YKL222C in *Saccharomyces cerevisiae* does not interact with SUB1 in the RNA polymerase III pathway and *ykl222c* mutant is sensitive to hydrogen peroxide

Michael Geddati, Emma DiMartini, Maggie Scott and Dr. Elaine Frawley

Bio Lab 131 L2

ABSTRACT

The RNA polymerase III pathway is important because the proteins involved help regulate transcription of DNA and activate other proteins that can protect the DNA from hydrogen peroxide, which allow the cells to continue proliferating. In this experiment, hydrogen peroxide was utilized to initiate this pathway to determine if *YKL222C* plays a role as a transcriptional factor. In order to determine if there is an effect of hydrogen peroxide on yeast cells with *YKL222C* and *ykl222cΔ::URA3*. These cells were placed on plates that were either exposed or unexposed to the 3mM of hydrogen peroxide. These plates were then observed under a microscope to determine the CFU. The data gathered displayed that yeast cells with *YKL222C* decreased in growth. *ykl222cΔ::URA3* had a similar amount of growth with and without the stress condition while the growth of the wild-type decreased in the presence of hydrogen peroxide. The morphology of the cells was unaltered as well. From the results obtained, it was understood that *YKL222C* is not found in this pathway since growth should have been increased when hydrogen peroxide is present. Due to the unexpected results of this experiment, another experiment could be conducted by using a different stress condition to determine the function of *YKL222C*.

INTRODUCTION

In the genome of the yeast species *Saccharomyces cerevisiae*, there are many genes whose functions have been determined but just as many that are yet to be determined. The purpose of this project is to find the unknown function of a particular gene, which will help other researchers and scientists with their own study of genes in this genome as well as genomes of other species. Most cells, including yeast cells, are constantly having their DNA and proteins damaged by many natural factors, such as oxidative stress. The presence of an oxidative stress, such as hydrogen peroxide, can activate the transcription of other genes and proteins which prevent DNA from degradation. These genes and proteins can be found in the RNA polymerase III pathway. In the RNA polymerase III pathway, proteins help regulate transcription of DNA and activate other proteins that can protect the DNA from hydrogen peroxide, which allow the cells to continue proliferating.

When there is DNA damage caused by hydrogen peroxide (H_20_2), there are breaks, called DNA double-strand breaks, in the DNA that need to be repaired (Driessens et al, 2006). With the aid of *RAD2*, *SUB1* is able to allow transcription to occur by interacting with other proteins, playing a role in peroxide resistance. However, SUB1 also requires other proteins that promote transcription. These proteins include *RB1*, *TFIIB*, and *SNAPc* (The Rat Genome Database, 2015). *RB1* codes for the retinoblastoma protein that acts as a repressor, controlling the rate of cell growth and also influences survival by activating other proteins (Giacinti & Gioradano, 2006). *TFIIB* is a transcription factor that promotes transcription by allowing RNA polymerase to bind to the promoter (Buratowski et al, 1993). *SNAPc* is a complex that binds to the proximal

elements, which are necessary for transcription to begin (EMBL-EBI). These key proteins aid in the process of transcription when cells become exposed to hydrogen peroxide.

In the genome of *S. cerevisiae*, *YKL222C* is a gene found in Chromosome XI with a length of about 2,118 base pairs (Saccharomyces Genome Database). Based on information from the Conserved Domain Database Search (CDD), a gene homologous in sequence was found that helped to find the possible structure of *YKL222C* (National Center for Biotechnology Information). *GAL4*, a DNA-binding transcription factor, contains a zinc-finger (MacPherson, 2006). Therefore, since *YKL222C* and *GAL4* are homologous in sequence, it is highly likely that the two structures are similar as well. Since zinc-fingers aid in the binding of the protein to DNA or RNA, it can be concluded that *YKL222C* codes for a protein that binds to either DNA or RNA (Laity et al., 2001). Another gene that genetically interacts with *YKL222C* is *SUB1*. *SUB1* is a transcriptional regulator that allows for the elongation of mRNA to occur by modifying RNA polymerase II, which catalyzes transcription. It also plays a role in peroxide resistance with the help of *RAD2,* which repairs the damaged DNA that could be caused by oxidative stress (Wang et al, 2004). Since *YKL222C* has a genetic interaction with *SUB1*, which binds to RNA, there is a high possibility that *YKL222C* first binds to DNA, where SUB1 can then interact with it while RNA is being created.

To determine how accurate the number of nuclear proteins actually are compared to the predicted number, the NucPred score was calculated through Stockholm Bioinformatics Center by using the protein sequence of *YKL222C*. The NucPred score displayed a score of 0.82, meaning that it is very likely that this gene is present in the nucleus (Heddad et al). In addition, the Yeast GFP Fusion Localization Database, which finds the possible localization of YKL222C,

showed that the gene was present in the nucleus (Yeast GFP Fusion Localization Database, 2006). From this evidence, it is highly likely that *YKL222C* is present in the nucleus, where it uses its zinc finger to bind to DNA and aids in the process of transcription when hydrogen peroxide is present.

From all of the information gathered, *YKL222C* may be a transcriptional regulator that binds to DNA, located in the nucleus. Since *YKL222C* shares a common sequence with *GAL4* and a genetic interaction with *SUB1*, it is highly likely that *YKL222C* contains zinc fingers that bind to DNA while *SUB1* plays a role with *RAD2* in the resistance of hydrogen peroxide in the RNA polymerase III pathway. Therefore, if hydrogen peroxide is present, then *YKL222C* will become activated, allowing it to bind to DNA, where *SUB1* can then bind to allow transcription. A possible outcome of this experiment is that the wild-type would increase when hydrogen peroxide is present while there would be a decrease when *ykl222c*Δ*::URA3* is present in the yeast cell. However, the null hypothesis would be that there is no effect due to the presence of hydrogen peroxide.

In order to conduct this experiment, *S. cerevisiae* colonies are plated with the stress condition, hydrogen peroxide, as well as plates without it. A comparison of the sizes of the colonies present on the plates with and without the stress condition will be used to measure the results. These results will help to determine if the hypothesis is supported or not. If there is more growth of the wild-type on the plate with hydrogen peroxide, then it would indicate that *YKL222C* plays a role as a transcriptional regulator in the RNA polymerase III pathway However, if there is no growth of the wild-type on the plate with and without the stress, then it would indicate that *YKL222C* does not play a role in peroxide resistance, unlike *SUB1*.

From this experiment, there was a decrease of the wild-type under hydrogen peroxide whereas *ykl222cΔ::URA3* was not affected and was able to grow to the same capacity both with and without hydrogen peroxide. There was no change in morphology when hydrogen peroxide was or was not present.

METHODS

Yeast strains and growth conditions

All yeast strains were obtained from Open Biosystems (Fisher Scientific Incorporated) or were constructed in this work. Yeast were grown using standard growth conditions (Sherman et al, 1989) unless otherwise indicated. Cultures were grown in synthetic complete media lacking uracil for selection of gene knockouts or on rich YPD media. Standard growth temperature was at 30ºC. Growth of yeast at elevated temperature was at 37ºC. Yeast BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) was considered WT and used for the construction of all *YKL222C* deletion strains used in this study.

YKL222C deletion construction

A deletion cassette containing the *URA3* gene flanked by *YKL222C* sequences was obtained by PCR amplification. Plasmid pRS416 was used as template and primers were designed to incorporate 60 nucleotides upstream of *YKL222C* at one end of the PCR product and 60 nucleotides downstream of *YKL222C* at the other. PCR product was used to transform yeast strain BY4741 using the Frozen-EZ Yeast Transformation II Kit (Zymogen Inc., T2001). Confirmation of deletion of *YKL222C* was determined by detection of PCR product using primers with homology to sequences internal to *URA3* and downstream of *ykl222cΔ::URA3* sequences used for integration.

CFU Spot Assays

Each strain used in this experiment was used to create a yeast suspension. The yeast suspensions were created by mixing each strain in a tube containing sterile water. The optical density at a wavelength of 600 for each suspension needed to be at 0.1 ± 10%, where it was about 0.089 for wild-type and 0.102 for *ykl222c*Δ*::URA3*. Each set of dilutions was replicated four times with each replicate having a dilution factor of 1x, 10x, 100x, 1000x, and 104x. The dilutions were placed on each of the four yeast plates with and without the stress condition under 30°C and 38°C. These plates were then incubated for two days.

Statistics

Microsoft Excel was used to analyze the data in a two-way ANOVA table. The logarithm of each value was first calculated. The average number of colonies and the standard deviations for each group were calculated. The sum of squares for the genotype, environment, residual, and total were calculated using DEVSQ for each appropriate data set. The sum of squares for GXE was then calculated by subtracting the remaining sum of squares in the table. The degrees of freedom of the genotype, environment, and the total category were calculated by subtracting one from the number of groups used to calculate the sum of squares in its appropriate category. The degree of freedom of GXE is the product of the degrees of freedom of the genotype and the environment, and the degree of freedom of the residual is the difference in the product of the number of groups in the genotype and the environment and the total number of data points in the entire data set. The mean squares were calculated by finding the quotient of the sum of squares and its degree of freedom for each category, excluding the total. The F-ratios were calculated by finding the quotient of the mean of squares and the mean of squares of the residual for each

category, excluding the residual and total. The p-values were calculated by implementing an f-distribution of the f-ratio, the degrees of freedom, and the degree of freedom of the residual for each category, excluding the residual and the total, which will determine the significance of the interaction.

Morphology Assay

Using the plates from the desired temperature, yeast cells from each plate were taken and mixed with sterile water. These samples were then examined under a microscope at a magnification of 40x to analyze the cells by determining the percentage of buds present, the size, and other notable phenotypes.

RESULTS

ykl222cΔ::URA3 leads to no effect on yeast growth to hydrogen peroxide

Mutations in the RNA polymerase III pathway are predicted to significantly decrease yeast growth in the presence of hydrogen peroxide. In order to make sure this outcome is accurate, yeast cells with *YKL222C* and *ykl222cΔ::URA3* were exposed to 3mM of hydrogen peroxide (Figure 1). It was observed that the wild-type strain is sensitive to hydrogen peroxide, where the average CFU in logarithmic form of the four replicates decreased from 3.9 to 3.4. However, the CFU average of yeast cells with *ykl222cΔ::URA3* remained the same at around 4.2 with and without hydrogen peroxide (Figure 2). The p-value for the genotype is less than 5%, which displays a significant impact by the genotype of the strain only (Table 1). Knowing information about the morphology could determine how hydrogen peroxide can affect these yeast cells.

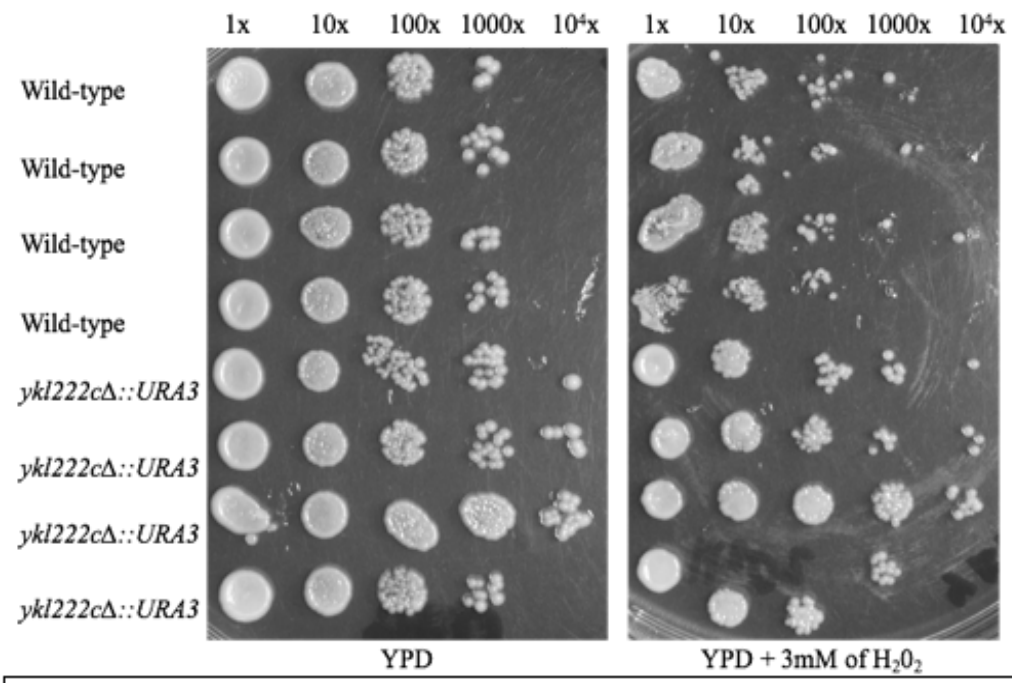

Figure 1. CFU of the wild-type in *S. cerevisiae* decreased in the presence of the 3mM of hydrogen peroxide at 38˚C. The wild-type strain, first four rows, contains YKL222C and lacks *URA3* while *ykl222cΔ::URA3* contains only *URA3*. A 10-fold dilution of the yeast was created. The dilution factors are shown at the top of each plate. The two plates either contained 3mM of H_2O_2 or it did not and were incubated for two days.

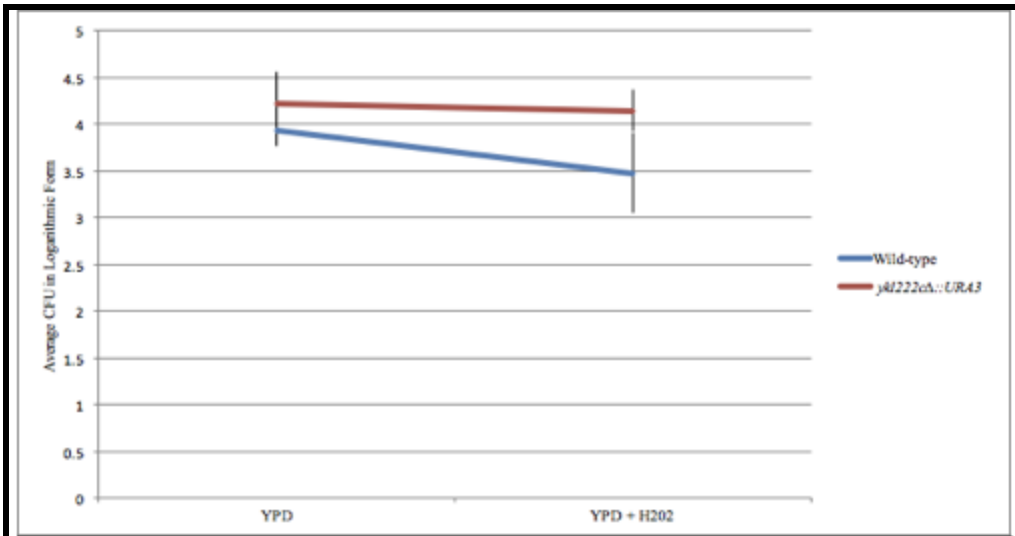

Figure 2. Wild-type of *S. cerevisiae* is sensitive to the 3mM of H_2O_2, where there was a decrease in the average CFU but no effect on yeast cells with *ykl222cΔ::URA3*. The wild-type strain contains YKL222C and lacks *URA3* while *ykl222cΔ::URA3* contains only *URA3*. The x-axis displays the environment (YPD or H_2O_2) and the y-axis shows the average CFU in logarithmic form. The standard deviations of the wild-type and *ykl222cΔ::URA3* with and without hydrogen peroxide are denoted as error bars.

ANOVA @ 38°C					
Source	SS	dF	MS	F-ratio	p-value
Genotype	0.927983407	1	0.927983407	10.05487057	0.008055529
Environment	0.191999185	1	0.191999185	2.080346419	0.174794683
GXE	0.236001181	1	0.236001181	2.557116124	0.135781075
Residual	1.107503155	12	0.09229193		
Total	2.463486929	15			

Table 1. The two-way ANOVA table displays that there is a significant effect of genotype. In the genotype category, there were two groups (wild-type and *ykl222cΔ::URA3*). A red box indicates a significant value; $p<0.05$ was the cutoff for significance.

No effect on morphology due to hydrogen peroxide

A morphology experiment was used in order to determine if *YKL222C* has a morphological effect by hydrogen peroxide. These cells were placed under a microscope to examine their morphology. There was no difference in the morphology of the cells. They remained the same size in the presence and absence of hydrogen peroxide (Figure 3).

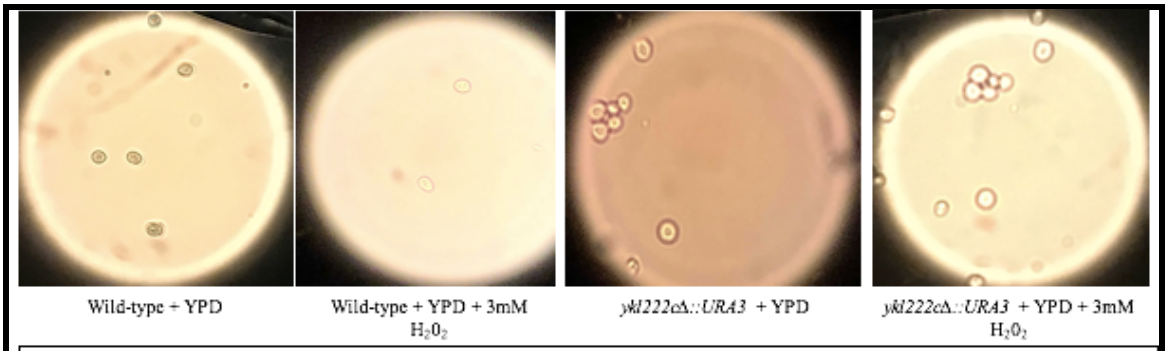

Figure 3. There is no effect on the yeast cells' morphologies that were and were not exposed to 3mM of hydrogen peroxide. The wild-type strain contains YKL222C and lacks *URA3*. These plates were incubated at 38˚C and these images were taken using a 40x magnification.

DISCUSSION

For this experiment, YKL222C is possibly a transcriptional regulator that binds to the DNA found in the nucleus. This hypothesis came about due to the fact that a gene homologous in its sequence is *GAL4*, which is a DNA-binding transcription factor that contains a zinc-finger (MacPherson, 2006). Since *YKL222C* interacts with *SUB1*, which is a transcriptional regulator that allows for the elongation of mRNA to occur by modifying RNA Polymerase III, *YKL222C* likely aids in the process of transcription as well. At the same time, *SUB1* catalyzes transcription and plays a role in peroxide resistance with the help of *RAD2,* which repairs the damaged DNA that could be caused by oxidative stress (Wang et al, 2004).

From the results collected, the 3mM of hydrogen peroxide only affected the wild-type, where there was a decrease in growth (Figure 1 and Figure 2). This correlation determines that hydrogen peroxide does not play a role as a transcription regulator since there should have been an increase in growth when *YKL222C* was present. In addition, only the genotype had a significant value, establishing that the growth of these cells was based solely on whether or not *YKL222C* was present and not the environment, which is the hydrogen peroxide (Table 1). These p-values determine that only the genotype of the gene affected growth of the cells. There was also no effect on morphology, showing that hydrogen peroxide does not affect the size of the yeast cells containing *YKL222C* or *ykl222c*Δ*::URA3*. Therefore, the results did not support what was expected to happen since there was a decrease in growth of the yeast cells with *YKL222C* and equal growth of yeast cells with *ykl222c*Δ*::URA3*. This experiment did not give results to support the idea that *YKL222C* is a transcriptional regulator. These unexpected results lead to questions regarding the function of *YKL222C*, the reasoning as to why the growth of the wild-type decreased when in contact with hydrogen peroxide, and why YKL222C is unnecessary for a cell to function. Furthermore, the RNA polymerase III pathway may not need *YKL222C* to function properly.

A possible further experiment is to use a different stress condition instead of hydrogen peroxide to determine how the growth of *S. cerevisiae* is affected. Using a different stress condition, such as caffeine or cycloheximide, could possibly affect growth in a different way than the way hydrogen peroxide did, perhaps in the way that we hypothesized. Using such a stress condition could initiate a different pathway that may determine a possible function of *YKL222C*.

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