in the redistribution of these phospholipids and proteins to create an asymmetrical distribution on either side of the inner membrane, which causes the membrane to curve (10). The complex interacts with multiple pathways in the cell that work to form the inner architecture of the mitochondria, including the translocase of the outer membrane (TOM), sorting and assembly machinery (SAM), Ugo1, porin (VDAC), and the mitochondrial intermembrane space assembly (MIA) pathway (11). It's also been shows that defects in the MICOS complex can lead to lethal diseases in humans. For example, a condition known as Barth syndrome causes cardiomyopathy and muscle weakness due to a defect in cardiolipin within the inner mitochondrial membrane which results in defective cristae formation. (9).

Van Laar, V. and his lab found that overexpression of the *MIC60* gene led to increased cellular respiration. His lab also showed that the knockout of the Mic60 protein led to increased dopamine-induced cell death within dopaminergic neuronal cells, which contributes to Parkinson's disease neuropathy (12). This shows that mutations within the MICOS complex can lead to serious and lethal human diseases due to their effects on the rate of cellular respiration.

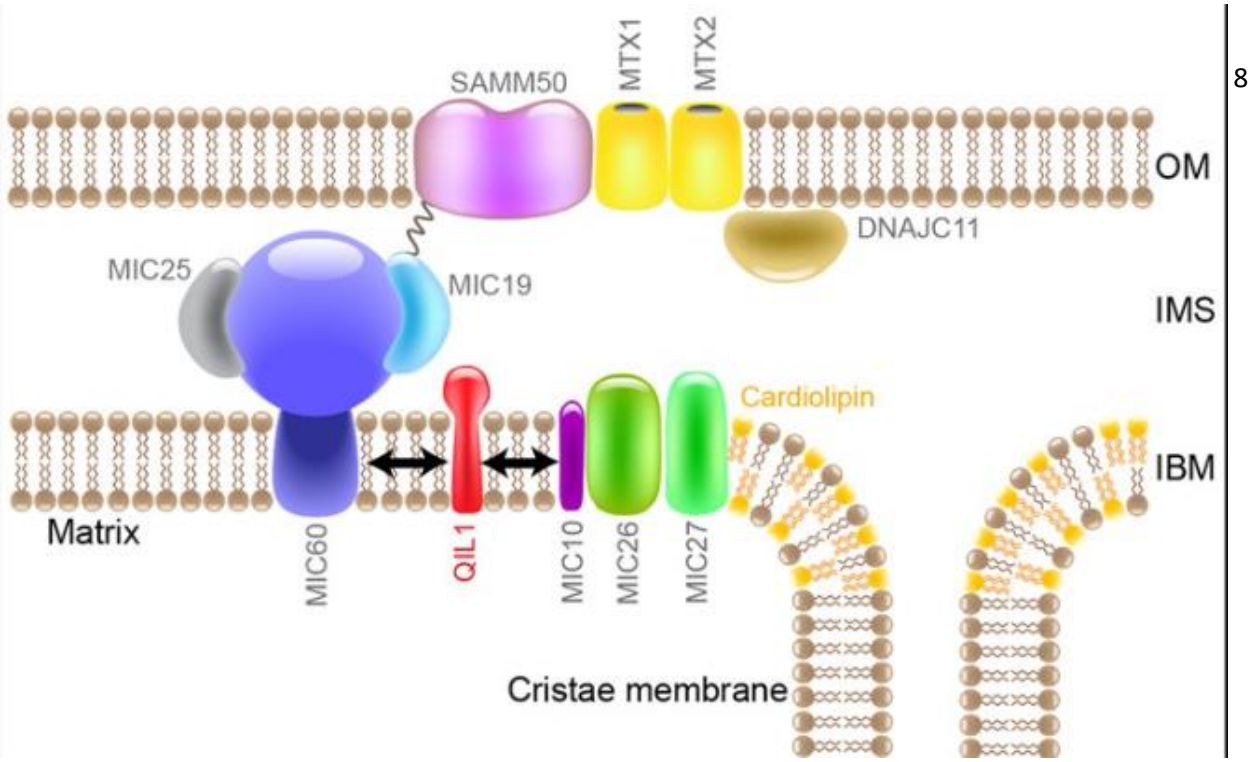

Figure 2. The MICOS complex. The image above shows where the MICOS protein complex is located in the mitochondria (Adapted from Guarani et al, 2015). Most of the complex is embedded in the inner mitochondrial membrane of the cristae junctions. These cristae junctions help to increase the surface area of the inner membrane, which allows more cellular respiration to occur. Without cristae junctions to increase the rate of respiration, the mitochondria wouldn't be able to keep up with the cell's need for ATP (1). The MICOS complex's role in the mitochondria is to help dictate the morphology of the cristae junctions. For this reason, the MICOS complex is significant to the cell's ability to produce enough energy for survival.

Mic19p

This research specifically focused on Mic19p within the MICOS complex and its significance on maintaining the integrity of mtDNA. Mic19p lies peripheral to the inner mitochondrial membrane and functions to control the distribution of Mic60p and connect Mic60p to the outer mitochondrial membrane and the Mic27p/Mic12p/Mic10 subcomplex (8). Its biochemical role is still to be completely determined, but it's thought to have roles in the maintenance of the morphology of the cristae junctions and the assembly of the MICOS complex through its oxidation (11).

It's been found that human Mic19p contains five cysteine residues, four of which form a coiled-coil helix coiled-coil helix (CHCH) substrate domain that is common within the mitochondrial intermembrane space assembly (MIA) pathway. The CHCH substrate domain is used to create a disulfide bond between Mic19p and Mia40p of the MIA pathway. Yeast Mic19p is similar in that it contains two cysteine residues that are thought to form a simplified MIA substrate motif (11). It was further shown that Mic19p levels decrease when Mia40p and other MICOS proteins are downregulated, suggesting that Mic19p is interdependent with Mia40p and the rest of the MICOS protein complex (11).

Mutations and Repair of mtDNA

There are many reasons why mtDNA may spontaneously mutate, including alkylation damage, hydrolytic damage, formation of adducts, mismatched bases, DNA strand breaks, and oxidative damage. These mutations can be repaired through direct reversal, mismatch repair, single-strand break repair, double-strand break repair, homologous recombination, nonhomologous end joining, or base excision repair. If the mutations are not repaired, they can cause diseases that can be fatal. Furthermore, mtDNA mutations can cause a variety of disorders other than mitochondrial diseases, including cardiovascular disease, diabetes, cancer, and neurodegenerative diseases (14).

Previous research has shown that mutations or deletions of other mitochondrial genes have had a negative impact on the integrity of the mtDNA and thus the functionality of the mitochondria. For example, Stein, A. and his lab demonstrated a decrease in mtDNA repair and stability due to the loss of Rad51p, Rad52p, and Rad59p (15). However, the effect of the loss of proteins within the MICOS complex on the stability of mtDNA is still unknown. The goal of this research is to determine the significance of the Mic19p on maintaining the integrity of mtDNA.

This knowledge will contribute to further research in the area of mtDNA mutations and their important pathological effects.

Project Goal

The goal of this research is to determine the significance of the MICOS protein complex on the integrity of the mitochondrial DNA and ultimately its role in maintaining the functionality of the mitochondria. Specifically, this project focused on the importance of Mic19p within the MICOS complex. A respiration loss assay was used to determine the effect of the loss of Mic19p on the rate of spontaneous mutations within the mtDNA by quantifying the frequency of cellular respiration loss. It was predicted that the loss of Mic19p would cause an increase in the rate of spontaneous mutations and therefore an increase in the frequency of respiration loss due to its important role in maintaining the stability of the mitochondrial genome.

Materials and Methods

Media

Yeast culture mediums were made by combining yeast extract, peptone, agar, distilled water, and various carbon sources. YPD media was made using 2% dextrose, YPG media was made using 2% glycerol, YPR media was made using 2% raffinose, and YPG + 0.2% Dextrose plates were made using 2% glycerol and 0.2% dextrose. The broth was thoroughly mixed and autoclaved to ensure sterility. After autoclaving, the media was poured into plates and stored at -4 degrees Celsius.

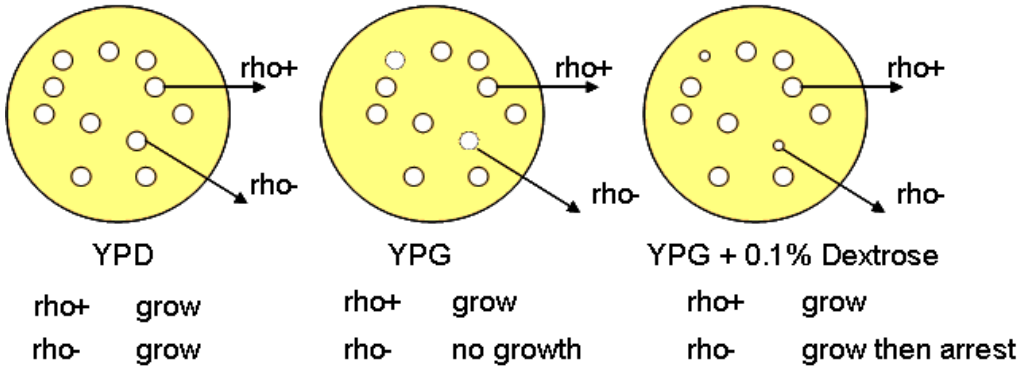

Figure 3. Media used in the respiration loss assay. The image above depicts the different growth media being used in the respiration loss assay. YPD plates contain the fermentable carbon source dextrose, while YPG and YPG + 0.2% dextrose plates contain the non-fermentable carbon source, glycerol. YPG ensures that all yeast that grows on it has a functional mitochondria because it only allows for aerobic respiration through oxidative phosphorylation. YPD allows for anaerobic respiration through glycolysis, which allows for spontaneous mutations to occur. YPG + 0.2% dextrose plates ensure that the non-mutated colonies grow to normal size while the mutated colonies arrest their growth at a petit size once the limited amount of dextrose has been used up.

Respiration Loss Assay

Wild type and mutant yeast strains were obtained from Christopher Prevost at Dr. Elaine Sia's genetic laboratory. All strains were stored at -75 degrees Celsius. The mutant strain *mic19Δ* has the MIC19 gene knocked out. First, the wild type strain *MIC19* was patched onto YPG and stored at 30 degrees Celsius to allow growth for 24 hours. Autoclaved toothpicks were used to transfer and streak *MIC19* onto YPD. The streaked plates were kept at 30 degrees Celsius to allow growth for three days. Sterile toothpicks were used to transfer individual colonies each into their own 1.5ml Eppendorf tube. Each colony was diluted by $2x10^{-5}$ and 100ml was plated onto YPG + 0.2% Dextrose. The YPG + 0.2% plates were stored at 30 degrees Celsius to allow growth for three days. Finally, the big and petit colonies were counted by hand and the frequency of respiration loss was calculated by using the formula: (# petit colonies/total colonies) x 100. This assay was repeated using the *mic19Δ* strain in place of *MIC19*. The assay was again repeated using each *MIC19* and *mic19Δ*, but the yeast was streaked on YPR instead of YPD.

Each strain was patched on YPG and stored at 30 degrees Celsius to allow growth for 24 hours. After being streaked on YPR, the plates were stored at 30 degrees Celsius to allow growth for four days due to slower growth exhibited when using raffinose instead of dextrose. Individual colonies were diluted $3x10^{-5}$ before being plated on YPG + 0.2% Dextrose. They were again stored at 30 degrees Celsius to allow growth for three days before being counted. Each different variation of the assay depending on the strain and carbon source used was repeated at least three times to ensure statistic significance.

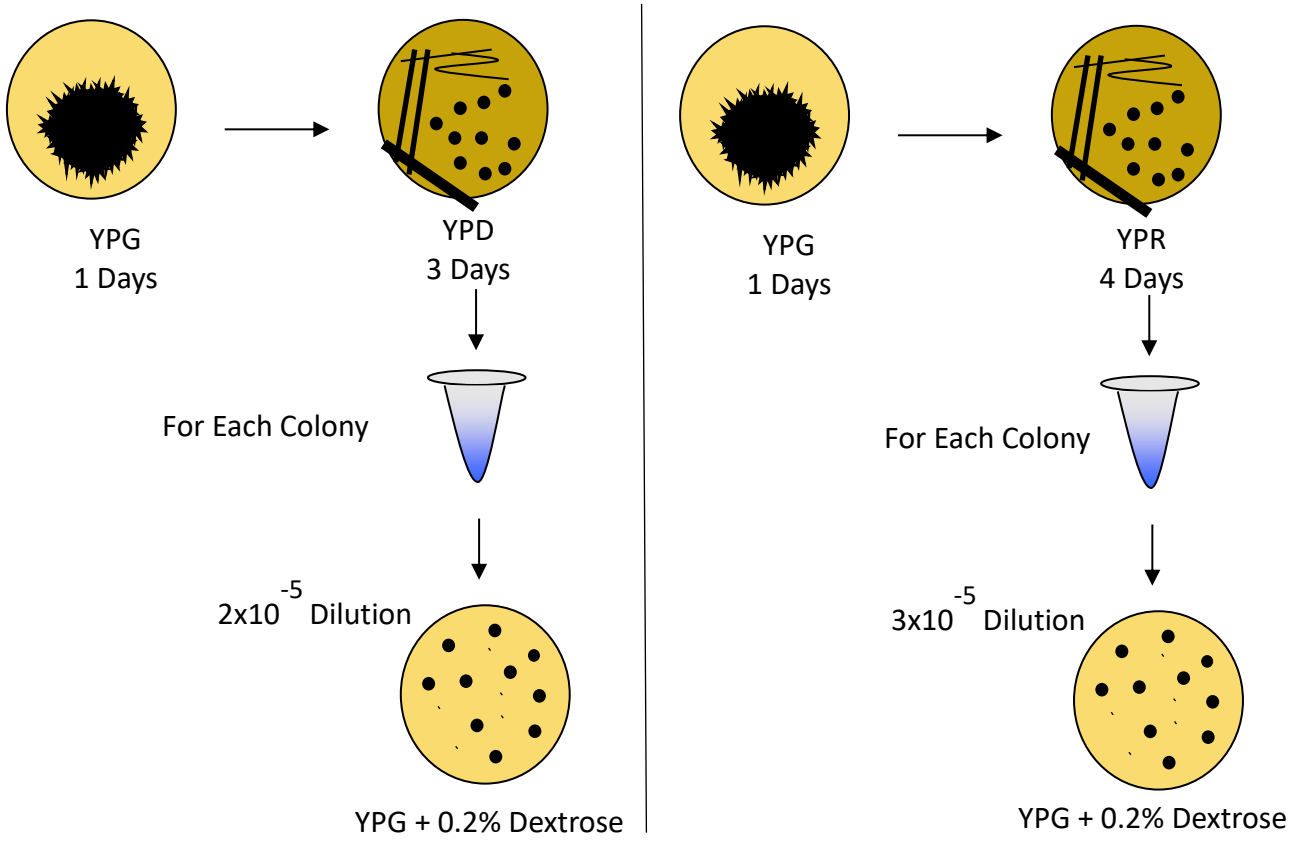

Figure 4. Respiration loss assay. The image above depicts the respiration loss assay. This assay is used to determine the frequency of spontaneous respiration loss is *S. cerevisiae* by quantifying the ratio of petit to big colonies when the strain is plated on YPG + 0.2% dextrose. On the left, the yeast is being streaked on growth media that contains dextrose. On the right, the yeast is being streaked on growth media that contains raffinose. When plated on YPG + 0.2% dextrose, colonies either grow to normal size if they haven't undergone spontaneous mutations or to petit size if they have undergone spontaneous mutations. This allows for a quantitative analysis of respiration loss.

Results

A respiration loss assay was performed to determine the effect of the loss of Mic19p on the integrity of the mitochondrial genome. Mitochondrial DNA encodes proteins that contribute to the functional success of the mitochondria. The mitochondria mainly functions in aerobic cellular respiration, thus an assay designed to allow for a quantitative analysis of cellular

respiration loss was used. When first patched on YPG, the yeast must use oxidative phosphorylation in order to break down the glycerol in the media. This ensures that every cell that grows on the patch contains a functional mitochondria to begin with. Then, the yeast was streaked on YPD. The dextrose in this media is a fermentable carbon source and allows the yeast to use their preferred method of cellular respiration through glycolysis. Since a functional mitochondria is not needed for the breakdown of dextrose through glycolysis, spontaneous mutations in the mitochondrial DNA are able to occur within the individual colonies that grow from the streak. Finally, each individual colony is plated onto YPG + 0.2% dextrose to allow the colonies with a functional mitochondria to grow to full size while colonies that have mutated and do not have a functional mitochondria arrest growth at a petit size once all the fermentable dextrose is used up. The ratio of big to petit colonies was compared and the frequency of respiration loss was determined. As such, a higher rate of respiration loss corresponds to a higher rate of spontaneous mutations within the mitochondrial genome. The assay was performed with both the wild type strain *MIC19* and a single-gene knockout mutant strain *mic19Δ* that represents the loss of Mic19p. The frequency of respiration loss was compared between *MIC19* and *mic19Δ* to determine if Mic19p plays a significant role in maintaining the stability of mtDNA. The assay was performed again with *MIC19* and *mic19Δ* using raffinose as a fermentable carbon source instead of dextrose to determine if different carbon sources affected the rate of spontaneous mutation.

Respiration Loss Assay Using Dextrose as a Carbon Source

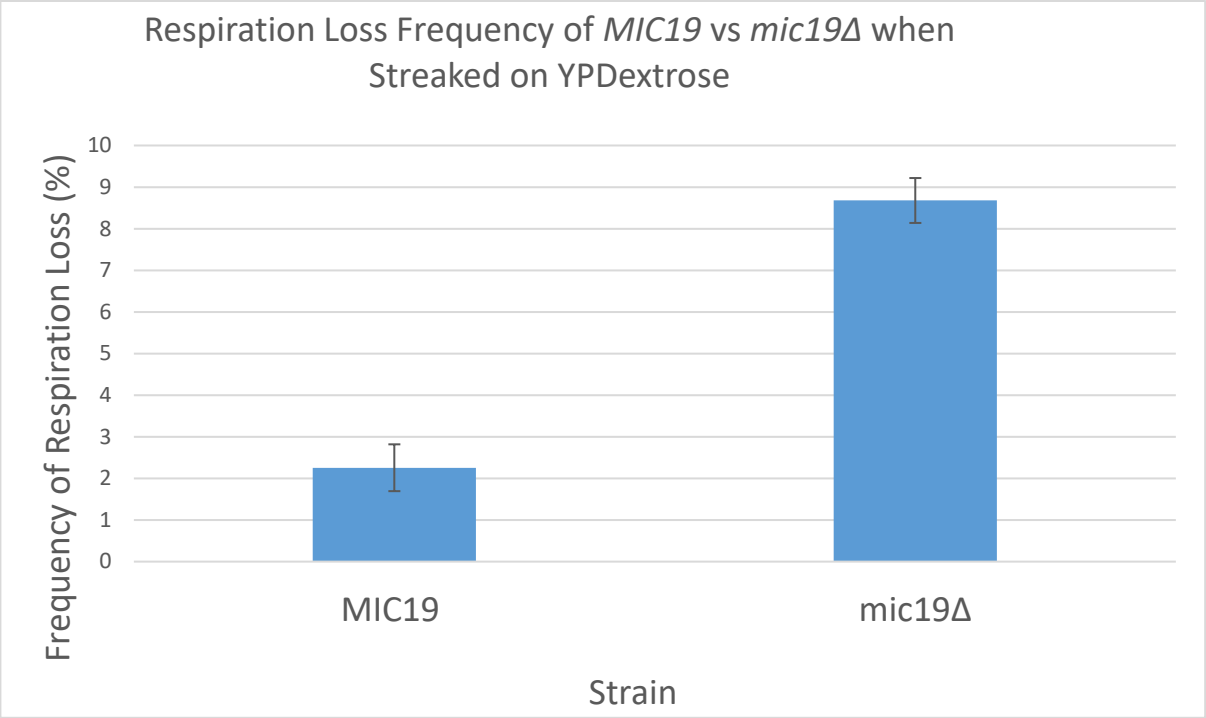

Figure 5. Respiration loss frequency of *MIC19* vs *mic19Δ* when using dextrose as a fermentable carbon source. The graph above depicts a comparison of the average frequency of respiration loss for *MIC19* and *mic19Δ* when using dextrose as a carbon source. The assay was performed at least three times for each strain to ensure statistic significance. Error bars shows the range of results for each assay.

First, dextrose was used as a fermentable carbon source during the streaking phase of the assay. When streaked on dextrose, the yeast was able to respire anaerobically, which allowed for spontaneous mutations of the mtDNA to occur. The assay was performed using dextrose with both *MIC19* and *mic19Δ*. *mic19Δ* demonstrated a 3.85-fold increase to 8.678% in spontaneous respiration loss compared to the 2.256% observed in *MIC19*.

Respiration Loss Assay Using Raffinose as a Carbon Source

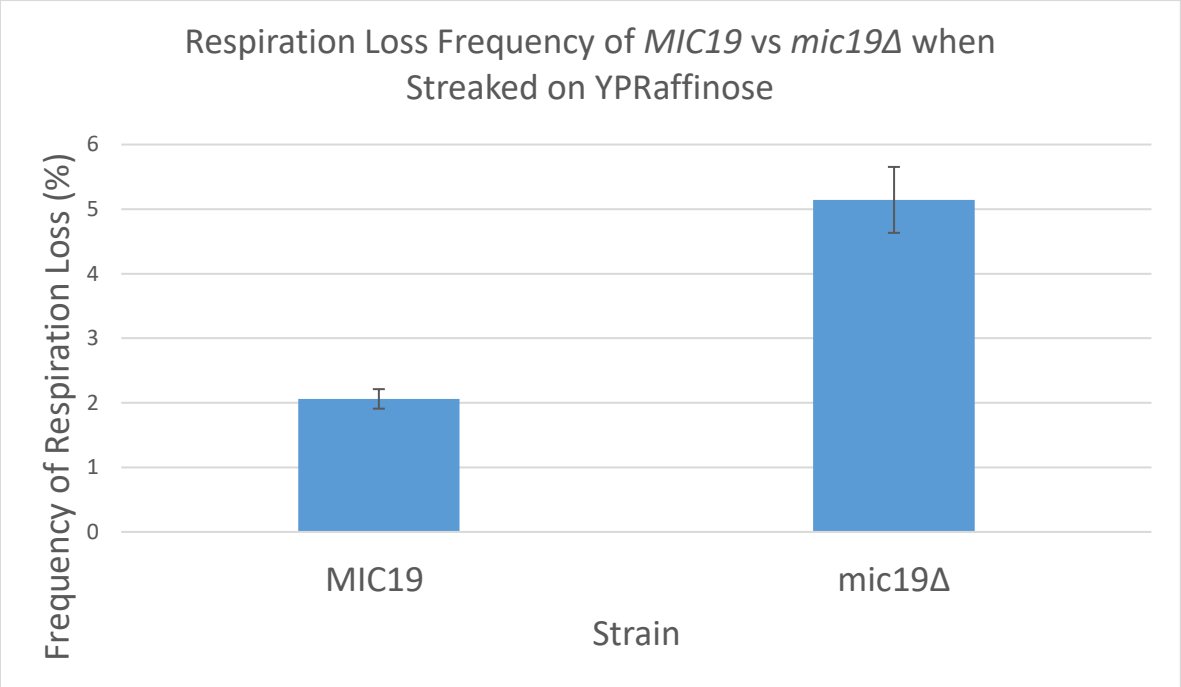

Figure 6. Respiration loss frequency of *MIC19* vs *mic19* when using raffinose as a fermentable carbon source. The graph above depicts a comparison of the average frequency of respiration loss for *MIC19* and *mic19Δ* when using raffinose as a carbon source. The assay was performed at least three times for each strain to ensure statistic significance. Error bars show the range of results for each assay.

The respiration loss assay was then repeated using raffinose as the carbon source during the streaking phase of the assay. The assay was again performed using *MIC19* and *mic19Δ* for comparison. This was done to determine the effect of using different fermentable carbon sources on the frequency of respiration loss. It was observed that yeast took a longer time to grow on a raffinose-rich media than on a dextrose-rich media. Differences in the structures of the carbon source being broken down during respiration can lead to different growth times, which can affect the success of mtDNA repair during growth and reproduction. When using raffinose as a carbon

source, *mic19Δ* demonstrated a 2.50-fold increase to 5.143% in spontaneous respiration loss compared to the 2.060% observed in *MIC19*.

Effect of Different Carbon Sources

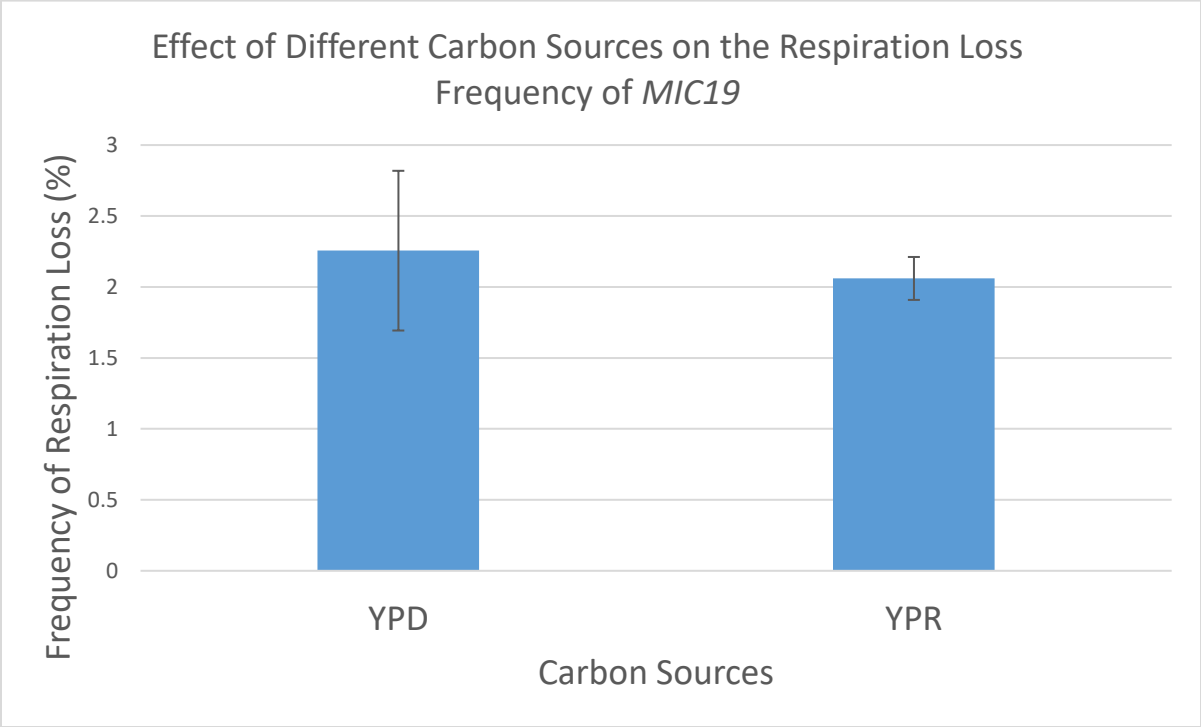

Figure 7. The effect of different carbon sources on the respiration loss frequency of *MIC19*. The graph above depicts a comparison of the average frequency of respiration loss for *MIC19* when using dextrose versus using raffinose as a carbon source. Error bars show the range of results for each assay. *MIC19* showed a decrease in respiration loss when using raffinose instead of dextrose as a fermentable carbon source.

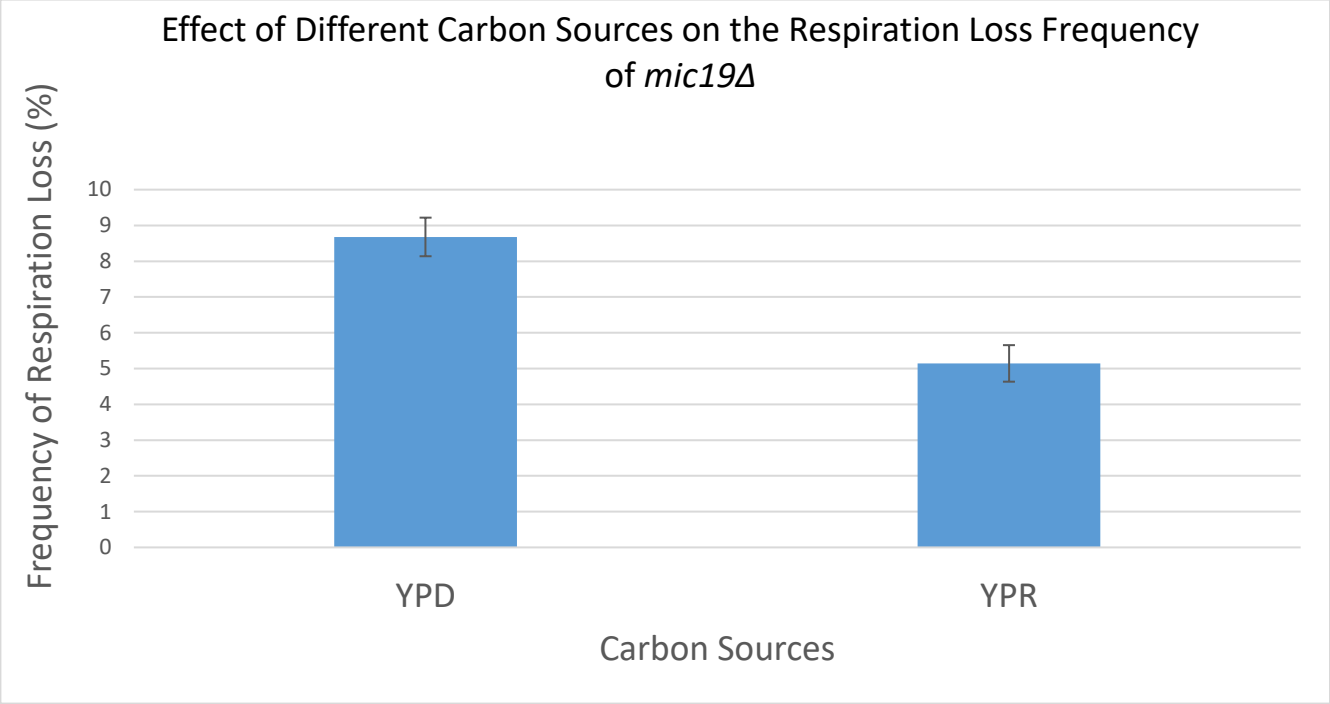

Figure 8. The effect of different carbon sources on the respiration loss frequency of mic19Δ. The graph above depicts a comparison of the average frequency of respiration loss for *mic19Δ* when using dextrose versus using raffinose as a carbon source. *mic19Δ* demonstrated a decrease in respiration loss when using raffinose instead of dextrose as a fermentable carbon source.

Results from using different carbon sources during the streaking phase of the respiration loss assay were compared for both the wild type and mutant strains. This allowed the rate of spontaneous mutations to be compared when the mutations occurred on either dextrose or raffinose. As seen in *Figure 7, MIC19* underwent more spontaneous mutations when grown on dextrose than on raffinose. When using dextrose as a carbon source, *MIC19* showed a 1.10-fold increase to 2.256% in spontaneous respiration loss compared to the 2.060% observed when streaked on raffinose. Again, the same trend was seen with *mic19Δ* as was seen with *MIC19* – *mic19Δ* underwent more spontaneous mutations when grown on dextrose than on raffinose. When using dextrose as a carbon source, *mic19Δ* showed a 1.10-fold increase to 2.256% in spontaneous respiration loss compared to the 2.060% observed when streaked on raffinose.

Summary of Results

Summary of Results from Respiration Loss Assay

Strain	Frequency of respiration loss when using dextrose (%)	Frequency of respiration loss when using raffinose (%)
MIC19	2.256	2.060
mic19Δ	8.678	5.143

Figure 9. Summary of results from the respiration loss assays. The above table depicts all results from the respiration loss assays performed in this study. The assay was performed using both dextrose and raffinose as carbon sources and results from MIC19 and mic19Δ were compared. Each assay was repeated at least three times to ensure statistic significance and the results from each assay were averaged. The results shown above are the averages from each variation of the assay.

When looking at a summary of the results, two main trends hold true. First, *mic19Δ* always demonstrated a higher frequency of respiration loss than *MIC19* no matter what carbon source was used. This shows that the loss of Mic19p correlates with a higher rate of spontaneous mutation within the mtDNA. Second, both *MIC19* and *mic19Δ* showed a decrease in frequency of respiration loss when using raffinose instead of dextrose. This shows that growing on raffinose allows the yeast to perform more DNA repair than when grown on dextrose.

Discussion

The goal of this study was to determine the significance of the MICOS protein complex on the integrity of the mitochondrial DNA. The MICOS complex functions to form cristae junctions within the inner mitochondrial membrane. These cristae junctions help to form a large

surface area across the membrane over which a proton gradient can exist in order for oxidative phosphorylation to occur successfully. This project focused on the importance of the Mic19p within the MICOS complex. A respiration loss assay was used to determine the effect of the loss of Mic19p on the rate of spontaneous mutations within the mtDNA by quantifying the frequency of cellular respiration loss. Furthermore, different carbon sources were used to determine their effect on the rate of spontaneous mutations during growth and reproduction. As predicted, *mic19Δ* demonstrated an overall increase in frequency of respiration loss compared to *MIC19,* showing that Mic19p plays a significant role in maintaining the integrity of mtDNA.

Respiration Loss Assay Using Dextrose as a Carbon Source

As seen in *Figure 5,* results from the respiration loss assay when using dextrose as a carbon source show that the mutant strain *mic19Δ* had a 3.85-fold increase is spontaneous respiration loss compared to the wild type strain *MIC19.* This shows that *mic19Δ* underwent more spontaneous mutation during the respiration loss assay than *MIC19,* demonstrating that the loss of Mic19p causes a decrease in respiratory function of the mitochondria. It's suspected that this is due to Mic19p's significant role in maintaining the stability of the mitochondrial genome, which is responsible for forming a fully functioning mitochondria. Therefore, when Mic19p is removed from the mitochondria, the mtDNA is more susceptible to spontaneous mutations compared to when Mic19p is present within the organelle.

Respiration Loss Assay Using Raffinose as a Carbon Source

Figure 6 shows that *mic19Δ* again demonstrated an increase in spontaneous respiration loss compared to *MIC19.* Specifically, *mic19Δ* demonstrated a 2.50-fold increase in respiration loss compared to that demonstrated for *MIC19*. This trend is consistent with the results from

using dextrose as a carbon source. This shows that the effect of the loss of Mic19p on the organelle's function to respire aerobically is the same when using either dextrose or raffinose as the carbon source. It's important to note that even though this trend is consistent, the fold increase in respiration loss in the mutant strain compared to the wild type strain is slightly less when using raffinose as a carbon source. This shows that the removal of Mic19p has less of an effect on respiration loss when streaked on raffinose to allow for spontaneous mutation.

Effect of Different Carbon Sources

Shown in *Figure 7*, *MIC19* demonstrated a 1.10-fold increase in respiration loss when using dextrose compared to using raffinose as a carbon source. This shows that streaking on dextrose allows for more spontaneous mutations to occur than when streaking on raffinose. This trend is likely due to the difference in growth times of yeast on the different carbon sources. For example, it was observed that the yeast took longer to grow on raffinose than they did on dextrose. A longer growth time on raffinose allows the yeast to have more time to repair its DNA when mutations have occurred, resulting in a lower frequency of respiration loss.

The same trend can be seen in *mic19Δ*. As seen in *Figure 8, mic19Δ* demonstrated a 1.69-fold increase in respiration loss when using dextrose compared to using raffinose as a carbon source. Again, the mutant strain took longer to grow on raffinose than it did on dextrose. This similarity in growth times and consistency in trends of respiration loss support the hypothesis that a decrease in spontaneous mutations when using raffinose is directly linked to growth time.

Future Work

Future work for this research will include repeating the respiration loss assay with different mutant strains. The research done so far has only focused on the effects of the loss of Mic19p within the MICOS complex, though there are five other proteins (Mic10p, Mic25p, Mic26p, Mic27p, and Mic60p) that must be studied. Using mutant strains that represent the loss of multiple MICOS proteins may also be useful in determining the significance of the MICOS complex on the stability of the mitochondrial genome.

The respiration loss assay should also be repeated using different fermentable carbon sources, such as fructose and galactose, to determine the effect of the type of carbon sources on the amount of spontaneous mutations generated. As more carbon sources are studied, it's important to also study the growth times of Saccharomyces cerevisiae on each carbon source to determine if there is a direct link to growth time and frequency of respiration loss. As stated before, trends when comparing the use of dextrose and raffinose suggest that a longer growth time may lead to more repair in mtDNA and thus a lower frequency of respiration loss.

Due to the MICOS complex's known role in forming the inner architecture of the mitochondria, it would also be beneficial to study any changes in the morphology of the organelle due to the loss of different MICOS proteins. This will contribute to determining the relative significance of each MICOS protein on the formation of cristae junctions within the inner mitochondrial membrane. A constructive method for this study may be to capture images of the inner architecture of the mitochondria in different mutant strains and comparing them to the inner morphology of the wild type strain.

Lastly, it would be beneficial to use an oxidative phosphorylation assay to further examine the molecular role of the MICOS complex. Mutant strains would again be compared to the wild type strain to determine the importance of each MICOS protein in the role of oxidative phosphorylation. This will help to further understand the significance of the MICOS complex on the function of cellular respiration and its role in the cell's survival.

Acknowlegements

I would like to thank Dr. Rey Sia for allowing me to be part of his research team. I will forever be grateful for the skills I have learned in the genetics lab during my time at SUNY Brockport. My work in the lab has taught me communication, research, and problem solving skills that I will take with me in my future endeavors.

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