Effect of Knocking Out YLR419W on S. cerevisiae Growth Following Proteotoxic, Heat, Osmotic, and Oxidative Stress

An Honors Thesis (HONR 499)

by

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I. Abstract

YLR419W is a yeast homolog for *DHX36,* a gene which encodes a G-quadruplex helicase, in humans. By studying how knocking out *YLR419W* affects *Saccharomyces cerevisiae* growth and survival following cellular stress, we can learn more about the role of the protein Ylr419w in in responding to cellular stress. I hypothesized that when *YLR419W* is deleted, *S. cerevisiae* will be less able to survive stress. To test this hypothesis, growth and survival under proteotoxic, osmotic, oxidative, and heat induced stress was tested using yeast growth assays. Deletion of *YLR419W* in experimental clone 1 moderately inhibited growth under proteotoxic stress, and did not inhibit growth under thermal stress, osmotic stress, and oxidative stress. However, the proteotoxic stress effects were not repeatable across yeast clone replicates, suggesting that Ylr419w is not required for the proteotoxic stress response.

II. Acknowledgments

This thesis would not have been possible without the support of my advisor, Dr. Philip Smaldino. I have greatly appreciated his encouragement and motivation throughout my two years working in the Smaldino lab. I would like to thank Dr. Smaldino for providing me with future direction and feedback after each experiment contained in this thesis.

I am extremely grateful for Kelsey Woodruff for collaborating with me on this project, teaching me how to conduct these experiments, and for patiently answering my constant questions. I would also like to thank Dr. Eric Rubenstein for helping foster this collaboration, and for providing feedback and guidance throughout these experiments.

Many thanks to the members of the Smaldino Lab; Siara Sandwith, Michael Resisner, Taylor Karns, Destinee Saunders, Joseph Huang, Tyler Osborne, Emily Schmidt, Hasna Alashi,

Evan Rogers, and Dylan Seiler. I appreciate each of them for listening to my research progress presentations, providing feedback, and encouraging me throughout this process.

I would like to thank both my friends and family for motivating me to complete the daunting task of writing a thesis. I would especially like to thank Siara Sandwith, William Lami, and Andi Fisher for assuring me that they wrote a thesis, and therefore I can too. I have appreciated them for proofreading this thesis, offering advice, and keeping me on track throughout this process.

Lastly, I would like to thank Ball State's Biology department and Honors College. I greatly appreciate both for encouraging undergraduate research, preparing me for this task over four years, and for supporting me throughout this thesis.

III. Process Analysis Statement

This thesis began as an extension on experiments previously conducted in the BIO 315 Methods in Cell Biology class at Ball State University. The class performed yeast growth assays on yeast peptone dextrose (YPD) plates containing hygromycin B. They used a wild type, control, and *ylr419wΔ* strain to compare how the knockout strain grew compared to the wild type strain. The results of the growth assays indicated that *YLR419W* confers resistance to hygromycin B. Kelsey Woodruff, a member of Dr. Rubenstein's lab at BSU, and I collaborated to validate and expand on these findings.

YLR419W is a yeast homolog for *DHX36*, a gene which encodes a G-quadruplex helicase, in humans. G-quadruplexes are structures formed in guanine-rich sequences in DNA and RNA that negatively regulate replication, transcription, and translation within a cell. G-quadruplex helicases, such as DHX36, unwind these structures. DHX36 binds and unwinds G-quadruplexes with great affinity, making it a possible target for therapeutics for many diseases. Because *YLR419W* is a yeast homolog for *DHX36*, studying the role of Ylr419w in yeast stress responses can inform our understanding of DHX36.

The aim of this study is to determine if knocking out *YLR419W* affects *Saccharomyces cerevisiae* growth and survival under stress. We hypothesize that when *YLR419W* is knocked out, *S. cerevisiae* will be less able to survive stress. In order to test this hypothesis, hygromycin B, heat, osmotic stressors, and hydrogen peroxide were chosen as stressors in these growth assays. Yeast were grown in the presence of each of these variables and analyzed to determine how growth was impacted by each stressor.

Wild type, an experimental control, and *ylr419w*Δ yeast were grown overnight. These yeast were then subjected to a 6-fold serial dilution and spotted onto YPD plates. Growth assays

were then incubated at 30°C for 2-5 days. A photograph of each of these growth assays was taken every day until growth was present in the last column of the plate. If after 5 days there was still no growth, then the plate was discarded. All growth assays were repeated in triplicate. Additional *ylr419w*Δ yeast clones were crossed in order to replicate these findings across multiple clones. Clone genotypes were confirmed using PCR.

Each growth assay procedure varied slightly based upon the stressor.

Proteotoxic Stress

Wild type, *rkr1*Δ*, hrd1*Δ*,* and *ylr419w*Δ yeast were grown overnight at 30°C and subjected to a 6-fold serial dilution. In this first experiment, wild type, *rkr1*Δ*,* and *hrd1*Δ are our control strains while *ylr419w*Δ is our experimental strain of interest. The control strains *rkr1*Δ and *hrd1*Δ were chosen for their role in conferring resistance to hygromycin B in *S. cerevisiae*. *rkr1*Δ yeast show a robust decrease in growth and *hrd1*Δ yeast show a moderate decrease in growth under proteotoxic stress. The wild type is the naturally occurring phenotype for *S. cerevisiae* and is resistant to hygromycin B at low levels. By including these controls, we can study the extent that hygromycin B impacts growth and ensure that the experiment ran as expected. These strains were then spotted onto YPD plates. These YPD plates included a range in the volume of drug added. The plates included 0 μg/mL, 25 μg/mL, 50 μg/mL, 75 μg/mL, 100 μg/mL, and 125 μg/mL hygromycin B. 0 μg/mL was included as a control plate to show "normal growth" in comparison to the plates containing an increasing concentration of hygromycin B. YPD plates with no hygromycin B, 25 μg/mL hygromycin B, and 50 μg/mL hygromycin B were incubated at 30°C for 2 days. YPD plates with 75 μg/mL were incubated for 3 days, while YPD with 100 μg/mL and 125 μg/mL hygromycin B were incubated at 30°C for 5 days. These growth

assays were imaged every day until growth was present in the last column of the plate. The variance in the number of days is to ensure growth is present in the last column of the plate before discontinuing the assay. Waiting until growth is present in the last column, the most dilute concentration of yeast plated, is to allow consistent comparison across plates. If after 5 days there was still no growth, then the plate was discarded.

Heat Stress

Wild type, *slt2*Δ, and *ylr419w*Δ yeast were grown overnight at 30°C, subjected to a 6-fold serial dilution and spotted onto YPD plates. These plates were incubated at 22°C, 30°C, 37°C, 39°C, and 42°C. YPD plates at 30°C, 37°C, and 39°C were incubated for 2 days. YPD plates at 22°C were incubated for 3 days, and YPD plates at 42°C were incubated for 4 days. These growth assays were imaged every day until growth was present in the last column of the plate. 30°C was included as a control plate to allow comparison of growth to that of yeast grown at increasing temperatures. Optimal growing temperature for *S. cerevisiae* is 28-33°C and thus 30°C was chosen as the temperature for incubation in all growth assays and the control for this experiment. 22°C was chosen to study how *ylr419w*Δ yeast grew at suboptimal temperatures, while 37°C, 39°C, and 42°C were chosen to study how *ylr419w*Δ grew under heat stress. The temperature of each incubator was monitored closely to ensure the temperature stayed consistent at the range stated.

Osmotic Stress

Wild type, *hog1*Δ, and *ylr419w*Δ yeast were grown overnight at 30°C and subjected to a 6-fold serial dilution. These yeast were then spotted onto YPD plates containing no stressor, 1.5

M NaCl, or 2.2 M sorbitol. YPD plates with no stressor, 1.5 M NaCl, and 2.2 M sorbitol were then incubated at 30°C for 3-4 days. “YPD plates + no osmotic stressor” was chosen as a control to allow comparison to YPD plates with an osmotic stressor. These growth assays were imaged every day until growth was present in the last column of the plate.

Oxidative Stress

Wild type, *fyv10*Δ, and *ylr419w*Δ yeast were grown in the presence of 0 µl H_2O_2, 0.6 µl H_2O_2, 2 µl H_2O_2, and 4 µl H_2O_2 at 30°C. These yeast were then subjected to 6-fold serial dilution, and spotted onto YPD. Yeast grown with 0 µl H_2O_2 were incubated at 30°C for 1 day while yeast grown at 0.6 µl H_2O_2, 2 µl H_2O_2, and 4 µl H_2O_2 were incubated at 30°C for 2 days. These growth assays were imaged every day until growth was present in the last column of the plate. The control strain *fyv10*Δ was chosen because Fyv10p has been shown to confer oxidative stress resistance.

Although before starting these experiments I knew that multiple replications were extremely important for reliable data, this thesis made this fact clear. Despite hours of work, ultimately the results of these experiments were not consistent across clone replicates. Although results were consistent within replicates using the same strain, results were inconsistent across different clones of the same knockout genotype. The data looked clean and clear in the original replicates performed by the Bio 315 class, in my growth assays, and again in my collaborator Kelsey Woodruff’s assays, but ultimately these results were not consistently replicated in other clones of the same strain. This thesis greatly emphasized the importance of validating results and maintaining healthy skepticism when conducting research.

The greatest challenge I encountered throughout this thesis was admitting when I needed help. Many of the experiments performed for this research project were completely new to me. Although I had an excellent collaborator, Kelsey Woodruff, to teach me how to perform these growth assays, I often struggled to ask questions or admit when I did not understand a concept. In my lab, and again in the Rubenstein lab, I was surrounded by people who seemed to know much more than me. Because of this, I often struggled to admit when I needed help due to fear of looking unintelligent to my peers. During this experience I was forced to admit when I needed help and to learn to not excessively stress over asking for help.

One thing that I learned about myself is that I learn best through tactile learning and repetition. I am not a visual or auditory learner in the slightest. However, I often try to learn new techniques from reading, then watching and listening to someone walk me through the process. This is great for a first introduction to a procedure, but through completing these experiments I found this was not the most practical or effective way for me to learn. I found that I learned a new technique best when I was briefly told what I was to do, then allowed to try it myself. My memory and understanding are best when I can physically perform the action or technique. Performing growth assays in triplicate was intended for accurate data, however this experimental design additionally served an enormous benefit for me as it allowed me to improve over time at the techniques necessary. From the first assay I completed until the last, I found that I felt dramatically more comfortable and competent. This realization about myself and my learning style will benefit me greatly as I continue my education into graduate school.

Overall, I believe my thesis signifies that these experiments will need to be further troubleshooted before accurate results can be analyzed. Due to time constraints and other projects occurring alongside this one, it was not practical to keep troubleshooting these assays.

Multiple attempts were made to create *ylr419w*Δ clone strains, however even after 12 clones were created and analyzed, no clear pattern emerged (Figures 5-6). Reasons why this may have been the case are discussed in the discussion and conclusion section of this thesis. Oftentimes experiments are 'abandoned' if they no longer are productive while other projects happening alongside them continue to demand more and more attention. This thesis is a reminder that experiments often do not go as planned. Sometimes we must decide whether it is more worthwhile to continue to pursue a project that would entail completely starting from scratch, or if our time would be better spent prioritizing more promising experiments. At the time this was a difficult decision to make, and I was disappointed to leave a project that I had become attached to, however it was no longer the best investment for me or my collaborator.

This thesis indicates that although we do not know how knocking out *YLR419W* affects *S. cerevisiae* growth and survival under stress, with further troubleshooting we can gain a better understanding of Ylr419w and therefore a better understanding of DHX36.

Effect of Knocking Out YLR419W on S. cerevisiae Growth Following Proteotoxic, Heat, Osmotic, and Oxidative Stress

IV. Background and Significance

G-quadruplexes are secondary DNA and RNA structures formed in guanine-rich sequences. When four guanines self-associate, they form a tetrad. Two or more of these tetrads then vertically stack to form a G-quadruplex structure[1]. G-quadruplexes negatively regulate replication[2], transcription[3], and translation[4] within a cell. G-quadruplex helicases unwind these structures in DNA and RNA, relieving the negative regulation[5]. *DHX36* is the major G-quadruplex helicase in human cells[6].

G-quadruplexes are involved in many diseases such as ALS[7], frontotemporal dementia (FTD)[8], and cancer[9], it is essential that we understand the enzymes that interact with these structures. Because *DHX36* binds and unwinds G-quadruplexes with great affinity[6], this enzyme may be a possible target for therapeutics for many diseases.

YLR419W is a yeast homolog for *DHX36*[10], a gene which encodes a G-quadruplex helicase, in humans[6]. By studying how knocking out *YLR419W* affects *Saccharomyces cerevisiae* growth and survival following cellular stress, we can learn more about the role of the protein Ylr419w in cell survival. *S. cerevisiae* is a suitable model organism because they are eukaryotic like humans, grow relatively fast, and show phenotypic changes allowing us to visualize the results of these experiments quickly. Yeast growth assays will be used to assess how a *YLR419w* deletion strain of yeast, (referred to hereafter as "*ylr419wΔ")*, compares to the wild type when grown with various stressors.

By studying *S. cerevisiae* under various stressors when *YLR419W* is knocked out, we can begin to determine the importance of *YLR419W* in the cellular stress response. Because *YLR419W* is the human homolog of *DHX36*[10], studying this enzyme's involvement in yeast stress responses can inform our understanding of DHX36. DHX36 plays a vital role in many diseases[789], so it is essential to study how this enzyme impacts a cell's ability to survive under stress. Therefore, understanding the role *YLR419W* in stress in yeast will inform how we understand DHX36 under stress conditions such as in disease in humans. I hypothesized that when *YLR419W* is deleted, *S. cerevisiae* will be less able to survive stress.

This project was completed in collaboration with Kelsey Woodruff of the Rubenstein lab at Ball State University.

V. Methods

The aim of this study is to determine if knocking out *YLR419W* affects *S. cerevisiae* growth and survival under proteotoxic, osmotic, oxidative, and heat induced stress.

*S. cerevisiae ylr419w*Δ strain genotypes were confirmed using PCR. The following primer sequences were used to genotype the *YLR419W* knockouts:

VJR420_YLR419W_T1: 5' TTTCTACCATGGTTGCTTTCG 3'

VJR421_YLR419W_B1: 5' TTGGCCCTTTTGTCATCTTC 3'

VJR259_KANB = 5' CTGCAGCGAGGAGCCGTAAT 3'

Additional *ylr419w*Δ yeast clones were created to confirm phenotypes across multiple clone lines. These clones were then genotyped via PCR to confirm *YLR419W* was successfully knocked out.

Wild type, experimental control, and *ylr419w*Δ yeast were grown overnight at 30°C, subjected to a 6-fold serial dilution, spotted onto YPD plates, and grown with a stressor. All experimental conditions were repeated in triplicate. Growth assays were imaged after 2-5 days of growth using a Bio-Rad Gel Doc XR+ Imager. Images of each of the three replicates were taken and a representative image was chosen from each condition. Specific experimental conditions for each stressor are outlined below.

Proteotoxic Stress

Wild type, *rkr1*Δ*, hrd1*Δ*,* and *ylr419w*Δ yeast were grown overnight, subjected to a 6-fold serial dilution and spotted onto YPD plates with 0 μg/mL, 25 μg/mL, 50 μg/mL, 75 μg/mL, 100 μg/mL, and 125 μg/mL hygromycin B. YPD plates with no hygromycin B, 25 μg/mL hygromycin B, and 50 μg/mL hygromycin B were incubated at 30°C for 2 days. YPD plates with 75 μg/mL were incubated for 3 days, while YPD with 100 μg/mL and YPD with 125 μg/mL hygromycin B were incubated at 30°C for 5 days. These growth assays were imaged every day until growth was present in the last column of the plate.

Heat Stress

Wild type, *slt2*Δ*,* and *ylr419w*Δ yeast were grown overnight, subjected to a 6-fold serial dilution and spotted onto YPD plates at 22°C, 30°C, 37°C, 39°C, and 42°C. YPD plates at 30°C, 37°C, and 39°C were incubated for 2 days. YPD plates at 22°C were incubated for 3 days, and YPD plates at 42°C were incubated for 4 days. These growth assays were imaged every day until growth was present in the last column of the plate.

Osmotic Stress

Wild type, *hog1*Δ, and *ylr419w*Δ yeast were grown overnight, subjected to a 6-fold serial dilution and spotted onto YPD plates containing no stressor, 1.5 M NaCl, or 2.2 M sorbitol. YPD plates with no stressor, 1.5 M NaCl, and 2.2 M sorbitol were then incubated at 30°C for 2-4 days. These growth assays were imaged every day until growth was present in the last column of the plate.

Oxidative Stress

Wild type, *fyv10*Δ, and *ylr419w*Δ yeast were grown in the presence of 0 μL H_2O_2, 0.6 μL H_2O_2, 2 μL H_2O_2, and 4 μL H_2O_2, subjected to 6-fold serial dilution, and spotted onto YPD. Yeast grown with 0 μL H_2O_2 were incubated at 30°C for 1 day while yeast grown at 0.6 μL H_2O_2, 2 μL H_2O_2, and 4 μL H_2O_2 were incubated at 30°C for 2 days. These growth assays were imaged every day until growth was present in the last column of the plate.

VI. Results

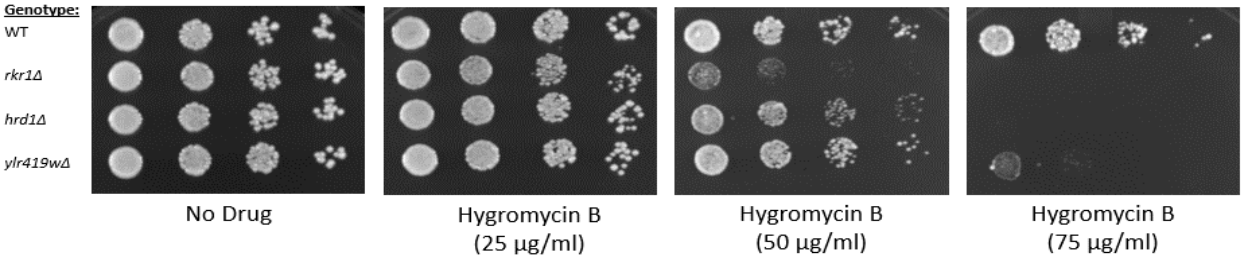

Figure 1: Deletion of *YLR419W* moderately inhibits growth under proteotoxic stress

Yeast of the indicated genotypes were subjected to a 6-fold serial dilution and spotted onto YPD plates with 0 μg/mL, 25 μg/mL, 50 μg/mL, 75 μg/mL, 100 μg/mL, and 125 μg/mL hygromycin B. YPD plates with no hygromycin B, 25 μg/mL hygromycin B, and 50 μg/mL hygromycin B

were incubated at 30°C for 2 days. YPD plates with 75 µg/mL were incubated for 3 days, while YPD with 100 µg/mL, and YPD with 125 µg/mL hygromycin B were incubated at 30°C for 5 days. No growth was observed on the 100 µg/mL and 125 µg/mL hygromycin B YPD plates after incubation for 5 days. The *ylr419wΔ* strain exhibited less growth compared to the wild type control strain under proteotoxic stress. This assay utilized the original strain created for these experiments, labeled as '*ylr419w*Δ' in **Figures 5-6**.

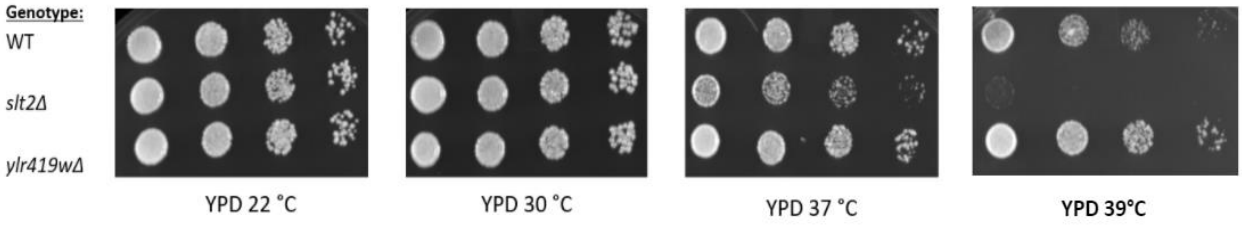

Figure 2: Deletion of *YLR419W* does not inhibit growth under heat stress

Yeast of the indicated genotypes were subjected to 6-fold serial dilution and spotted onto YPD plates at 22°C, 30°C, 37°C, 39°C, and 42°C. YPD plates at 30°C, 37°C, and 39°C were incubated for 2 days. YPD plates at 22°C were incubated for 3 days, and YPD plates at 42°C were incubated for 4 days. These growth assays were imaged every day until growth was present in the last column of the plate. No growth was observed at 42°C after incubation for 4 days. This assay utilized the original strain created for these experiments, labeled as '*ylr419w*Δ' in **Figures 5-6**.

Figure 3: Deletion of *YLR419W* does not inhibit growth under osmotic stress

Yeast of the indicated genotypes were subjected to a 6-fold serial dilution and spotted onto YPD plates containing no stressor, 1.5 M NaCl, or 2.2 M sorbitol. YPD plates with no stressor, 1.5 M NaCl, and 2.2 M sorbitol were then incubated for 2-4 days at 30°C. This assay utilized the original strain created for these experiments, labeled as '*ylr419w*Δ' in **Figures 5-6**.

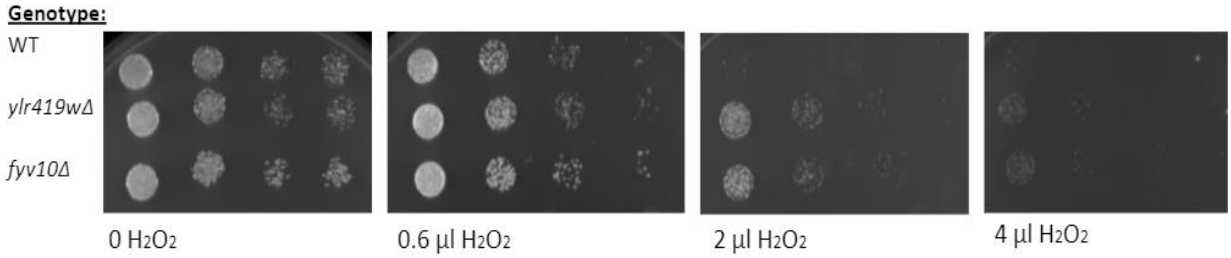

Figure 4: Deletion of *YLR419W* does not inhibit growth under oxidative stress

Yeast of the indicated genotypes were grown in the presence of 0 µL H_2O_2, 0.6 µL H_2O_2, 2 µL H_2O_2, and 4 µL H_2O_2, subjected to 6-fold serial dilution, and spotted onto YPD. Yeast grown with 0 µL H_2O_2 were incubated at 30°C for 1 day while yeast grown at 0.6 µL H_2O_2, 2 µL H_2O_2, and 4 µL H_2O_2 were incubated at 30°C for 2 days (all assays pictured here at day 1). This assay utilized the original strain created for these experiments, labeled as '*ylr419w*Δ' in **Figures 5-6**.

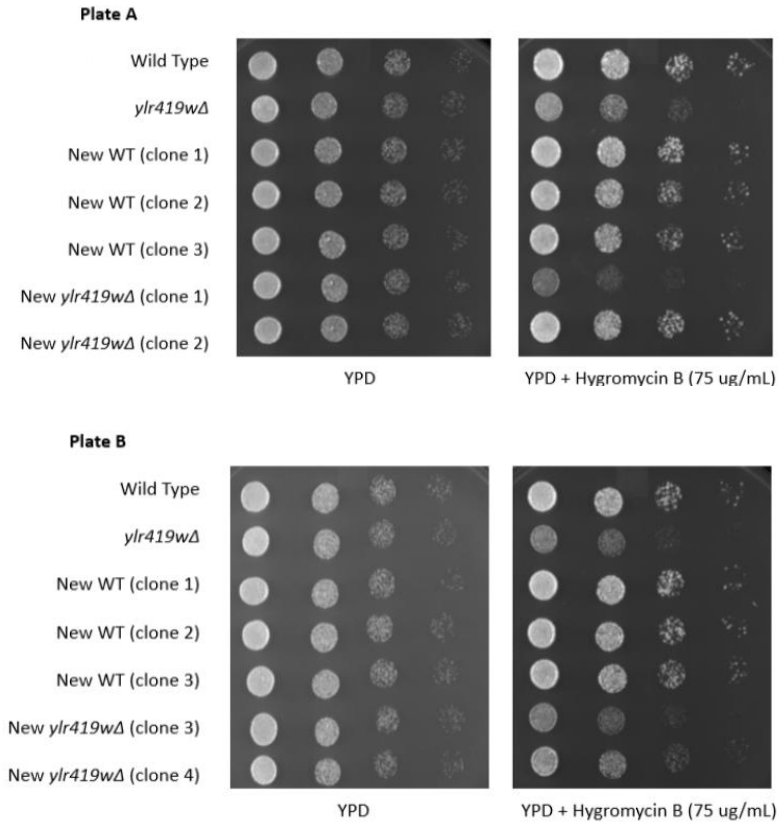

Figure 5: Deletion of *YLR419W* inconsistently inhibits growth under proteotoxic stress

Yeast of the indicated genotypes were subjected to a 6-fold serial dilution and spotted onto YPD plates with no hygromycin B and YPD + 75 ug/mL hygromycin B. Three out of four clones showed defective growth in presence of hygromycin B, comparable to what was seen in the original experimental strain (labeled here as *ylr419w*Δ).

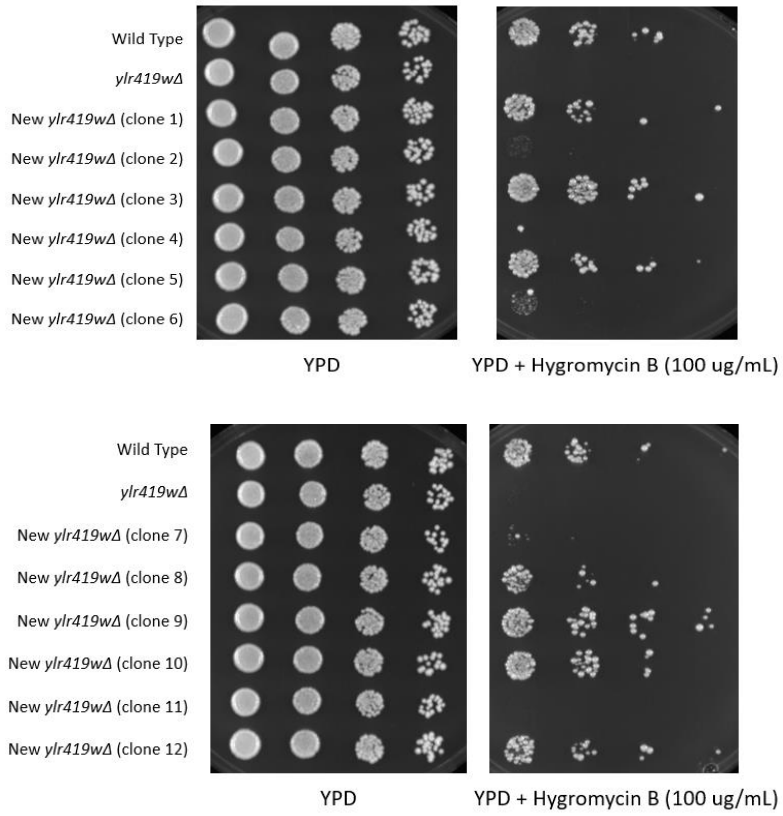

Figure 6: Deletion of *YLR419W* inconsistently inhibits growth under proteotoxic stress

Yeast of indicated genotypes were subjected to a 6-fold serial dilution and spotted onto YPD plates with no hygromycin B and YPD + 100 ug/mL hygromycin B. Newly generated strains were inconsistently sensitized to hygromycin B. Six out of the twelve clones showed defective growth in presence of hygromycin B, comparable to what was seen in the original experimental strain (labeled here as *ylr419w*Δ).

VII. Discussion

Deletion of *YLR419W* moderately inhibits growth under proteotoxic stress (**Figure 1**). Hygromycin B is a drug that causes the misreading of mRNA leading to the accumulation of misfolded and nonfunctional proteins[11]. By growing *S. cerevisiae* on plates containing increasing concentrations of hygromycin B, we can analyze how this organism is able to degrade these

misfolded proteins without the protein Ylr419w. Hrd1 and Rkr1 deletion strains were used as controls because of their known sensitivity to proteotoxic stress, allowing us to compare their growth to the Ylr419w deletion strain. The *hrd1*Δ strain has been documented to show a moderate decrease in growth, while the *rkr1*Δ strain demonstrates a more robust decrease in growth[11]. The effects of *Ylr419W* deletion on *S. cerevisiae* growth on increasing concentrations of hygromycin B, was used as a readout of aberrant and misfolded protein degradation to study the role of Ylr419w in proteotoxic stress. This experiment suggests Ylr419w contributes to increased survival and growth under proteotoxic stress.

Contrary to my hypothesis, the results in **Figure 2** indicate that deletion of *YLR419W* does not inhibit growth under heat stress. *ylr419w*Δ yeast grew relatively the same as the WT strain at 22°C, 30°C, and 37°C. *ylr419w*Δ yeast grew better at 39°C than the WT strain, while both strains failed to grow at 42°C. *slt2*Δ was chosen as a control because of its documented role in heat stress[12]. This experiment suggests Ylr419w does not facilitate growth or survival under heat stress. The 39°C assay suggests that yeast without Ylr419w grow better under heat stress than yeast with the Ylr419w protein.

Deletion of *YLR419W* does not inhibit growth under osmotic stress **(Figure 3).** *ylr419w*Δ yeast grew relatively the same as the WT strain when grown on YPD plates containing 2.2 M sorbitol and grew slightly better than WT when grown at 1.5 M NaCl. *hog1*Δ was chosen as a control because of its known role in osmotic stress; without Hog1, yeast are more sensitive to changes in osmolarity[13]. This assay suggests Ylr419w does not facilitate growth or survival under osmotic stress. The 1.5 M NaCl assay suggests that yeast without Ylr419w grow better under osmotic stress than those with the Ylr419w protein.

Deletion of *YLR419W* does not inhibit growth under oxidative stress **(Figure 4)**. *ylr419w*Δ yeast grew better than the WT and relatively the same as the *fyv10*Δ strain at 2 μL H_2O_2 and 4 μL H_2O_2. *fyv10*Δ was chosen as a control because *fyv10*Δ yeast have increased resistance to the effects of H_2O_2 and oxidative stress[14]. This experiment suggests Ylr419w does not facilitate growth or survival under oxidative stress. This assay suggests that yeast without Ylr419w have increased resistance to the effects of H_2O_2 and oxidative stress than those with the Ylr419w protein.

Deletion of *YLR419W* inconsistently inhibits growth under proteotoxic stress **(Figures 5-6)**. Four new additional *ylr419w*Δ clone strains were crossed and genotyped via PCR to confirm *YLR419W* was successfully knocked out, however these clones showed varied responses under proteotoxic stress. Three out of the four new *ylr419w*Δ clones (clones 1, 3, and 4) showed defective growth in the presence of hygromycin B, comparable to what was seen in the original experimental strain (labeled here as *ylr419w*Δ) and in **Figure 1**. Clone 2 showed normal growth under proteotoxic stress when compared to the wild type. YPD + 75 ug/mL hygromycin B and YPD + 100 ug/mL hygromycin B plates were chosen because these conditions showed the clearest differences between the experimental and control strains in the original assay testing for the effects of proteotoxic stress (**Figure 1**). Three new wild type clones were created as a control, and each showed similar growth to the original WT strain. This assay was run in triplicate, but due to these inconsistencies the assay seen in **Figure 6** was run as well.

Twelve new *ylr419w*Δ clone strains were crossed and genotyped via PCR to confirm *YLR419W* was successfully knocked out, however these clones showed varied responses under proteotoxic stress, similar to the results seen in **Figure 5**. Six out of the twelve new *ylr419w*Δ clones (clones 2, 4, 6, 7, 8, and 11) showed defective or decreased growth in presence of

hygromycin B, comparable to what was seen in the original experimental strain (labeled here as *ylr419w*Δ) and in **Figure 1**. Six out of the twelve new *ylr419w*Δ replicates (clones 1, 3, 5, 9, 10, and 12) showed normal growth under proteotoxic stress when compared to the wild type. These newly generated *ylr419w*Δ strains were inconsistently sensitized to hygromycin B; deletion of *YLR419W* inconsistently inhibits growth under proteotoxic stress (**Figures 5-6**).

These inconsistent results indicate that the *ylr419w*Δ strains were likely not true knockouts. This could have been due to the targeting construct used to knockout *YLR419W* being inserted elsewhere in the genome by "mistake". Another possible explanation is that one of the parental yeast strains that was crossed to create the *ylr419w*Δ strains had accumulated a separate mutation that went undetected. This mutation was then passed to some of the offspring and would explain the varying phenotypes seen in **Figures 5-6**.

Deletion of *YLR419W* in experimental clone 1 moderately inhibited growth under proteotoxic stress, and did not inhibit growth under thermal stress, osmotic stress, or oxidative stress (**Figures 1-4**). Proteotoxic stress results were not replicable however across additional *ylr419w*Δ clone strains (**Figures 5-6**). Further testing is needed in order to determine the role of *YLR419W* in the growth and survival of *S. cerevisiae.*

VIII. Conclusion

I hypothesized that when *YLR419W* is deleted, *S. cerevisiae* would be less able to survive stress. However, deletion of *YLR419W* in *S. cerevisiae* caused inconsistent growth results across clone replicates. Although knocking out *YLR419W* in experimental clone 2 moderately inhibited growth under proteotoxic stress (**Figure 1**), these results were not repeatable in other clone lines (**Figures 5-6**). This likely indicates that the *ylr419w*Δ strains were not true *YLR419W* knockouts.

To troubleshoot these assays, new *ylr419w*Δ strains can be crossed from new parental strains to achieve a true knockout. These knockout strains can again be tested via PCR and the resulting phenotypes compared to ensure consistent sensitization. The variables used here, proteotoxic stress, thermal stress, osmotic stress, and oxidative stress can be utilized in growth assays using these newly generated strains. Additional growth assays using arsenate, UV light, and varying pH concentrations can be conducted to further test how Ylr419w impacts cell growth and survival. Based on the data seen here in **Figures 1-6**, we cannot conclusively determine how deletion of *YLR419W* affects cell survival. However future assays can be used to better understand the function of *YLR419W* in yeast, and thus better understand the human homolog *DHX36*.

IX. References

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