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The Significance of the Nuclear Gene, SGS1, in Mitochondrial Genome Stability in Saccharomyces cerevisiae

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The Significance of the Nuclear Gene, SGS1, in Mitochondrial Genome Stability in *Saccharomyces cerevisiae*

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By

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Abstract

The homologs that humans share with *Saccharomyces cerevisiae* render yeast an ideal model organism to investigate the potential importance of genes in humans. *SGS1* is a nuclear gene for a RecQ helicase in yeast, known to play a role in homologous recombination in nuclear genome repair. The research in question is intended to evaluate if *SGS1* has a similar importance in mitochondrial genome repair. These conclusions can be employed to better understand the disease phenotypes that humans present as a result from mitochondrial malfunction. A respiration loss assay showed that *SGS1* knockout strains have a ~2.2 fold increase in spontaneous respiration loss frequency, indicating that *SGS1* plays a role in mitochondrial genome stability. A direct-repeat mediated deletion assay proves *SGS1* is involved in homologous recombination in mitochondria due to an ~1.6 fold decrease in rate of homologous recombination in *SGS1* knockout strains. With a p value of 0.66, no significant difference was observed in an induced direct-repeat mediated deletion assay between wild type and *sgs1Δ* strains, implying that *SGS1* does not play a fundamental role in double strand break repair. Future experimentation could include additional knockout strains testing other genes known to be involved in nuclear homologous recombination repair and double knockout strains to assess the relative order of active proteins involved in genetic repair mechanisms.

Introduction

Yeast as a Model Organism

Saccharomyces cerevisiae, as with all model organisms, are used in research because the information learned from them can be applied to higher organisms. Studying a gene in yeast, for example, can provide insight into organisms with homologs. In other words, the role of a gene in yeast may have a very similar role in a human because the genes are very similar therefore, yeast that lack this gene may exhibit a relatable mutant phenotype to humans lacking this gene. Genetic similarities such as these allow knowledge to be gained about organisms that are not ethical or available for experimental research. Hence, using organisms that share genetic similarity are often the initial steps in medical advancement.

There are a number of reasons to use yeast as a model organism in research. It requires minimal care and can be frozen indefinitely between experiments without damage. Yeast grows relatively fast and can therefore provide a much greater amount of data in the amount of time a mouse could, for example. For our molecular genetics laboratory in particular, yeast has many more unique characteristics that make it an optimal model organism. There are many repair mechanisms that organisms can exploit when genetic damage presents itself. Yeast tends to prefer repair via homologous recombination which is a characteristic that we can track in experimentation. Yeast is also unique in its preference to ferment as opposed to undergo oxidative phosphorylation as a means to produce energy for metabolism. By manipulating the sugar sources available to the yeast, it is possible to control whether it will produce ATP through fermentation or oxidative phosphorylation. By exploiting this preference for ATP production, experimentation can monitor the function or lack of function from the mitochondria relatively easily.

The Mitochondrion

The organelle that enables eukaryotes to perform oxidative phosphorylation is the mitochondrion. Mitochondria are often nicknamed the “powerhouse” of the cell due to their role in producing ATP. For many organisms, mitochondria are responsible to supply a majority of the energy that fuels all metabolic activity within a cell. *Saccharomyces cerevisiae* is relatively unique for its preference for fermentation over oxidative phosphorylation if the appropriate sugar source is available. Oxidative phosphorylation has the capacity to produce a net of 34 ATP upon completion versus fermentation’s net of 2 ATP after glycolysis. Mitochondria are composed of two membranes and the inner-most one is highly folded forming what are termed cristae. These cristae greatly increase the amount of surface area of the membrane that can fit within the outer membrane. The space between the membranes is appropriately called the intermembrane space and within the inner membrane is the matrix. The inner membrane is an essential participant in the electron transport chain, the final step in

oxidative phosphorylation. The electron transport chain gradually creates an increasingly higher proton gradient between the membranes that is ultimately coupled to ATP synthesis via the protein ATP synthase. The mitochondrion plays a number of additional roles for the cell, for instance, releasing cytochrome C to induce a caspase cascade and apoptosis if necessary.

Mitochondrial Genome

A common misconception is that the nucleus is the only location of genetic information in the cell. Mitochondria also contain a relatively small genome, compared to that of the nucleus, that will be passed maternally in sexual reproducing organisms. This genetic information codes for proteins involved in the ATP production within the mitochondria. Human mtDNA is approximately 16.6kb long consisting of two rRNAs, 22 tRNAs, and 13 proteins (Alexeyev *et al.* 2013). Of these 13 electron transport chain proteins, seven are involved in complex I- NADH dehydrogenase, three are destined for complex IV- cytochrome c oxidase, two are part of complex V, or ATP synthase, and one becomes a subunit of complex III- cytochrome b (Alexeyev *et al.* 2013). Human mtDNA is dense, containing minimal intergenic, noncoding regions and with many genes overlapping (Alexeyev *et al.* 2013).

Although mitochondrial genomes have commonalities that differentiate it from nuclear DNA, mtDNA between organisms can also differ. The mtDNA of *S.cerevisiae* is approximately 85.8kb long, coding for three subunits of cytochrome c oxidase, apocytochrome b, three subunits of ATPase, and pVAR1 (Langkjær *et al.* 2003). In addition to these proteins, the mtDNA encodes the small and large rRNAs and the 9S RNA, a part of RNase P (Langkjær *et al.* 2003). A slightly different universal code for yeast mtDNA makes translation vary, for example, AUA codes for methionine instead of the typical AUG (Langkjær *et al.* 2003). Contrary to human mtDNA, yeast mtDNA has a great amount of intergenic regions, comprising upwards of 62% of the genome (Langkjær *et al.* 2003). Other characteristics of *S.cerevisiae* mtDNA include a low GC content and the useful direct and indirect repeats found primarily within the intergenic

space (Langkjær *et al.* 2003). These repeats are manipulated in assays directed towards measuring homologous recombination, such as the DRMD described later.

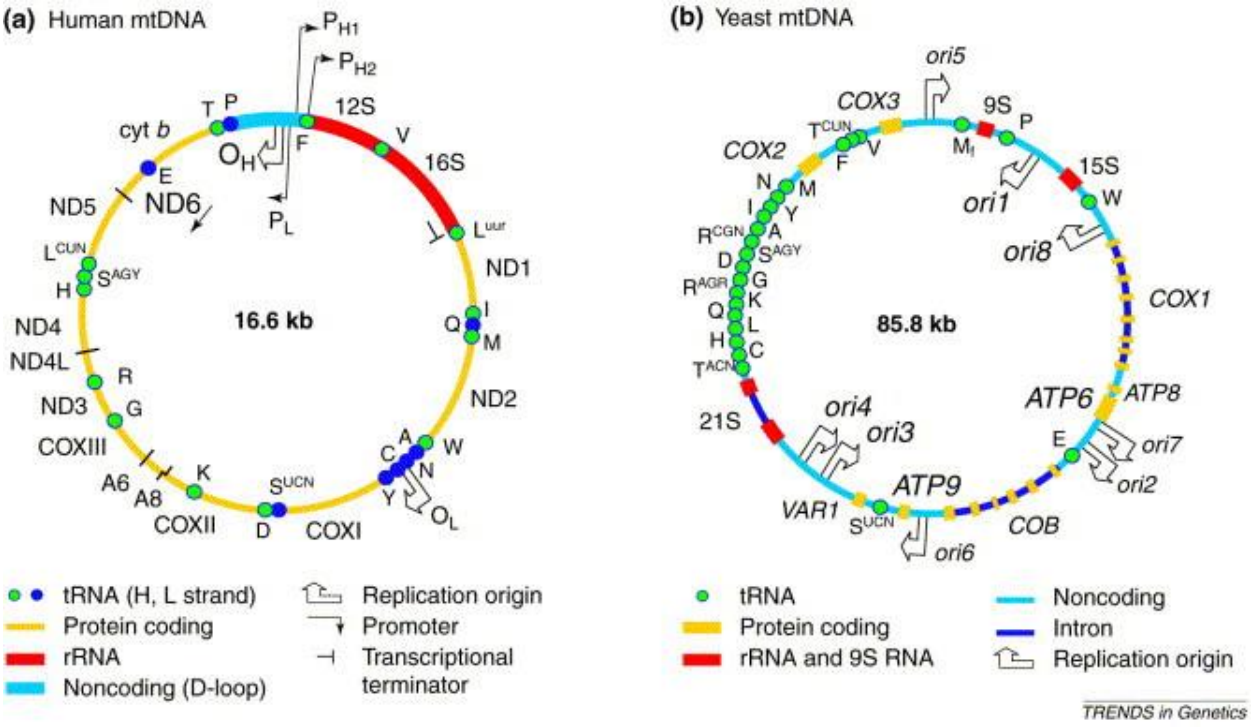

Figure 1. **Comparing Mitochondrial Genomes.** Above are visuals designed to compare human mtDNA and yeast mtDNA. Both are circular and lack histone proteins for compact assembly. The varying amount of noncoding, intergenic material is evident with human mtDNA containing very little relative to yeast. The letters at each tRNA symbol correspond to the amino acid that is involved. As evident in the figure, yeast mtDNA is thought to contain several more origins of replication and each is associated with a promoter. Adapted from Howard Jacobs' *Making Mitochondrial Mutants* (2001).

mtDNA Repair

Much like nuclear DNA, mitochondrial DNA is organized into nucleoproteins, the proteins complexed with the genetic material differ, though. Although it is also condensed and packaged like nuclear DNA, its location has a closer proximity to potential reactive oxidative species, which most likely accounts for its greater susceptibility to damage from endogenous sources. There are a number of repair mechanisms that play a role in maintaining mtDNA integrity, some methods more frequently than others and some more useful in the study of yeast than others. Alkylation, hydrolytic damage, chemically induced adducts, mistakes in replication,

oxidative damage, and DNA breaks all necessitate specific and functional repair mechanisms. Some chemical mutagens are thought to preferentially target mitochondrial DNA upon exposure (Alexeyev *et al.* 2013). Direct reversal is one type of repair that can be utilized in yeast and is not considered an active repair mechanism in higher eukarya (Alexeyev *et al.* 2013). Nucleotide excision repair is a relatively well-known method of repair, but research is suggesting that adducts typically destined for nucleotide excision repair pathway are left unrepaired in mitochondrial DNA unless they can be attended to by an alternative pathway (Alexeyev *et al.* 2013).

Mismatch Repair

Mismatch repair, or MMR, is a known mode of repair frequent in nuclear genomes that targets wrongly paired nucleobases. Mismatch repair resolves inappropriate base pairing that occurs within double-strand DNA. This improper pairing could be the result of DNA polymerase slippage while the strand is undergoing DNA replication or it can be caused by deamination, oxidation, and alkylation, which can occur spontaneously or be induced for experimentation purposes (Alexeyev *et al.* 2013). In the nucleus, the majority of MMR is conducted by MSH and MLH proteins. In the nucleus, it is known that the mismatched bases would be recognized by one protein complex which will induce the recruitment of a second complex, in *S.cerevisiae*, Mlh1-Pms1 (Alexeyev *et al.* 2013). The template strand is preserved while the complementary strand undergoes excision and resynthesis (Alexeyev *et al.* 2013). Although MMR can occur in the mitochondria as well, it occurs independently of most MSH proteins and is thought to rely more on the Y-box binding protein 1 (Alexeyev *et al.* 2013). MLH1 was recently found to be present in the mitochondria, but a minimal amount of other proteins fundamental to MMR in the mitochondria are currently understood (Alexeyev *et al.* 2013). MTH1 serves as an alternative mechanism to try and limit the need for mismatch repair. Since damaged dNTPs , the molecules incorporated into the DNA sequence during DNA synthesis, pose a threat to the integrity of the DNA, MTH1 has the ability to minimize this threat by hydrolyzing triphosphates

into monophosphates that will not potentially be incorporated into a DNA strand being synthesized (Alexeyev *et al.* 2013). This is termed sanitation of the dNTP pool and although it is not ideal or truly a *repair* mechanism, it functions to maintain some integrity of the genome. It can reduce the chances of incorrect MMR prior to mismatches occurring that could further jeopardize the DNA.

Homologous Recombination

Homologous recombination is an exchange between identical or similar sequences of DNA. It is most well-known for its role in the chiasmata during meiosis, but it also serves as a repair mechanism. It is evident in organisms as simple as *E. coli* and yeast and as complex as humans. Homologous recombination is not the only way cells can execute repair, but in organisms such as budding yeast, it is a preferred method. Since cells have additional modes of repair and genetic diversity is not pertinent for the survival of a single organism, it may be mistaken that homologous recombination is an inessential element. On the contrary, homologous recombination is such an essential event that evolution has preserved a number of proteins and complexes that function to ensure the appropriate timing and location of it (Modesti and Kanaar 2001). The need for functional homologous recombination is clear because failure to accomplish this precision diminishes reparation abilities and is associated with greater frequency of cancer and other syndromes in humans (Modesti and Kanaar 2001). Despite having alternative routes to fixing lesions in the DNA, if loss of homologous recombination is linked to predispositions to cancer and other ailments, it is clear that the significance of its role is likely the reason for its conservation across so many species. In their review of homologous recombination regulation, Krejci *et al.* demonstrated how a variety of proteins that participate in repair mechanisms can influence invasion, double Holliday junction resolution, D-loop intermediates, and whether resolution of intermediates results in crossover or not (2012).

Consistent with the conservation of homologous recombination, the majority of relevant proteins in one organism are homologs or paralogs to those employed in other

organisms. The importance of this event has kept it relatively unchanged across organisms of all levels. In both yeast and humans, Rad51 and the RPA complex compete for attachment to DSBs, with RPA often maintaining a greater affinity (Krejci *et al.* 2012). Although RPA inhibits HR, it can cause temporary cell-cycle arrest and liberate DNA from potentially obstructive secondary structure, steps that can in turn, prove to be essential for HR (Krejci *et al.* 2012). Protein mediators assist Rad51 in overcoming the strength of RPA bound to ssDNA to permit its own binding and subsequently, HR initiation. In yeast, Rad55 and Rad57, paralogs of Rad51 and Rad52, are common mediators that help load Rad51 onto DNA and can establish co-filaments that are more resistant to anti-recombinase activity (Krejci *et al.* 2012). In humans, Rad52 nucleates Rad51 so it can be loaded on RPA bound ssDNA and can subsequently sequester RPA to inhibit it from reattaching to ssDNA (Krejci *et al.* 2012). With RPA requisitioned, the break can begin to undergo the process of homologous recombination repair with the MRX complex binding to the broken ends. The MRX complex must recruit a number of proteins, including Sgs1p in yeast and BLM in humans, to the site of a break so it can contribute to long range resection at the 3' overhang (Bernstein *et al.* 2013). This is the first step in HR where *SGS1* contributes and is a step that differentiates HR from NHEJ because NHEJ requires little to no resection at all (Bernstein *et al.* 2013). Mediator proteins do have domains that could play additional roles in HR, for example DNA annealing, but those previously mentioned are most well-known for their roles as mediators. More complex mediators can promote early phases of HR like branch migration, but later prevent Rad51 binding to DNA. These include Rad54 and Rdh54/Tid1 in humans and Uls1 in budding yeast (Krejci *et al.* 2012). In yeast, there are proteins that are primarily anti-recombinase, such as Srs2. Direct homologs in humans are yet to be found, but similarities have been found with BLM, RecQ5, and FANCJ (Krejci *et al.* 2012). Fml proteins and the FANCM are translocases in yeast and humans, respectively. They both target D-loops, encourage branch migration, and essentially promote SDSA versus DSBR, limiting the frequency of crossover products (Krejci *et al.* 2012). To put it in perspective, similar proteins may be involved in telomere maintenance and their deficiencies can be linked to telomere loss and detrimental chromosomal rearrangements (Krejci *et al.* 2012). Helicases, like Sgs1p

in yeast, form complexes (with Top3 and Rmi1 in this instance) to limit aberrant invasion events and resolve HR intermediates like double Holliday junctions in a non-crossover manner (Krejci *et al.* 2012). Sgs1 has a human homolog BLM and orthologues including WRN and RTS- all of which are related to cancer predispositions and/or premature aging syndromes. Both Sgs1p and BLM tend to establish hemicatenane structures via branch migration of two duplexes that topoisomerase III can dissolve with non-crossover products (Krejci *et al.* 2012). This is in part why ***sgs1Δ*** display significantly increased crossover (Krejci *et al.* 2012). All the elements of homologous recombination need to function properly in order for continuous double-stranded DNA to be restored. Whether the intention for homologous recombination is to induce genetic variation, maintain telomere length, or repair DSBs, the failure to accomplish it effectively can result in prolonged cell cycle pausing or even cell death.

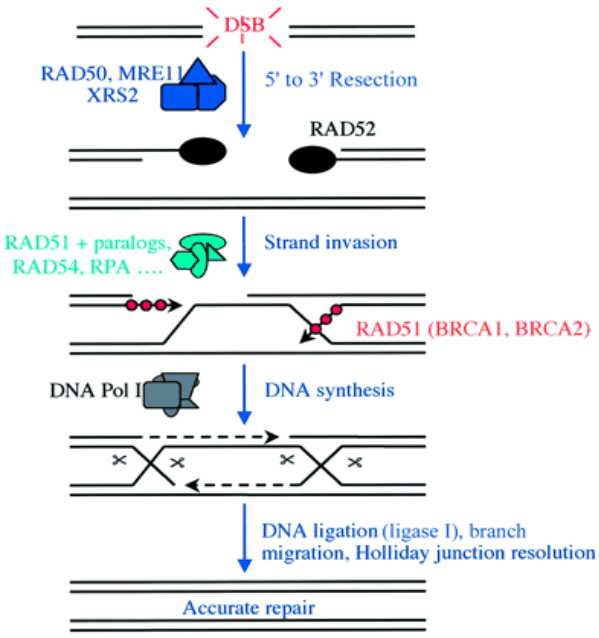

Figure 2. **Homologous Recombination.** The figure above is a representation of homologous recombination repair in yeast when a double-strand break is detected. This is yeast's preferred method of repair, but it can also be employed in higher eukarya. *SGS1* is primarily involved in the resection of the 3' end and the final step of Holliday junction resolution. Adapted from Stephen Jackson's *Sensing and repairing DNA double-strand breaks* (2013).

Nonhomologous End Joining

Nonhomologous end joining (NHEJ) is an alternative repair mechanism for double-strand breaks. In this case, DSBs can be resolved by rejoining two ends of DNA that may not share any homology and there is no invasion of similar strands to code from. This route may be preferred by some organisms versus homologous recombination for a number of reasons including the size and organization of the genetic material and the presence, or lack thereof, of certain relevant proteins. In yeast, for example, NHEJ appears to be efficient only in the organism's haploid state and is insufficient unless the two ends of DNA share some homology (Jackson 2002). Although the relative importance of repair mechanisms varies across organisms, NHEJ has been conserved throughout evolution from yeast to humans. Several proteins act as surveillance for abnormalities in the genome. ATM and ATR code for common proteins in humans that act to detect DSBs and Tel1p along with Mec1p function similarly in yeast (Jackson 2002). Ku, a heterodimer of Ku70 and Ku80, is one of the first responders to a double strand break in both yeast and humans and is not sequence specific- an important characteristic for acting on DSBs. Its catalytic function is minimal, but it assists in stabilizing both the exposed DNA ends and additional proteins that are attending to the break (Jackson 2002). In vertebrates, Ku attracts DNA-PKcs, a serine/threonine kinase, triggering a cascade of events starting with the recruitment of a nuclease (Jackson 2002). While Artemis and DNA-PKcs collaborate to act as a nuclease breaking bonds between nucleotides and nucleic acids in higher eukarya, yeast must employ other proteins for this function, possibly Mec1p (Jackson 2002). In addition to DNA-PKcs, when Artemis is phosphorylated, it uses a 5' endonuclease, a 3' endonuclease, and proteins that resolve hairpin motifs in order to eliminate any single stranded DNA ends that may have been produced from degradation or resection (Lieber *et al.* 2012). Yeast must have proteins that can also accomplish this, but one reason NHEJ is not as common to yeast may be because these participants are not as successful as Artemis and this complex. A major processing complex in humans is MRE11-RAD50-NBS1 with the analogous Mre11p-Rad50p-Xrs2p in yeast, but studying these have proved to be an obstacle because cells lacking these either cannot perform

NHEJ or are completely unviable (Lieber *et al.* 2012). In both humans and yeast, ligase IV is necessary to rejoin the DNA ends after processing, but in humans it functions alongside XRCC4 which appears to stabilize it (Lieber *et al.* 2012). There are other ligases that exist within these organisms that alone do not suffice for NHEJ, for example, DNA ligase I can potentially produce some ligation if there is a substantial amount of microhomology between the ends by chance. The exact role of polymerase in NHEJ is not well understood besides its likely role post DNA processing.

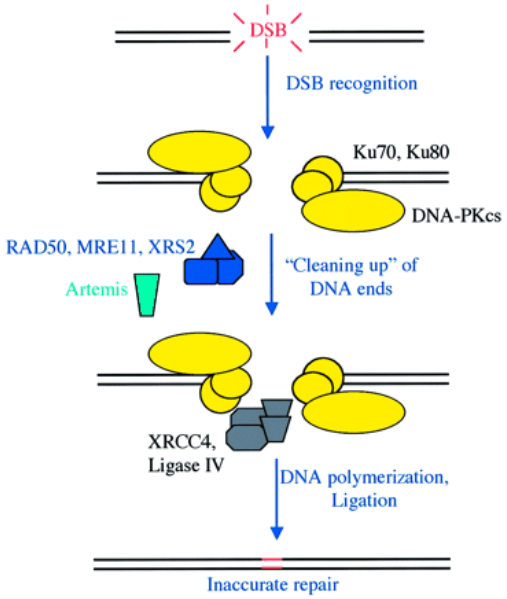

Figure 3. **Nonhomologous End Joining.** This demonstrates the important participants in yeast NHEJ when a double-strand break is detected. It is relatively similar to the process in vertebrate NHEJ. Adapted from Stephen Jackson's *Sensing and repairing DNA double-strand breaks*.

RecQ Helicases

One of the many crucial components of homologous recombination repair, in particular, is the RecQ family of helicases. Genes that code for helicases are some of the numerous sequences that are evident across all levels of living organisms and have been preserved through evolution. The RecQ family of helicases are no exception to this from simple organisms like yeast that only have one RecQ helicase gene in their genome to humans that have five RecQ helicases discovered to this date (Sharma *et al.* 2006). The RecQ family of helicases can be distinguished from other helicases by their

conserved motif approximately at amino acid 450, the RecQ C-terminal (RQC) motif, and their helicase and RNaseD C-terminal (HRDC) motif. Helicases are capable of utilizing the energy in ATP to translocate and unwind the double stranded DNA (Sharma *et al.* 2006). Sensibly, this makes helicases important in the progression of DNA replication and protein production because necessary proteins, such as polymerase, rely on a helicase for making DNA accessible for transcription. The role of helicases go beyond DNA replication, they play a pivotal part of resolving intermediates of homologous recombination. Therefore, their function has the capacity to influence both genetic variation and repair. RecQ helicases have displayed branch migration of the recombination intermediates double Holliday junctions and three-stranded D-loops in vitro (Sharma *et al.* 2006). They also could be responsible for resolving stalled or collapsed replication forks.

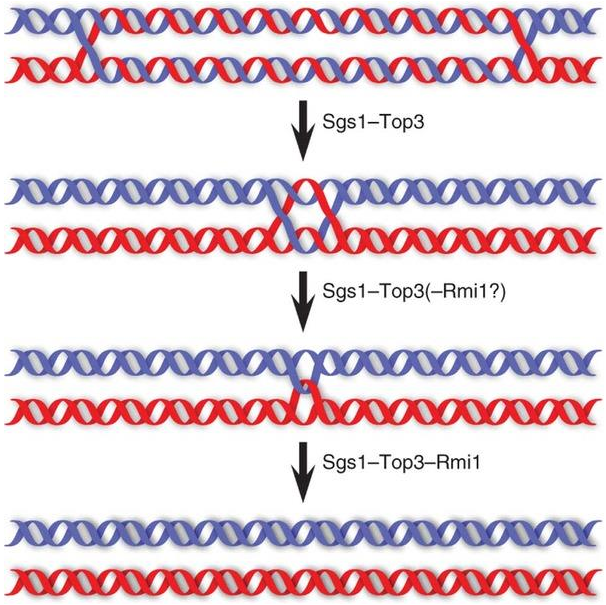

Figure 4. **Double Holliday Junction Resolution.** The image above is a representation of Sgs1p, with additional support proteins, working to resolve double Holliday junctions formed between homologous chromosomes. As depicted, *SGS1* can play a role in gradually mediating the dissolution of crossing over between chromosomes. Adapted from Petr Cejka's *Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3* (2010).

The RecQ Helicase *SGS1*

SGS1 is the single RecQ helicase in *Saccharomyces cerevisiae*, or budding yeast, and has human homologs including *BLM*, *WRN*, and *RECQ4*. The gene is coded for in the nuclear genome, but due to its protein function, its effects are not exclusive to the nucleus. As previously discussed, this conservation of genes does not only have meaningful implications for evolutionary study, but also allows model organisms to be utilized as a means to learn more about human RecQ family of helicases. *SGS1* displays functionally similar effects on Holliday junctions as *BLM*, *WRN,* and even *E. coli's RecQ* (Sharma *et al.* 2006). Since RecQ helicases participate in repair mechanisms that involve homologous recombination, they play a part in maintaining the integrity of the genome. The genetic material is subject to constant change and damage necessitating reliable repair systems. The diverse responsibility of homologous recombination makes it possibly to also influence genomic stability in other ways, for example, its role in telomere maintenance. Sgs1p has shown to be essential in telomere maintenance in cells that lack telomerase- a protein that has the capacity to counteract the loss of telomere length that accommodates cell division (Sharma *et al.* 2006). *SGS1* unites with other proteins in order to encourage polymerase association or can cleave forks in order to resume replication.

Thus far, research on Sgs1p has focused primarily on its role in nuclear genomic function. *SGS1* is a pivotal participant in genomic stability of the nucleus. Resection and double-strand break repair are important when the genome has been compromised, especially in yeast, an organism that has a preference for homologous recombination as a repair mechanism. Zhu *et al.* have proved that a major consequence of the *SGS1* gene being effectively knocked out is an increase in crossover recombination (2008). This is most likely due to the role of *SGS1* in resolving double Holliday junctions in a noncrossover manner. Loss of genes coding for proteins active in homologous recombination can weaken the genomes ability to undergo repair, thus the genome loses resiliency and becomes much more vulnerable. These problems can translate to diminished cell cycle efficiency because cycle checkpoints often reference the quality of the genome as one attempt to protect the organism from potentially harmful genetic

blueprints. When studying cell cycle progression of single and double knockout ***sgs1Δ*** strains, 90% of single knockouts exhibited extended arrest at the G2/M checkpoint in the cell cycle (Zhu *et al.* 2008). The much less frequent cell cycle arrest in double knockout mutant strains indicates that, in this case, losing more proteins was correlated with the cell damage to go undetected more readily (Zhu *et al.* 2008). Losing a single gene has already proven to jeopardize the organism, but the previous describes that double mutants that included ***sgs1Δ*** could pose a greater threat due to delayed cell cycle checkpoint recognition. Being more resistant to cell cycle checkpoints could lead to significant mutant phenotypes going undetected and a greater possibility of transmitting these changes to progeny.

Although the nucleus has been the focus of previous research regarding *SGS1*, it is important to investigate its significance in the mitochondria. Since *SGS1*, like other RecQ helicases, are likely to be fundamental in mitochondrial genome stability, it would be valuable to allot some attention to RecQ helicases within the mitochondria, in addition to, the nucleus. Many of the phenotypes corresponding to human diseases could be directly derived from abnormalities in the mitochondria and the loss of mitochondrial stability. It is already known that deficiencies in other helicases are linked with premature aging disorders, predispositions to cancer, and more like Bloom Syndrome and Werner's Syndrome.

This Research

These experiments are intended to evaluate the role of *SGS1*, specifically, in mitochondrial genome repair. *SGS1* is already known to be an active RecQ helicase in the nucleus of yeast, but its worth in the mitochondrion is relatively undetermined at this point. The goal is to evaluate if *SGS1* has a role in mtDNA stability and if so, specifically what pathways are impacted that lead to this change in genomic stability. Then homologous recombination repair will be studied comparing wild type and *SGS1* knockout strains to see if that is an action that *SGS1* plays a significant role in. Since there are human health disorders associated with mitochondrial genome instability,

studying the function of homologous proteins could contribute to the knowledge about human diseases and benefit our ability to combat them.

Materials & Methods

Media

The yeast strains relevant to this study are presented in Table 1, below. Liquid growth media used included YPD broth, composed of yeast extract, peptone, and 2% dextrose, and S+Raff-U-A, a synthetic media lacking uracil and arginine, with ammonium sulfate, yeast nitrogen base, and 2% raffinose. The agar media used included YPG (yeast extract, peptone, and 2% glycerol), YPD (yeast extract, peptone, and 2% dextrose), and YPG+0.2% dextrose. The related synthetic agar media involved SD-Arg, which lacked arginine, SD-Trp, which lacked tryptophan, and SD-U-A, which lacked both uracil and arginine. These synthetic agars otherwise shared the recipe of ammonium sulfate, yeast nitrogen base, and dextrose.

Table1: Relevant yeast strains

DFS188	*MATa ura3-52 leu2-3, 112 ly2 his3 arg8::hisG*
KOY101	DFS188 ***sgs1Δ***::kanMX
LKY196	DFS188 Rep96::*ARG8^{m}::cox2* Rep96*::URA3::trp1*
KOY201	LKY196 ***sgs1Δ***::kanMX
KOY202	LKY196 ***sgs1Δ***::kanMX
CSY080	LKY196 Rep96::*ARG8^{m}::cox2* w/ E234
CSY086	LKY196 Rep96::*ARG8^{m}::cox2* w/ E240
KOY439	LKY196 ***sgs1Δ***::kanMX w/ E234
KOY470	LKY196 ***sgs1Δ***::kanMX w/ E240

High Efficiency Yeast Transformation

The desired strain was inoculated into a YPD broth directly from the -80°C freezer and incubated at 30°C for approximately 24 hours. 1mL of this culture was diluted by combining it with 49mL of YPD broth. This was incubated 3 hours, until the OD_{600} was approximately 0.3. The 50mL culture was centrifuged for 7 minutes and the supernatant was discarded. After resuspending the cells in 1mL in sterile, deionized water, they were centrifuged again, this time for 7 seconds. The supernatant was aspirated and the cells were resuspended in 500uL 0.1M LiOAc, TE. If necessary, the cells could have been left at 4°C overnight at this point. In new microfuge tube, 100uL of the cells suspended in LiOAc, TE, 15uL of 10mg/mL salmon sperm carrier DNA, and 5uL of the appropriate PCR knockout cassette. This mixture was incubated at 30°C on the shaker for 30 minutes. Occasionally the tubes were also inverted manually. 1mL of 40% PEG, 0.1M LiOAc, TE was added and mixed by pipetting up and down. It was incubated for another 30 minutes at 30°C on the shaker with occasional manual shaking. The mixture was then heat shocked at 42°C for 15 minutes. After it was centrifuged for 7 seconds and the supernatant was aspirated, the pellet was resuspended in 1mL of sterile deionized water and 200uL was plated on each plate of selective media.

sgs1Δ::kanMX Strain Construction and Confirmation

A yeast deletion bank library supplied a *sgs1Δ::kanMX* strain. The disruption allele was amplified with polymerase chain reaction (PCR) and isolated. This PCR disruption cassette was then selected for by the appropriate *SGS1* forward and reverse primers. Non-coding sequences approximately 550bps in length that flank *SGS1* were included in the amplified sequence as a means to target the integration of this cassette into a genome. The high efficiency yeast transformation protocol was employed to transform the wild type DFS188 and LKY196 with the PCR disruption cassette, introducing the *sgs1Δ* allele. Exclusively used for the induced DRMD assay, high efficiency yeast transformation was also utilized to introduce the functional E234 and

dead E240 plasmids to strains previously transformed as *sgs1Δ*. PCR was used to both confirm the presence and absence of the mutant allele in the genomic DNA with primers that amplified the anticipated size fragments of DNA. The samples were run on an electrophoresis agarose gel adjacent to the Hyperladder I. The proper deletion would be verified using the *SGS1* forward primer SGS -494F (refer to Table 2 for specific primer sequence) and *kanMX* reverse primer kanMX 373R. The absence of wild type would be verified by the same *SGS1* forward primer, but in conjunction with the internal *SGS1* reverse primer SGS 79R.

Table 2:

Primer Name	Genetic Sequence
SGS -494 F	5' CAAACTGATCAGCGTTCGGTCC 3'
SGS 79R	5' CTTTGTCTTCCTGTAAAGTCGCC 3'
kanMX 373R	5' CGGATGTGATGTGAGAACTGTATC 3'

Primer Sequences. The table above provides the sequence and relative orientation of the primers employed in the relevant PCR amplification. SGS -494F and kanMX 373R are used to confirm the presence of the mutant allele, while SGS -494F and SGS 79R would only amplify the wildtype *SGS1*.

The *sgs1Δ* strains were also assessed for the presence of the appropriate reporters using specific growth media. The strains designed for experimentation were patched on SD-Arg and SD-Ura. The LKY196 *sgs1Δ::kanMX* with E234 and E240 were similarly examined for the presence of reporters using particular growth media. Prior to introducing the plasmids, the strains were grown in SD-Trp to assure the URA3 gene had been recombined out. Once transformed with the plasmids, the strains were grown on SD-Ura to indicate the plasmids were taken up by the cell. Growing on specific media indicated that reporters are present and functional in the genome, but PCR will confirm if the cassettes have integrated in the anticipated region.

Respiration Loss Assay

The frequency of spontaneous respiration loss in wild-type versus *sgs1Δ* strains were compared using the respiration loss assay. The strain was patched on YPG from the -80°C freezer. It was incubated for approximately 24 hours at 30°C before being streaked for singles on YPD and incubated at 30°C for a following 3 days. A series of 3 eppendorf tubes were prepared for a serial dilution of a single colony: the first containing 100uL sterile dH_2O, the second two with 500uL. A colony was put into the eppendorf of 100mL sterile water and vortexed. 5mL of this solution was then diluted into the following 500mL sterile water and vortexed. The last step of the dilution was to transfer another 5mL of the second solution into the third eppendorf, with 500mL sterile water and vortex it. Subsequent to dilution, 100uL from the third eppendorf tube was plated onto YPD+0.2% dextrose and this was repeated for 15 colonies per strain. After 3 days at 30°C, the colonies were counted and differentiated based on size. The petite colonies are considered to have lost respiration capability and ceased growth upon dextrose depletion. The large colonies maintained respiration abilities post fermentation and continued to grow. For each plate, the number of petite colonies was divided by the total number of colonies, to calculate respiration lost frequency. For each round of the assay, the median frequency of wild-type and mutant respiration loss is calculated and are then averaged to use in comparison.

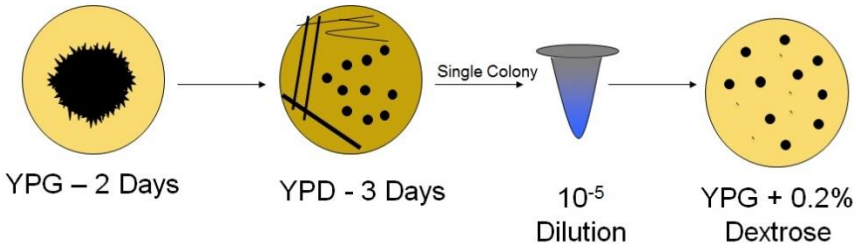

Figure 5. **Respiration Loss Assay.** The flow chart above is a representation of how the respiration loss assay is performed. The strain in question is patched onto YPG for a minimum of one day, then streaked onto YPD. After three days, a single colony undergoes a serial dilution and plated onto YPG + 0.2%. Fifteen separate plates are prepared per strain and assay. This assay will measure the frequency of spontaneous respiration loss in strains of yeast.

Direct-repeat Mediated Deletion Assay

Spontaneous direct-repeat mediated deletion events (DRMD) were measured in the wild-type and *sgs1Δ* strains. By using multiple plating mediums, both the nuclear and mitochondrial activities were being evaluated. The desired strains were patched onto SD-U-A from the -80°C freezer and incubated at 30°C overnight. From these patches, the yeast was streaked for singles on YPD and incubated at 30°C for the following three days. A set of 3 eppendorf tubes were prepared for a serial dilution with 100uL of sterile dH_2O in the first and 500uL of sterile dH_2O in the remaining two. A colony was added to the first tube and underwent the serial dilution, this was repeated for 15 colonies per assay. 100uL from the first tube were plated on YPG, 100mL from the second was plated on SD-Trp, and 100uL from the third tube was plated on YPD to serve as a cell count. After 3 days of incubating at 30°C, the plates were counted. A threshold size was determined and all colonies meeting the threshold were counted on the YPG. Every single colony that grew on both the SD-Trp and YPD plates were counted. The medians of the SD-Arg and YPG plates were used in Lea and Coulson protocol to calculate the rates for each assay (Lea and Coulson 1949).

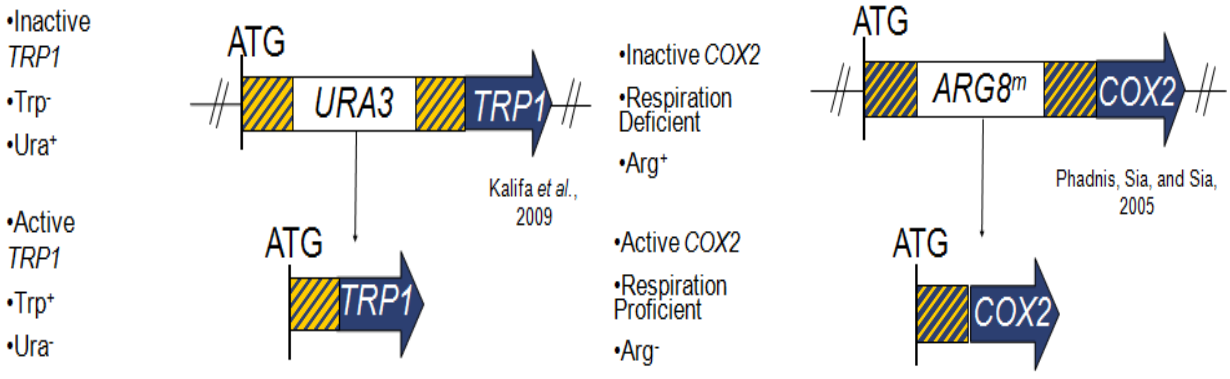

Figure 6. **Nuclear and Mitochondrial Reporters.** The figure above illustrates the nuclear and mitochondrial reporters that make direct-repeat mediated deletion assays possible. The left displays the nuclear reporter that is comprised of the *URA3* gene flanked by 96bp repeats and the *TRP1* gene. The *URA3* gene is strategically inserted 99bp into the *TRP1* gene and after the start codon, rendering it functionally inactive. Similarly, in the mitochondria, the $ARG8^m$ gene is 99bp into the *COX2* gene and is flanked by 96bp repeats. Homologous recombination has the potential to excise the *URA3* gene and $ARG8^m$ restoring the transcription of the *TRP1* and *COX2* genes in the nucleus and mitochondria, respectively.

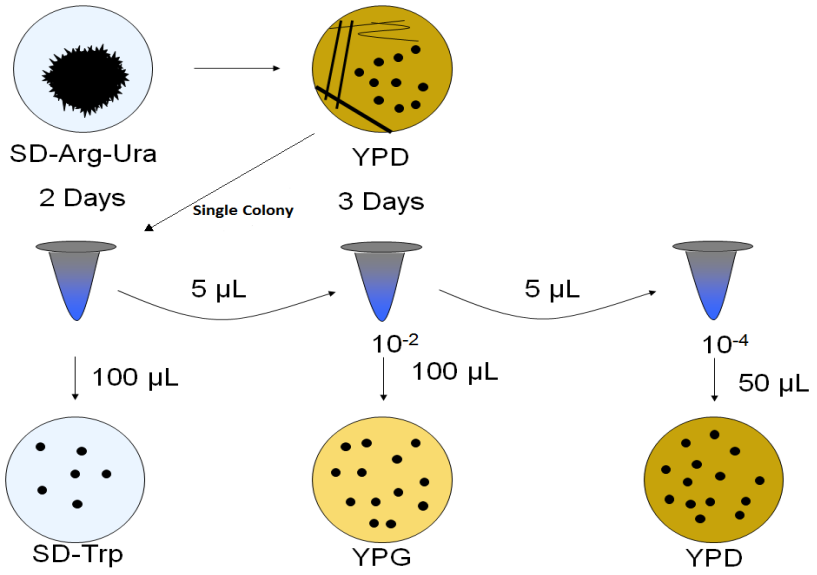

Figure 7. **DRMD Assay.** The flow chart above outlines the procedure on the direct-repeat mediated deletion assay. The subject strain is plated ono SD-U-A for a minimum of one day prior to being streaked on YPD for three days. A single colony undergoes a serial dilution and each of the three dilutions are plated onto separate media. The YPD plates will provide a cell count, growth on the YPG plates is indicative of mitochondrial homologous recombination events, and growth on SD-Trp is indicative of nuclear homologous recombination events.

Lea & Coulson Calculations

The rate of homologous recombination in DRMD was determined by the calculation protocol designed by Lea and Coulson. To calculate the number of cell divisions, the number of colonies that grew on YPD were counted and averaged. This average was subsequently multiplied by two and divided by two to establish a range. Any set of plates where the YPD count was outside of this range were omitted and regarded as outliers. If necessary, the average of YPD colonies was recalculated. The average number of colonies was then multiplied by 20,000 as a means to determine the approximate number of cell divisions on each plate. The median colony counts were determined for both YPG and SD-Trp, this number for SD-Trp was its r_0. To calculate the r_0 for YPG, the median was multiplied by 100. Individually, each range that these r_0's fell between was found on r_0 column of the Lea & Coulson table and the smaller r_0 of the range was subtracted from the experimental r_0. This number was divided by the

difference between the r_0 values that the experimental r_0 was found to have fallen between and then multiplied by 0.1. This number was added to the r_0/m value displayed the table that corresponds to the smaller r_0 of the range. This r_0/m was divided by r_0 to determine the number of mutations. Mutations per cell division were found by dividing this number by the total cell count of given agar plate (Lea and Coulson 1949).

Induced Direct-repeat Mediated Deletion

The induced homologous recombination events in both wild-type and *sgs1Δ* strains were measured for the nucleus and mitochondria through the induced direct-repeat mediated deletion assay. The relevant strains were patched on SD-U-A from the -80°C freezer. After growing for one night at 30°C, cells are inoculated in 20mL of S+Raff-U-A and grown for another night at 30°C on the shaker. Approximately 1-2mL of this was diluted into 50mL of S+Raff-U-A until an OD_{600} between 0.07-0.08 is achieved. Every two hours, the OD_{600} was measured until reaching between 0.1-0.2. When this OD_{600} was reached, this solution was the pre-induced culture. 22.5mL from each of this pre-induced culture was then added to 2.5mL of 20% galactose and 25uL of 1000x L-arginine and put at 19°C on a shaker from 16-18 hours to induce a double-stranded break. A serial dilution with two eppendorf tubes of 500uL of sterile dH_2O was also prepared for the pre-induced culture. 50mLof the culture was pipetted into the first eppendorf and vortexed, then 5mL was diluted into the second eppendorf tube and vortexed. 100uL of the diluted culture was plated on YPD. This was repeated for six plates for each strain both with the functional and dead plasmids. After growing at 30°C for two nights, the YPD plates were replica plated onto SD-Arg and YPG. All three kinds of plates were grown for one more night at 30°C. The YPD plates were counted to get a cell count. The colonies that grew on YPG were counted and the colonies that did not grow on SD-Arg were counted.

The culture incubated at 19°C for 16-18 hours was also subjected to a serial dilution through two eppendorfs with 500uL of sterile dH_2O. 5mL of the culture was pipetted into the first eppendorf tube and vortexed, then 5mL was diluted from that into

the second eppendorf and vortexed. 50mL of this post-induced diluted culture was also plated on YPD and repeated for 6 separate dilutions and plates. The exact same procedure for the pre-induced replica plating and counting was repeated for the post-induced. For both the pre-induced and post-induced data, the following calculations are performed: The data was adjusted by subtracting the colonies that were unable to grow on both YPG and SD-Arg from the total count of colonies of YPD. The total number of colonies that grew on YPG was divided by the adjusted total cell count to obtain the percentage of homologous recombination that restored the ability to respire. The percentage from the pre-induced is subtracted from the percentage of the post-induced to correct for cells that developed the ability to respire spontaneously.

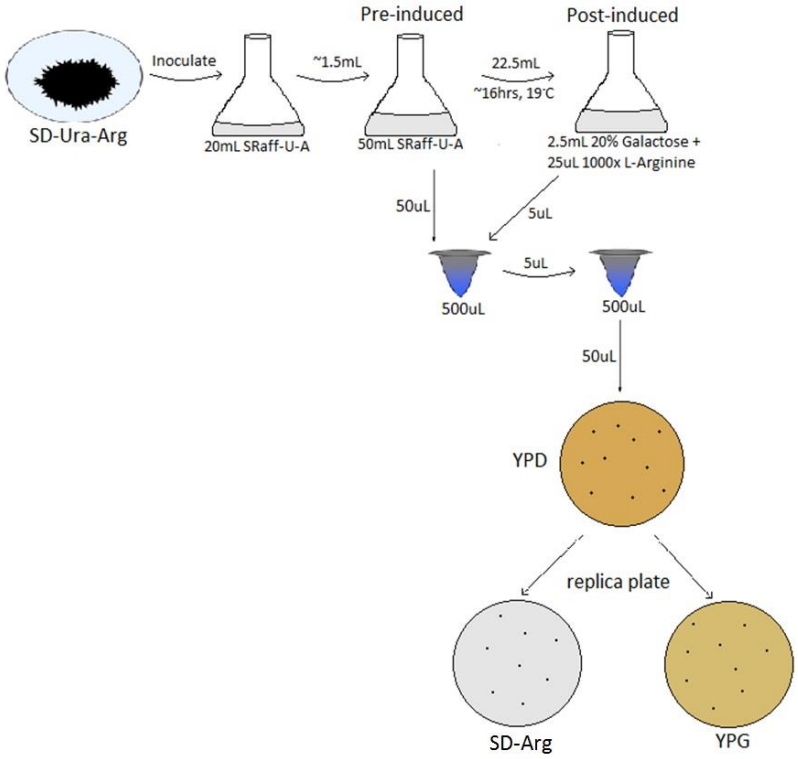

Figure 8. **Induced DRMD Assay.** The figure above is a flow chart that outlines the procedure for the induced direct-repeat mediated deletion assay used to measure homologous recombination repair of double-strand breaks. The subject strain is patched on SD-U-A for a minimum of one night prior to being inoculated into S+Raff-U-A. Once this solution is saturated, it is diluted into S+Raff-U-A and grown to the appropriate OD_{600}. Samples of this solution undergo a serial dilution and are plated onto YPD while some is incubated at 19°C for 16-18 hours. This solution is then plated onto YPD in a relatively similar manner. After two days of growth on YPD, both sets of plates are replica plated to SD-Arg and YPG. The data from these plates will indicate the effectiveness of homologous recombination repair following an induced double-strand break.

Results

sgs1Δ::kanMX Strain Construction and Confirmation

The generation of a complete deletion of a gene by one-step transplacement is the necessary first step to evaluating the phenotype of a mutant strain relative to a wild type strain. It involves encouraging recombination and selecting for a mutation at a specific locus thereby creating a strain of yeast that has effectively lost the function of a certain gene, in this instance, *SGS1.* A culture of wild type yeast was diluted further into YPD broth and incubated. After washing in sterile, deionized water, the cells are suspended in LiOAc, TE. A mixture of this solution, carrier DNA, and a PCR knockout cassette was incubated and PEG, LiOAc was subsequently added. After further incubation and heat shock, the cells were resuspended in sterile, deionized water and plated on specific media. This *sgs1Δ* strain of DFS188 background, designed for the respiration loss assay, was plated on YPG. The colonies that grew depicted functional mitochondria, they were patched onto YPG, and frozen down in 20% glycerol for storage. Growth on YPG indicates that a glycerol sugar source was used in oxidative phosphorylation to produce energy for the organism. The *sgs1Δ* strain of LKY196 background intended for the DRMD assays were plated on SD-U-A to confirm the appropriate reporters were present. The colonies that thrived on this media were then patched on SD-U-A and also frozen down in 20% glycerol for storage. High efficiency yeast transformation was also used to introduce the function and nonfunctional (E234 and E240) plasmids to the *sgs1Δ* LKY196 strain necessary for the induced DRMD.

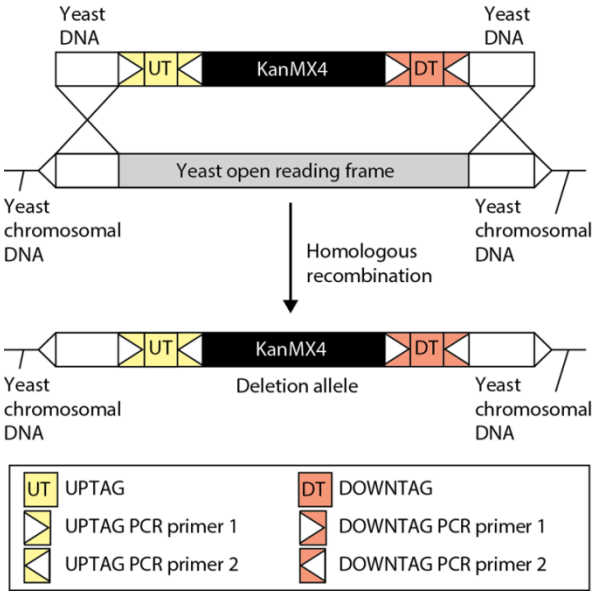

Figure 9. **kanMX Knockout Cassette.** The above is a visual of how a kanMX knockout cassette incorporates into a genome via homologous recombination. The sequences that flank the kanamycin resistance gene are homologous to those within the gene in question, in this case, *SGS1*. When the cassette integrates into the host genome, it interrupts a coding region of the original gene and the strain can be selected for based on kanamycin resistance. In the relevant research, the kanMX cassette inserts into the *SGS1* gene, thus this yeast can no longer transcribe a function al Sgs1p. Adapted from Kara Bernstein's *Resection Activity of the Sgs1 Helicase Alters the Affinity of DNA Ends for Homologous Recombination Proteins in Saccharomyces cerevisiae* (2013).

Two major modes of confirmation were employed to prove the presence or lack of specific mutations within the strains. One mechanism of verification was plating or patching on selective media that would only permit growth if the genetics of the strain contained specific components. *sgs1Δ* DFS188 grew on YPG ensuring the strain was originally capable of performing oxidative phosphorylation because the only sugar source available to the organism is glycerol which is used by their mitochondria for oxidative phosphorylation. It also grew on SD-Arg and SD-Ura, indicating intact *ARG8*^{m} and *URA3* because growth on media lacking an essential amino acid indicates that the yeast has the capability to produce it themselves. The strains for DRMD and induced DRMD assays were patched onto SD-U-A from the -80°C freezer where growth indicated that both of the genes, *URA3* and *ARG8*^{m}, were intact. For induced DRMD, the second mode of confirmation was via PCR and gel electrophoresis on an agarose

gel. Whole cell PCR was used for each of the strain samples, appropriate primers were employed to amplify target genes, and they were run adjacent to Hyperladder I of known molecular weights. When *sgs1Δ* DFS188 and *sgs1Δ* LKY196 were amplified with SGS -494 F and kanMX 373R, bands were apparent on the gel at approximately 900bp. When they were amplified with the primers SGS -494 F and SGS 79R, no bands were evident besides that correlating to the entire genome. To ensure the *sgs1Δ* LKY196 strain incorporated the E234 and E240 plasmids, prior to the transformation, the yeast had to demonstrate they grew on SD-Trp and did not grow on SD-Ura, exhibiting loss of *URA3*. Following the transformation, they grew on SD-Ura due to the fact that the plasmids carried a reporter that coded for uracil production. This is a reliable indicator that the strains incorporated the plasmids versus a PCR and gel electrophoresis.

Respiration Loss Assay

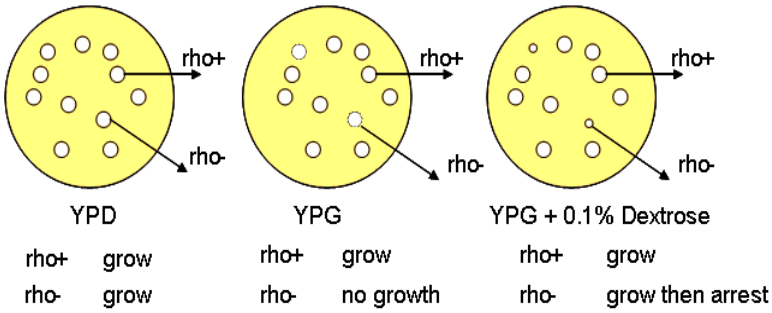

Figure 9. **Respiration Proficient versus Deficient Colonies.** The figure above is a visual representation of how the frequency of spontaneous respiration loss in yeast is measured using the respiration loss assay. The YPD and YPG plates are not actually prepared in the assay, they are included here as tools to understand how the sugar source can effect growth. The YPG + 0.2% dextrose is the media plated on for this assay. A total cell count can be calculated by counting every colony and the small colonies are indicative of cells that lost oxidative phosphorylation capabilities and therefore, mitochondrial function. The percentage of petit colonies represents a frequency of spontaneous loss of mitochondrial genome stability.

The respiration loss assay is performed as a means to calculate the frequency of spontaneous respiration loss. When the yeast has exhausted all of the dextrose in the media, it can no longer perform its preferred form of energy production- fermentation. The colonies that have not lost mitochondrial function will continue to grow off the

glycerol via oxidative phosphorylation. For the respiration loss assay, the strain was patched onto YPG for a single night and then streaked on YPD for 3 days. A single colony underwent a serial dilution and was plated on YPG+0.2% dextrose. This was repeated for 15 colonies from each strain per assay and then they were grown for three days. The respiration loss frequency was calculated by the petit and large colonies being counted separately and the total petites being divided by the total number of colonies for each plate.

The wild type strain displayed a 2.10% respiration loss while the mutant strain showed 4.56% respiration loss. In other words, the consequence of *sgs1Δ* was an approximately 2.2 fold increase in respiration loss.

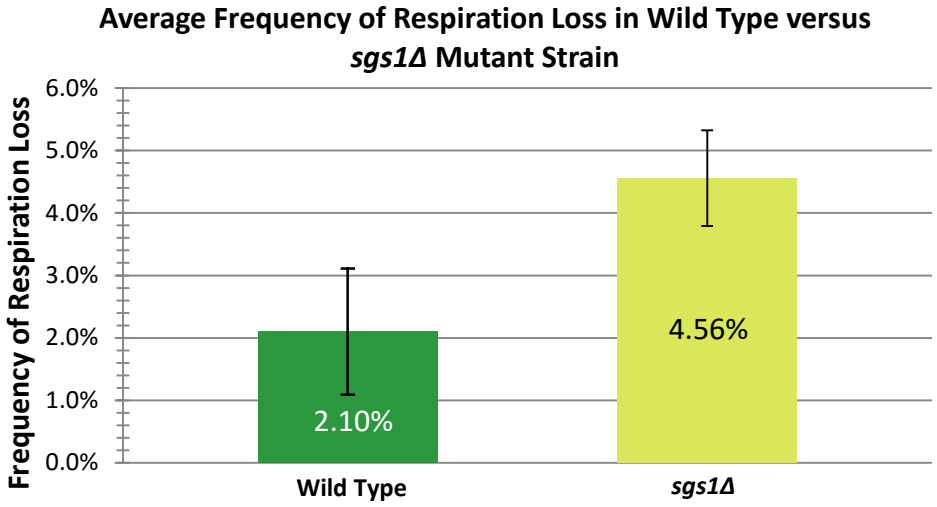

Figure 10. **Frequency of Spontaneous Respiration Loss.** The figure above is a graphical display of the difference between wild type respiration loss and the *sgs1Δ* DFS188. The mutant strain demonstrated a 2.2 fold increase to 4.56% spontaneous respiration loss compared to the 2.10% seen in wild type strains.

Direct-repeat Mediated Deletion Assay

The DRMD assay is used as a means to measure the rate of homologous recombination events in wild type versus *sgs1Δ* yeast strains. The strains were patched on SD-U-A and streaked on YPD. Colonies underwent a serial dilution and volumes

from the most concentrated to the least were plated on SD-Trp, YPG, and YPD respectively. The colonies that grew on SD-Trp were counted and result from nuclear homologous recombination events. The colonies that grew on the YPG plate were also counted and these result from homologous recombination in the mitochondria and the colonies that grew on the YPD plate provided a cell count for calculations. Using the Lea and Coulson calculation technique, it was determined that the *sgs1Δ* strains showed a homologous recombination rates of $28.9^{x10\ -7}$ and $34.4^{x10\ -7}$, for the nucleus, approximately 2.4 and 2.8 fold increases compared to the wild type's $12.2^{x10\ -7}$. The mitochondria showed that the *sgs1Δ* strains had homologous recombination rates of $1432.4^{x10\ -7}$ and $1616.5^{x10\ -7}$, approximately 1.5 and 1.7 fold decreases from the wild type's $2442.6^{x10\ -7}$.

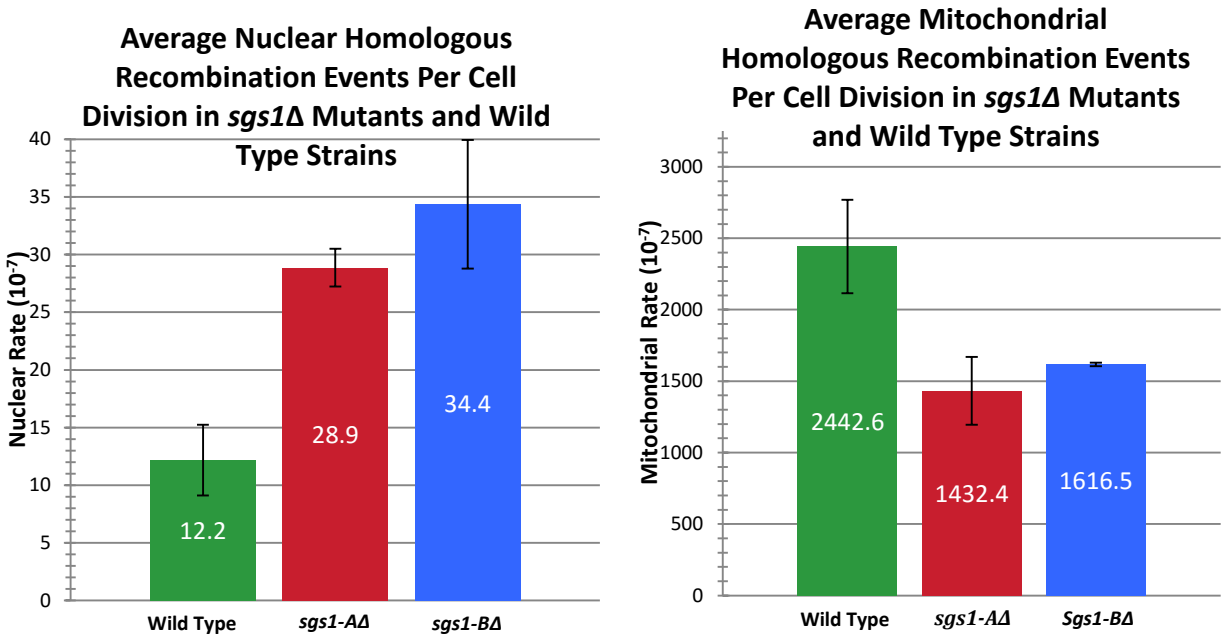

Figure 11. **Rate of Homologous Recombination in Nuclei and Mitochondria.** The above is a graphical representation of wild type and *sgs1Δ* strains rate of recombination events in the nucleus and mitochondria on the left and right, respectively. The nucleus showed a 2.4 and 2.8 fold increase in rate of recombination with the loss of Sgs1p. In the mitochondria, a 1.5 and 1.7 fold decrease in rate of recombination was measured with the loss of Sgs1p.

Induced Direct-repeat Mediated Deletion Assay

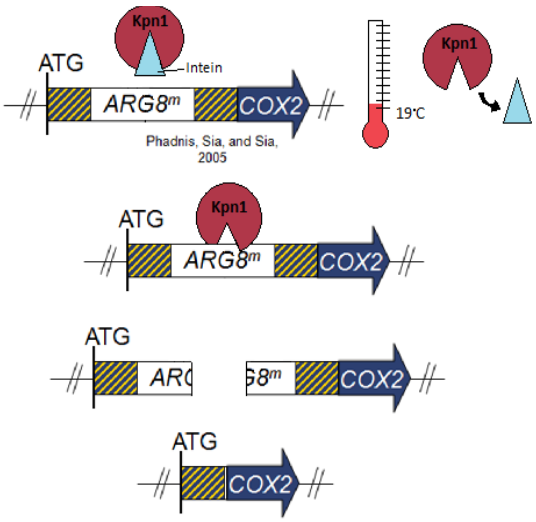

Figure 12. **Temperature Induced Double Strand Break.** This illustration reflects how the double-strand break is generated in the induced DRMD assay. A temperature sensitive intein binds to the restriction endonuclease, *Kpn*, rendering it inactive. At 19°C the intein releases *Kpn*, permitting it to become active. *Kpn* then cuts both strands of the helix in a specific gene, in this example *ARG8*^{m}, causing the yeast to be unable to produce the corresponding protein. Homologous recombination is indicated by the restoration of gene function of the adjacent gene, in this example, *COX2*. If *COX2* transcription is restored, the yeast will grow on YPG and it can be inferred that homologous recombination occurred.

The induced DRMD assay narrows down the methods of homologous recombination repair that Sgs1p prove to be important for. The strains were patched onto SD-U-A and inoculated in S+Raff-U-A and allowed to grow. These cultures were subsequently diluted and grown to the proper OD_{600} when the pre-induced samples were plated onto YPD. After 16-18 hours at 19°C, the post-induced samples were plated on YPD as well. After two nights of growth on YPD, both the pre-induced and post-induced plates were replica plated onto SD-Trp and YPG. All of the colonies on YPD and YPG were counted. The colonies that showed no growth on SD-Arg were counted and the corresponding colonies that displayed no growth on both YPG and SD-Arg were counted.

The YPD plates provided a cell count. For the pre-induced plates, the number of colonies that were Arg^{-} YPG^{-} were subtracted from the total number of colonies counted on YPD, giving the new total of colonies that are in question. The number of colonies

that were YPG$^+$ were divided by this new total to provide a baseline recombination frequency. For the post-induced, the Arg$^-$ YPG$^-$ were also counted and subtracted from the total colonies counted on the YPD plates. The number of YPG+ colonies were, again, divided by this new total. The baseline recombination frequency was then subtracted from this number to determine the percentage of recombination events induced by the double-strand break. The wild type and mutant strains with the inactive E240 plasmid displayed 2.60% and 0.11% rate of double strand break repair, respectively. The wild type strain with the functional E234 strain showed a 57.24% rate and the mutant strain with the functional E234 strain showed a 55.27% rate. The p value calculated was ~0.66.

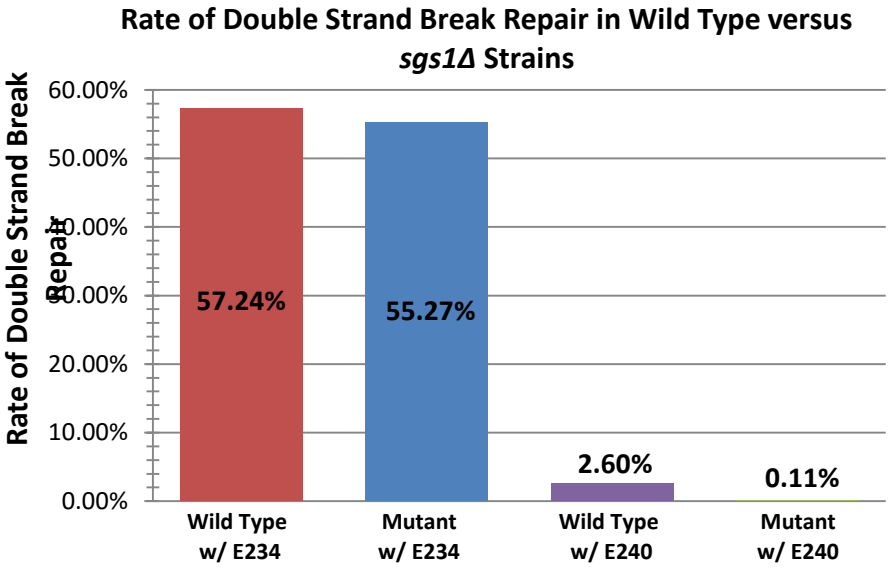

Figure 13. **Rate of Double Strand Break Repair.** The above is a graphical display of the rate of double strand break repair in wild type and *sgs1Δ* strains with the E234 and E240 plasmids. There is no significant difference between the wild type and mutant strains with the E234 plasmid. The wild type and mutant strains with the inactive E240 plasmid have minimal rates altogether.

Discussion

RecQ family helicases are known to be involved repair mechanisms, specifically through their role in end resection, and resolving double Holliday junctions. Previous research of *SGS1* has primarily focused on its presence in the nucleus, but efficient

genomic repair is also essential in the mitochondria to maintain mtDNA stability. The focus of this study is on the significance of *SGS1* in the mitochondria. The respiration loss assay simply identifies if *SGS1* is significant for mitochondrial genome stability. DRMD narrows the focus to homologous recombination in general and the induced DRMD allows us to concentrate on specific homologous recombination schemes. Thus far, this research suggests that *SGS1* does have a significant role in mitochondrial stability. The loss of Sgs1p is correlated with a decreased rate of recombination events, but not necessarily in double-strand break repair. *SGS1* also appears to be involved in homologous recombination at direct repeats within the nucleus, but in the opposite fashion. *SGS1* may play a limiting role for recombination when regarding the nucleus.

sgs1Δ::kanMX **Strain Construction and Confirmation**

PCR was employed following transformations to confirm the presence or absence of the ***sgs1Δ*** allele. The SGS -494F and SGS 79R primers were used to amplify an approximate 573bp segment of the genome that would only be visible on an agarose gel if the *SGS1* gene were present. If *SGS1* had been effectively knocked out, the SGS 79R primer would not anneal and no segment would be amplified. The SGS 494F primer was also partnered with the kanMX 373R primer to amplify a region of the DNA that was only present if the mutant allele had inserted into the proper location. The amplified sequence would begin in the same location, but only be completed if the kanMX 373R primer could bind and amplify from the opposite direction. This is only possible if the cassette containing *kanMX* had integrated into the genome. A band at ~900bp confirmed that the kanMX knockout cassette had, in fact, integrated into *SGS1*.

By plating yeast on particular media, inferences about the genetic characteristics of the strains could be made. Prior to respiration loss assays, both the wild type and mutant strains were patched on YPG. This provided the knowledge that this assay began with yeast containing functional mitochondria because the only way for it to grow in the presence of glycerol, was via oxidative phosphorylation. The DRMD strains had to show growth on both SD-Ura and SD-Arg to demonstrate that these reporters were

intact to begin with and homologous recombination had not yet restored *TRP1* or *COX2*. Designing the induced DRMD mutant strains mandated a transformation with the ***sgs1Δ*** allele and a plasmid. The mutant allele was confirmed with PCR and gel electrophoresis and growth on SD-Ura established the presence of the plasmid. Wild type LKY196 and the ***sgs1Δ*** mutant previously prepared grew on SD-Trp and then underwent transformation with the E234 and E240 plasmids. Growth on SD-Trp implied that the *URA3* gene had been recombined out of the genome, restoring *TRP1* function. The plasmid carried an allele for *URA3* therefore, the yeast that could subsequently grow on media lacking uracil again implied the plasmid was present in them. By selecting for yeast that lost *URA3* prior to transformation, yeast that took up the plasmids could be tracked by their ability to produce uracil once again following transformation.

sgs1Δ strains displayed an increase in respiration loss frequency compared to wild type

After greater than three trials of respiration loss assays, ***sgs1Δ*** strains were found to have a 2.2 fold increase on spontaneous respiration loss compared to wild type DFS188. Respiration loss is a naturally occurring, spontaneous event in yeast where a mutation effectively eliminates the organism's ability to perform oxidative phosphorylation whether it's caused by a point mutation, deletion, et cetera. This assay will not specifically identify between mutations or complete losses, it indicates loss of function in general. When mitochondrial genome stability is diminished and oxidative phosphorylation is no longer an option, yeast would be left with the option to ferment as a means to obtain energy, but this is only possible with the appropriate sugar source. Based on the 2.2 fold increase in spontaneous respiration loss from wild type's average of 2.10%, it can be inferred that Sgs1p plays a significant role in maintaining mitochondrial stability and the absence of this gene will lead to more frequent respiration loss.

There is a great amount of redundancy in mtDNA compared to nuclear DNA. These conclusions do not indicate whether the entire mitochondrial genome has been

compromised or if a single mutation could have conferred this damage. Since *SGS1* is a RecQ helicase and is known to be involved in repair mechanisms of the nuclear genom, it could be inferred that it plays a similar role in mitochondrial genome repair. The results from the respiration loss assay verify that *SGS1* is, in fact, important for a repair mechanism that if faulty will cause a greater frequency of respiration loss. The loss of this protein can be detrimental to yeast when faced with endogenous or exogenous sources of genetic damage because it is evident that ***sgs1Δ*** strains have decreased mitochondrial stability and therefore, a greater propensity to lose oxidative phosphorylation capabilities. To try and indicate, more specifically, what repair mechanisms SGS1 is important for, DRMD and induced DRMD assays were subsequently performed.

sgs1Δ strains show an increase in nuclear recombination events

Two distinct ***sgs1Δ*** strains displayed increases in nuclear recombination events compared to wild type, one by a 2.4 fold increase and the second by a 2.8 fold increase. These events are measured based on the yeast's ability to grow on an agar lacking tryptophan, SD-Trp. If the colonies are capable of growing, it can be inferred that recombination restored the organism's ability to transcribe the gene for tryptophan, a characteristic it was previously incapable of because of the *URA3* gene. Recombination that leads to *URA3* being excised out can lead to *TRP1* transcription being re-established. The increase in recombination events to 28.9 and 34.4 ($x\ 10^{-7}$) from wild type's 12.2 ($x\ 10^{-7}$) indicates that Sgs1p is involved in recombination events in the nucleus and somehow may limit its rate. Since the rate of recombination increases with the loss of Sgs1p, it can be inferred that this protein impedes on the rate of recombination, at least in the nucleus, when present and functional.

sgs1Δ strains show a decrease in mitochondrial recombination events

Two distinct *sgs1Δ* strains showed decreases in mitochondrial DRMD events compared to wild type, one by a 1.5 fold decrease and the second by a 1.7 fold decrease. Recombination events were measured based on the yeast's ability to grow on YPG. Growth on this media indicates recombination that restored *COX2* transcription and therefore, the yeast's ability to perform oxidative phosphorylation and respire in the presence of a glycerol sugar source. This was previously not possible due to the *ARG8*^{m} gene that was interrupting the transcription of *COX2*. The recombination rate decreased from the wild type's 2,442.6 (x 10^{-7}) to the mutant's 1,432.4 and 1,616.5 (x 10^{-7}) indicates that *SGS1* plays a significant role in recombination in the mitochondria. Loss of Sgs1p is correlated with the loss of homologous recombination for these specific genes suggesting that *SGS1* assists in this process that can be pivotal for the cell. It is known that homologous recombination is a preferred mechanism of repair in yeast, therefore, knowing *SGS1* somehow contributes to HR, we can infer that losing *SGS1* function could prove to be detrimental for cell viability when exposure to damage causing agents are so uninhibited. DRMD measures homologous recombination events in general. As a means to determine the specific recombination methods that are significant, induced DRMD assays were subsequently performed.

Double strand break repair via homologous recombination is reduced in *sgs1Δ* strains

Wild type and mutant strains with functional E234 plasmids display no significant difference with a p value of 0.66. The minimal difference between wild type strains and those lacking *SGS1* implies that this gene is not important for the mode of homologous recombination double strand break repair because the lack of the gene has no significant change in rate of repair. The wild type strain with E234 showed a 57.24% rate and the mutant strain a 55.27% rate. The inactive E240 plasmid was a control that should not have resulted in any rate of double strand break repair. Close to what was expected, the wild type strain with the E240 plasmid had a rate of 2.60% and the mutant

strain had a rate of 0.11%. The wild type strain with E240 having a rate of 2.60% could be the result of an experimental or counting error and a greater number of trials should reduce this value towards the anticipated 0%. This data leads to the conclusion that although *SGS1* is a fundamental role in homologous recombination, as evident by the direct-repeat mediated deletion assay, this role in recombination is not due to participation in double strand break repair.

Future Work

There are two approaches to future experimentation that would be beneficial to build upon my conclusions. Based on the results sated above, *SGS1* has proven to play a role in mitochondrial genome stability and specifically, homologous recombination. Figure 2 is a visual of nuclear homologous recombination repair in yeast. In a means to determine how similar this pathway is to that in the mitochondria, subsequent experimentation can create a knockout strain of another protein that is active in nuclear homologous recombination, such as *BRCA1*. If this mutation displayed mutant phenotypes that are derived from mitochondrial defects, it can be inferred that this gene, like in the nucleus, plays a role in mitochondrial homologous recombination. This experimentation would be most informative if the subject gene were exclusive to homologous recombination, as opposed to *RAD50*, for example, that is known to be involved in both homologous recombination and nonhomologous end joining. If the gene is involved in numerous pathways, then conclusions cannot easily be drawn about these phenotypes being clearly derived from HR being impacted. Additionally, a subsequent double mutant strain would expand upon this even more. Continuing with the previous *BRCA1* example, if a double knockout of *SGS1* and *BRCA1* were created the single knockouts could be compared to the results to determine a relative order of when the proteins may function in homologous recombination. If the double knockout mutant phenotype has more similarities to the phenotype of the ***sgs1Δ*** strain, it is likely that *SGS1* plays a role prior to *BRCA1.* Contrariwise, if the double knockout shows results closer to that of ***brca1Δ*** strains, then *BRCA1* is more likely to have an active role first

regarding homologous recombination. Both of these methods would investigate deeper into mitochondrial homologous recombination and determine how similar it may or may not be to the already well-known pathway in the nucleus.

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