

The interconnection between oxidative stress, genomic instability, mitotic asymmetry, and chronological life span in *Saccharomyces cerevisiae*

by

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## ABSTRACT

Cellular aging in *Saccharomyces cerevisiae* can lead to genomic instability and impaired mitotic asymmetry. Here, we focus on the role of oxidative stress on genomic instability and mitotic asymmetry. We treated yeast cells from a collection of natural isolates with hydrogen peroxide ( $H_2O_2$ ), and monitored the frequencies of loss of heterozygosity (LOH) in response to  $H_2O_2$  concentration. We found that the increase of  $H_2O_2$ -dependent genomic instability occurs before a drop in viability. This leadoff is inversely proportional to cells' ability to maintain homeostasis despite substantial  $H_2O_2$ -induced DNA damage. We previously observed that elevation of genomic instability generally lags behind the drop in viability during chronological aging. Hence, hydrogen peroxide treatment and chronological aging lead to opposite timing of genomic instability with regards to viability. This contrast argues that the effect of oxidative stress on genome integrity is well suppressed up to the dying-off phase during chronological aging. We then found that the leadoff of genomic instability to viability is negatively correlated with chronological life span, with an R-squared of 0.54 and a p-value of 0.024, indicating that cells' ability to maintain homeostasis despite substantial  $H_2O_2$ -induced DNA damage is positively correlated with chronological life span. Surprisingly, this leadoff is positively correlated with a measure of endogenous mitotic asymmetry with an R-squared of 0.43 and a p-value of 0.054, indicating a trade-off between mitotic asymmetry and cell's ability to fend-off  $H_2O_2$ -induced oxidative stress. Overall, our results demonstrate strong associations between oxidative stress, genomic instability, and mitotic asymmetry within the context of aging.

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## Introduction

Aging is a phenomenon found in all eukaryotic organisms. Benjamin Gompertz, a British mathematician circa the early nineteenth century, first quantitatively defined biological aging as the exponential increase of mortality rate over time (GOMPERTZ 1825). In essence, this is a statistical definition asserting that the probability of dying increases with age (DEFOSSEZ *et al.* 1998). To most biologists, aging is a phenotype that can be seen as declining of fitness over time.

Aging is generally believed to be a complex trait that is influenced by many genes, as argued by the antagonistic pleiotropy theory (WILLIAMS 1957) and the disposable soma theory (KIRKWOOD 1977). From the evolutionary perspective, natural selection likely acts upon young individuals, and advantages early life will inevitably lead to detrimental effects later in life (WILLIAMS 1957). Huntington Disease, for example, is a genetic disease characterized by the decline in the functionality of the central nervous system. Because of its late onset, individuals with the disease can pass deleterious alleles to progeny before their natural death (CONNALLY 1984; FARRER *et al.* 1984). This kind of trade-off between early and late life is also the central argument of the disposable soma theory (KIRKWOOD 1977). Hence, aging is a conserved fundamental biological phenomenon because of differential selection on individuals in age-structured populations during evolution (CHARLESWORTH 1994).

The effect of calorie restriction (CR) on life span is conserved across several domains of life. CR has been shown to extend life span in yeast, nematodes, rodents, and humans. One argument for the ubiquitous effect of CR is that most species have only sporadic access to nutrients in their natural environment. Hence, natural selection

favors those individuals that can delay reproduction and prolong survival in response to limited nutrients during the boom/bust cycles of evolution (HARRISON and ARCHER 1989; HOLLIDAY 1989; KOUBOVA and GUARENTE 2003).

A thirty-six year follow-up study in 2000 non-smoking Japanese-American men also supports the proposal that CR can extend life span. It showed that caloric intake that was 15% less than the average extended life span (WILLCOX *et al.* 2004). The Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) is a longitudinal study conducted in humans to determine whether CR data aligns with reduced health-related consequences of aging. Phase I of CALERIE revealed that humans with 25% less caloric intake over a 6-month period had reduced levels of LDL, substantial weight loss in subjects, and fewer DNA damages caused by oxidative stress. All of these factors, when elevated, have been linked to the development of cardiovascular disease and other age-related diseases (DAS *et al.* 2007).

These studies also align with data that show a correlation between obesity and premature death. Dietary habits that involve excess caloric intake are associated with shorter life span whereas individuals that have moderate eating habits live longer (STANFEL *et al.* 2009).

All living organisms are comprised of a single or multiple cells. The processes undergone by individual cells will ultimately affect the organism as a whole. If these processes cause a decline in cell fecundity, this can lead to detrimental consequences for the entire organism.

The free radical theory of aging is an accepted mechanistic explanation for aging in eukaryotic organisms (HARMAN 1956). This theory suggests that biological systems age because free radicals react with macromolecules and disturb key pathways that are vital to maintaining the overall functional and genomic integrity of cells (YU *et al.* 2012).

Reactive oxygen species (ROS), a form of free radicals, are natural by-products of the mitochondrial respiratory chain.  $H_2O_2$ , for example, is generated via oxidase-catalyzed reactions. The endogenous level of ROS also plays a role in signaling transduction, defense, and other normal cell functions (BLAGOSKLONNY 2008). Thus, low levels of  $H_2O_2$  and ROS can be beneficial to the cell (RAHMAN 2007; WEINBERGER *et al.* 2010) (Figure 1).

However, superoxide,  $H_2O_2$ , and singlet oxygen can also oxidize lipids, proteins, and nucleic acids. Over time, the accumulation of these molecules can lead to oxidative stress (MORADAS-FERREIRA *et al.* 1996). The non-replacement of impaired cellular sites induced by these destructive molecules can result in altered metabolic homeostasis and abnormal cell growth, which can onset the aging process (HARMAN 1956; RISTOW and SCHMEISSER 2011). The positive correlation between age and the increased probability of developing disease can serve as evidence of detrimental effect of the loss of genomic integrity (GRAVEL and JACKSON 2003; McMURRAY and GOTTSCHLING 2003; McMURRAY and GOTTSCHLING 2004).

Cellular aging is the basis of physiological aging, in consistence with the free radical theory. The budding yeast, *Saccharomyces cerevisiae*, is an effective model to study cellular aging. *S.cerevisiae* is a eukaryotic fungus that has served as a paradigm

in aging research. The life span of budding yeast can be quantified under experimental conditions over short periods of time.

Budding yeast have a replicative life span (RLS) and a chronological life span (CLS). RLS and CLS are distinguished by the ways in which life span is measured. CLS measures the amount of time required for a single mother cell to stop replication. RLS refers to the number of times a cell undergoes the cell cycle (DEFOSSEZ *et al.* 1998; QIN and LU 2006; WEI *et al.* 2008).

In yeast, CR can extend both replicative and chronological life spans. Wei *et al.* reported a 10-fold increase in the life span of calorie restricted *S. cerevisiae* mutants that had deleted *RAS2* and *SCH9* genes. The life span extension effect of *RAS2* and *SCH9* is shown to be mediated by Rim15 (WEI *et al.* 2008; WEINBERGER *et al.* 2010).

Most of the known genes related to life span are conserved in both humans and yeast; some of which include Sir2 and Tor1. The TOR (Target of Rapamycin) pathway has been shown to be involved in regulating cell growth, mitotic division, as well as nutrient response in both yeast and human (WEI *et al.* 2008).

Loss of heterozygosity has become a commonly used method for detecting loss of genome integrity in yeast (McMURRAY and GOTTSCHLING 2003; McMURRAY and GOTTSCHLING 2004). Heterozygosity on the *MET15* locus (*MET15*<sup>+/-</sup>) is achieved by knocking out one copy of the wild-type allele using a kanamycin-resistance marker. LOH can be monitored in *Saccharomyces cerevisiae* only when the *MET15*<sup>+/-</sup> is converted into a homozygous recessive form (*MET15*<sup>-/-</sup>) following mitotic division. When yeast strains are plated on medium containing lead, LOH occurs resulting in black sectors in cream-colored colonies. Thus, a *MET15*<sup>-/-</sup>-containing strain forms completely

black colonies. Colonies may have a brown tint, depending on the yeast strain used. Both dominant forms of the *MET15* gene (*MET15<sup>+/+</sup>*) and *MET15<sup>+/-</sup>* yield white or cream-color colonies. As a result, only fifty percent of LOH events are observed because these two genotypes are phenotypically indistinguishable. The number of cells that did not undergo LOH at the *MET15* locus is an indication of robustness, with respect to that specific locus (HIRAOKA *et al.* 2000; McMURRAY and GOTTSCHLING 2003). Thus, LOH can be used as a sign of genomic alteration on the *MET15* locus (Figure 2).

H<sub>2</sub>O<sub>2</sub>-induced damage triggers LOH through mitotic recombination (MR) when double-strand breaks are present on DNA. If DNA damage in one chromosome is detected, an intact allele on its homologous chromosome can replace the damaged allele by recombination. As a result, the goal of MR is to restore the genotype (HIRAOKA *et al.* 2000; McMURRAY and GOTTSCHLING 2003).

To study the role of ROS on LOH, we altered the intracellular level of H<sub>2</sub>O<sub>2</sub> through high exogenous H<sub>2</sub>O<sub>2</sub> levels and monitor its effect on LOH, and examined the characteristics of the H<sub>2</sub>O<sub>2</sub> dose-dependent changes of genome instability and viability in a collection of yeast natural isolates (Figure 3).

## Materials and Methods

### Yeast strains and Culturing

Strains with *Met15<sup>+/-</sup>* locus were grown overnight at 30°C in 5mLs of YPD using autoclaved glass tubes. Strains used with heterozygous *Met15<sup>+/-</sup>* were described previously (QIN *et al.* 2008)(Table 1). Following incubation, a spectrophotometer was used to determine saturation of yeast in the glass tubes at an absorbance of 600 nm



( $A_{600}$ ). The yeast culture was diluted to  $A_{600}$  0.6 in fresh YPD in new autoclaved glass tubes with a final volume of 4~6 ml. This diluted culture was grown in a 30°C shaker for an additional two hours, during which generally the absorbance reaches between 0.8 and 0.9. Cells were then harvested, transferred to 1.5 ml Eppendorf tubes, and centrifuged at maximum speed for 5 minutes. Following YPD decantation, cells were washed in an equal volume of double distilled water, vortexed, and centrifuged. Cells were washed two additional times. Cells were sonicated for 4 minutes. As control, some yeast samples were also sonicated using a point-sonicator.

### **H<sub>2</sub>O<sub>2</sub> Treatment**

The protocol used for this project follows the H<sub>2</sub>O<sub>2</sub> sensitivity test used in Yu et al. Stock solutions of 2X H<sub>2</sub>O<sub>2</sub> containing 0.3%, 0.2%, 0.15%, 0.1%, 0.075%, 0.05%, 0.025%, 0.01%, and 0.005%, and 0% H<sub>2</sub>O<sub>2</sub> were prepared. For each dilution, reaction was carried out in a 1.5 ml Eppendorf tube in which 4 µl of a 10X dilution of yeast cells, 16 µl of ddH<sub>2</sub>O, and 20 µl of the appropriate hydrogen peroxide dilution were added. The experiment was conducted under sterile conditions near a Bunsen burner. The Eppendorf tubes were vortexed and wrapped in parafilm. The tubes were incubated in a shaker for 3 hours at 30°C. The reaction was terminated by adding 960 µl of water (final dilution 50X) and chilled on ice. Eppendorf tubes were sonicated in a water bath for 2 minutes (Figure 4). 250 µl of each reaction mix was spread onto large MLA plates using sterile glass beads. If small plates are used, 150 µl of each sample of treated cells should be added to each plate. Plates were spread in triplicates for each H<sub>2</sub>O<sub>2</sub>

concentration (Figure 5). Plates were placed in a 30°C incubator overnight or for two additional days depending on observed growth.

### Counting Colonies

Images of each MLA plate were taken using a Colony Doc-It Imaging Station. Colonies were assessed for any notable characteristics and counted by color-sectoring patterns using a Bantex Colony Counter. The number of fully black, fully white, half black, quarter black, three-quarter black, quarter-quarter black, and others were documented. Color-sector patterns that were less than one-eighth were ignored (Figure 6).

### Data Analysis

As colonies were counted, all results were documented on formatted charts. Original data were then recorded in excel document with the information on strains, absorption values at  $A_{600}$ , dilution, date,  $H_2O_2$ percentage, number of white colonies, number of black colonies, number of half black colonies, number of quarter black colonies, number of three-quarter black colonies, number of quarter-quarter black colonies, the number of other color-sector patterned colonies, and any additional observations. The *R Language and Environment for Statistical Computing* used for data analysis.

## Results

The interconnection between oxidative stress, genomic instability, mitotic asymmetry, and chronological life span in *S. cerevisiae* was addressed using exogenous  $H_2O_2$  to induce an oxidative stress response. LOH assays on lead-containing plates were used to detect and quantify LOH during a yeast CLS. The primary objective of the study was to compare the  $H_2O_2$  dose-response curves of LOH and viability with the viability change in normal aging.

### **$H_2O_2$ -dose dependent changes of viability and LOH**

The  $H_2O_2$  dose-dependent change in viability and LOH are generally sigmoid (Figure 7). Half-black colonies indicated LOH occurred after cells have divided on MLA plates. The ratio of half-blacks versus full blacks can be viewed as an indicator of asymmetric partition of oxidative damage during mitosis. We observed much higher occurrence of half-black colonies in  $H_2O_2$ -induced LOH than those occurred in chronological aging, suggesting that elevating intracellular  $H_2O_2$  level can lead to breakdown of mitotic asymmetry.

Qin et al. measured biological aging with a logistical model using the ratio  $T_g/T_c$ .  $T_g$  represents the midpoint of the genome instability, which is measured by LOH.  $T_c$  represents the midpoint of chronological life span. With respect to the biological survival curve, the midpoint of genome instability comes after the midpoint of chronological life span (QIN *et al.* 2008). Thus, the biological survival curve will likely display a greater frequency of strains with  $T_g/T_c$  ratios at 1.0 or greater (Figure 8, 9). The logistical model for the hydrogen peroxide dose-response curve uses the ratio  $C_b/C_v$ .  $C_b$  represents the

middle concentration of black colonies, which is a measure of genome instability.  $C_v$  represents the middle concentration of viability. With respect to the dose-response curve,  $C_b$  usually comes before  $C_v$  in the strains used (Figure 8). The inverse of  $C_b/C_v$  thus represents the capability of cells to maintain viability after the increase of  $H_2O_2$ -induced LOH. For most natural isolates, this  $C_b/C_v$  ratio is lower than 1.0 (Figure 8, 9). This comparison suggests that endogenous levels of  $H_2O_2$  must be held low up to the dying-off phase during chronological aging.

A regression analysis suggested that strain sensitivity to  $H_2O_2$  varies. There is a significant association between CLS and the  $C_b/C_v$  ratio with a p-value of 0.024, and an R-squared value of 0.54. A longer CLS corresponds to a smaller  $C_b/C_v$ . A  $C_b/C_v$  value less than 1.0 indicates that  $C_b$  comes before  $C_v$ , and thus a greater dose of  $H_2O_2$  is required to kill the cell. Thus, strains with lower ratios are more tolerant to hydrogen peroxide with respect to viability. A  $C_b/C_v$  value greater than 1.0 indicates that  $C_b$  comes after  $C_v$ , and thus cells are more sensitive to hydrogen peroxide treatment. YPS128 seems to have the most sensitive response to hydrogen peroxide treatment whereas M13 seems to be substantially more tolerant to  $H_2O_2$  treatment (Figure 10).

### **Trade-off between tolerance to oxidative stress and mitotic asymmetry**

A regression analysis revealed that there is a positive correlation between  $C_b/C_v$  and  $L_0$ , which represents mitotic asymmetry. Mitotic asymmetry is the ratio of the number of daughter cells that have undergone LOH and the number of mother cells that have undergone LOH. The p-value representing this data is 0.055. This significant

association is supported by a relatively high R-squared value of 0.43. Cells with a smaller  $C_b/C_v$  seem to have a lower  $L_0$  (Figure 11).

## Discussion and Conclusions

Results from the  $H_2O_2$  dose-response curve are consistent with the biological survival curve in that cells with a higher tolerance ROS have longer chronological life spans (QIN *et al.* 2008). However, we report that the biological survival curve and the  $H_2O_2$  dose-response curve exhibit contrasting switching patterns, which suggests that there is opposite timing of genomic instability with regards to viability. Additionally, mitotic asymmetry is positively correlated with chronological lifespan.

### ***Assessment of Materials and Methods***

Previous results revealed that cells in their stationary phase were more resistant to oxidative stress. Strains were treated in their log phase so that differences in responsiveness to hydrogen peroxide would be more apparent. It would be more challenging to compare robustness or tolerance to hydrogen peroxide if all strains were resistant to oxidative stress.

After growth, the samples were sonicated to break clumps of cells and ensure uniform segregation of the cells. Cells were re-sonicated following  $H_2O_2$  treatment because the cells might clump together during the final incubation period. Without re-sonification, there would have been a higher number of half-black colonies on plates compared to fully black colonies, as shown by a previous protocol.

## **Assessment of Results**

We report that an increase in black colonies is relative to the viability drop. In biological aging, ROS must be low enough such that DNA damage is suppressed before there is a substantial drop in viability. Conversely, H<sub>2</sub>O<sub>2</sub> dosage has more of an immediate effect on the robustness of the cell. Viability drops more rapidly in H<sub>2</sub>O<sub>2</sub>-treated cells because ROS levels are increased via the external elevation of H<sub>2</sub>O<sub>2</sub> and the inhibition of superoxide dismutase (SOD) activity (WEINBERGER *et al.* 2010) (Figure 1).

Results also indicated that cells with better mitotic asymmetry have a longer life span. It is rare that budding produces two identical daughter cells. Daughter cells may harbor the same genetic information, but may have an uneven distribution of proteins and other intracellular molecules following cytokinesis. Mother cells may be able to harbor damages within themselves, thus limiting the amount of potentially toxic protein aggregates that are passed to daughter cells. An alternative possibility is that damages in daughter cells are returned to the mother cells before complete separation of the cells (LIU *et al.* 2010). This may explain why daughter cells with a lower mitotic asymmetry have a longer chronological lifespan and are able to maintain their replicative potential.

Future directions include testing gene deletion mutants with H<sub>2</sub>O<sub>2</sub>. *MSN2/4* has been shown to be vital to the pathway for extending CLS in yeast. This gene product functions by up-regulating genes that allow the cell to tolerate stress. SOD activity, for example, is increased and extends life span during this process, but can also reduce CLS if it is expressed excessively (MEDVEDIK and SINCLAIR 2007). Low concentrations of H<sub>2</sub>O<sub>2</sub> has been shown to increase CLS by increasing SOD activity. An increase in CLS

by  $H_2O_2$  is further increased in the stationary phase in yeast (MESQUITA *et al.* 2010). If the SOD gene is deleted, we should see similar  $C_v$  and  $C_b$  patterns to the ones reported in this current project. Under the same experimental conditions, deleting SOD and eliminating its action may also increase superoxide levels.

Further, we can assess the effect of CR or rapamycin on  $H_2O_2$ -induced LOH patterns. CR also increases respiration and boosts mitochondrial functions, decreases proton leakage and ROS production in the mitochondria (BARROS *et al.* 2004; LIN *et al.* 2002) and attenuates the accumulation of oxidative damage (REVERTER-BRANCHAT *et al.* 2004).

CR in the CLS paradigm was also found to increase cell's resistance to heat and oxidative stresses, prevent protein oxidative damage, reduce the level of iron and of lipid peroxidation, through high levels of catalase (Ctt1) and superoxide dismutase enzymes (Sod1, Sod2) (REVERTER-BRANCHAT *et al.* 2004). Hence, further study on CTT1, SOD1, SOD2, *isc1* mutants may be informative.

Finally strains will be treated with paraquat (methyl viologen) instead of  $H_2O_2$  in order to directly induce superoxide production (HANSSON and HAGGSTROM 1986). Paraquat was not used before because it is unstable in water, which would have been difficult to work with. It will be informative to compare LOH and viability pattern when cells are treated with these inducers. Superoxide levels when cells are treated with  $H_2O_2$  and paraquat will also be measured directly using a fluorescent probe.

***Implications on for Human Aging***

We have shown that oxidative stress, mitotic asymmetry, and the length of chronological lifespan are interconnected factors that contribute to understanding of cellular aging (Figure 12). Cellular aging is the basis of physiological aging in humans. For example, abnormal redox activities of hemoglobin and myoglobin have been linked with hypertension and kidney dysfunction, both consequences of aging (REEDER 2010). The chronological aging of yeast is a model of senescence in non-dividing cells. Hence, this study provides useful information on the cellular senescence in non-dividing cells in humans, such as erythrocytes and neurons.



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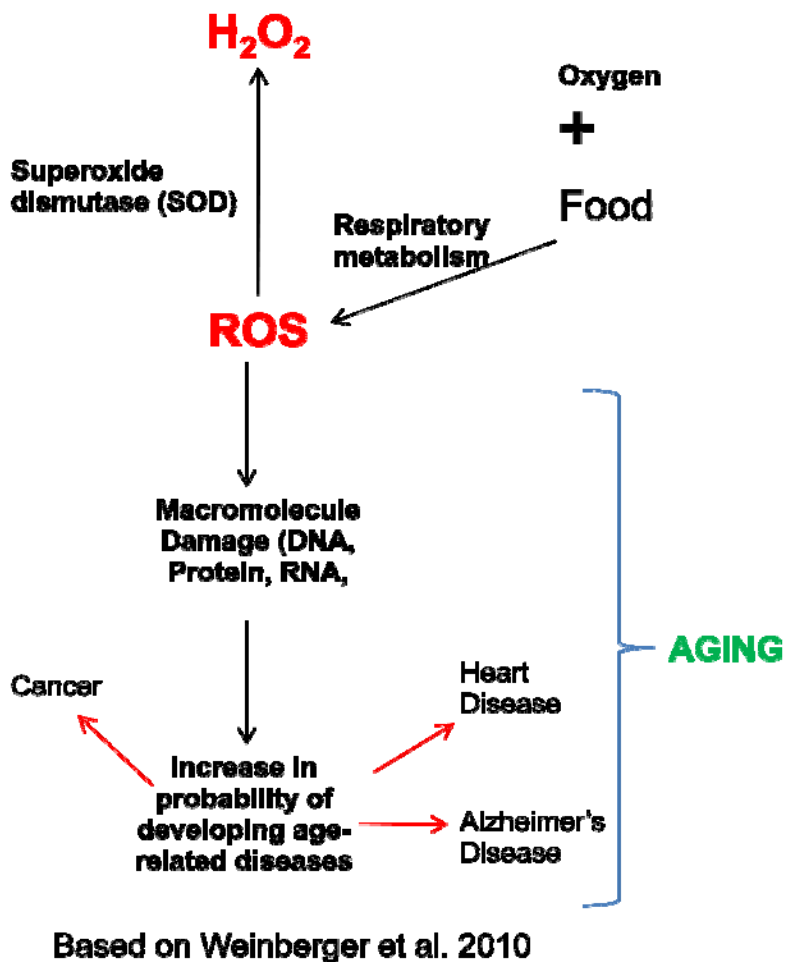
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**Table 1. Yeast strains in this study were derived from Qin et al. 2008.**

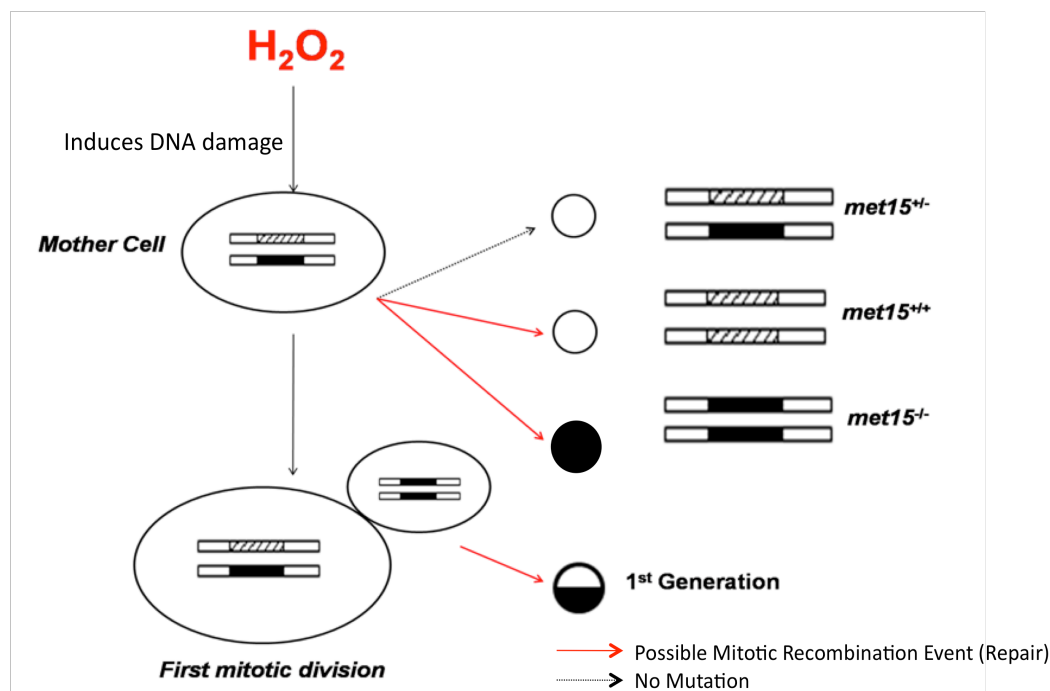
Strain	Description	Source
101S Met15 <sup>+/-</sup>	101S (Parental Strain )	Qin et. al 2008
M8 Met15 <sup>+/-</sup>	M8 (Parental Strain)	Qin et. al 2008
M5 Met15 <sup>+/-</sup>	M5 (Parental Strain)	Qin et. al 2008
M34 Met15 <sup>+/-</sup>	M34 (Parental Strain)	Qin et. al 2008
YPS163 Met15 <sup>+/-</sup>	YPS163 (Parental Strain)	Qin et. al 2008
M2-8 Met15 <sup>+/-</sup>	M2-8 (Parental Strain)	Qin et. al 2008
YPS128 Met15 <sup>+/-</sup>	YPS128 (Parental Strain)	Qin et. al 2008
M13 Met15 <sup>+/-</sup>	M13 (Parental Strain)	Qin et. al 2008
M1-2 Met15 <sup>+/-</sup>	M1-2 (Parental Strain)	Qin et. al 2008
M32* Met15 <sup>+/-</sup>	M32 (Parental Strain)	Qin et. al 2008
SGU57 Met15 <sup>+/-</sup>	SGU57	This study

**Table 2. Summary of key terms and variables.**

Terms and variables	Explanation
CLS	Chronological life span is a measure of amount of time taken for a single <i>S. cerevisiae</i> mother cell to stay alive.
RLS	Replicative life span measures the number of times required for a mother cell to stop undergoing cell division.
MR	Mitotic Recombination refers to the exchange of genetic information between homologous chromosomes in a somatic cell.
ROS	Reactive Oxygen Species are a group of molecules or atoms that have a free radical. ROS are a natural by-product of metabolic processes, but can be elevated under certain conditions and can damage macromolecules.
LOH	Loss of heterozygosity can be used to measure genomic integrity in cells. It occurs in genes that have one expressed and one unexpressed allele. In subsequent generations, the expressed allele becomes non-functional.
MA	Mitotic asymmetry refers to the generation of two dissimilar daughter cells following mitotic division.
<i>MET15</i> locus	A genetic locus at which LOH is detected via the knock-out of one allele using a kanamycin-resistance marker.
C <sub>b</sub>	A variable in the H <sub>2</sub> O <sub>2</sub> dose-response curve that represents the middle concentration black colonies on MLA plates.
C <sub>v</sub>	A variable in the H <sub>2</sub> O <sub>2</sub> dose-response curve that represents the H <sub>2</sub> O <sub>2</sub> concentration at which cell viability decreases by half.
T <sub>g</sub>	Based on the biological survival curve, T <sub>g</sub> represents the time at which there is a 50% decrease in genomic integrity ( <b>QIN et al. 2008</b> ).
T <sub>c</sub>	Based on the biological survival curve, T <sub>c</sub> represents the midpoint of CLS ( <b>QIN et al. 2008</b> ).
L <sub>0</sub>	A ratio that measures the frequency of LOH events in daughter cells by the frequency of LOH events in mother cells.

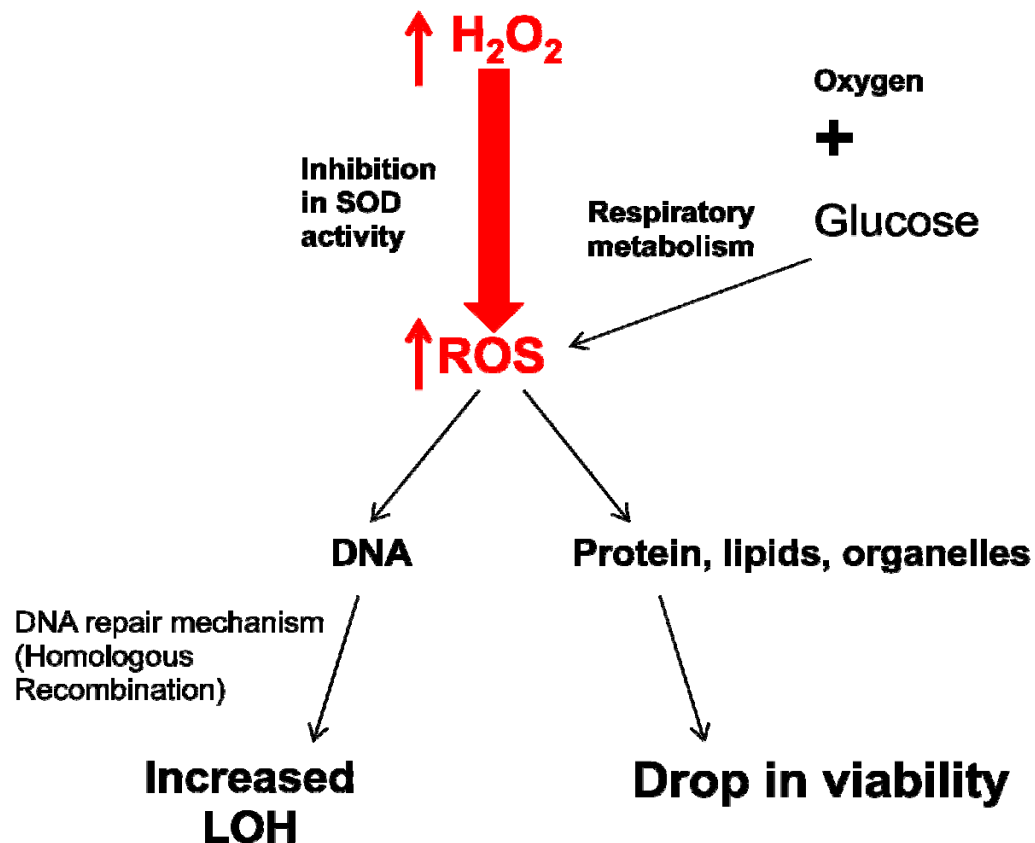


**Figure 1. Reactive oxygen species (ROS) are accepted mechanistic causes of aging.** ROS are natural by-products of the respiratory metabolic breakdown of food. There are endogenous levels of ROS in cells. Superoxides are naturally converted into  $H_2O_2$  via superoxide dismutase activity (SOD). Thus, natural levels of ROS and  $H_2O_2$  can be beneficial to the cell. Damage caused by ROS can accumulate over time and is considered to be the mechanistic cause of aging.



**Figure 2.** LOH was used to measure genome integrity. A kanamycin-resistance marker was used to knock-out one copy of the *MET15* gene to yield a heterozygous genotype for that locus. In the mother cell, the chromosome with the dashed segment represents the wild type *MET15* gene and the chromosome with the black segment has the gene knocked-out. During CLS, a mother cell may produce daughter cells without LOHs on the target locus, whereby white colonies form. White-colored colonies may also form if LOH occurs and yield daughter cells *MET15*<sup>+/+</sup> genotype. Only 50% of the LOH events are observed because *MET15*<sup>+/-</sup> and *MET15*<sup>+/+</sup> are indistinguishable. Fully black colonies are homozygous recessive at the *MET15* locus (*MET15*<sup>-/-</sup>) and represent LOH as a result of a LOH event that is most likely linked to mitotic recombination.

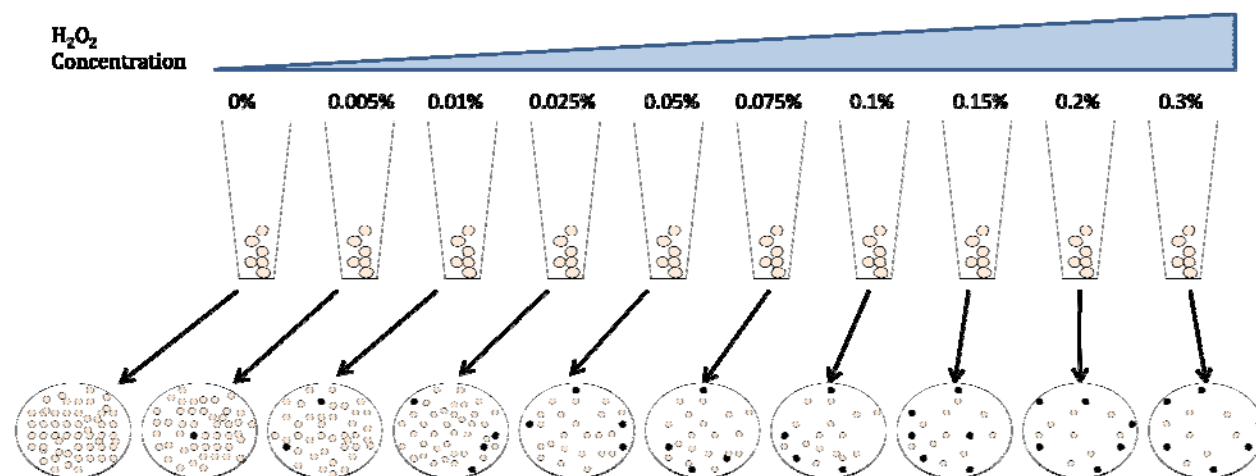




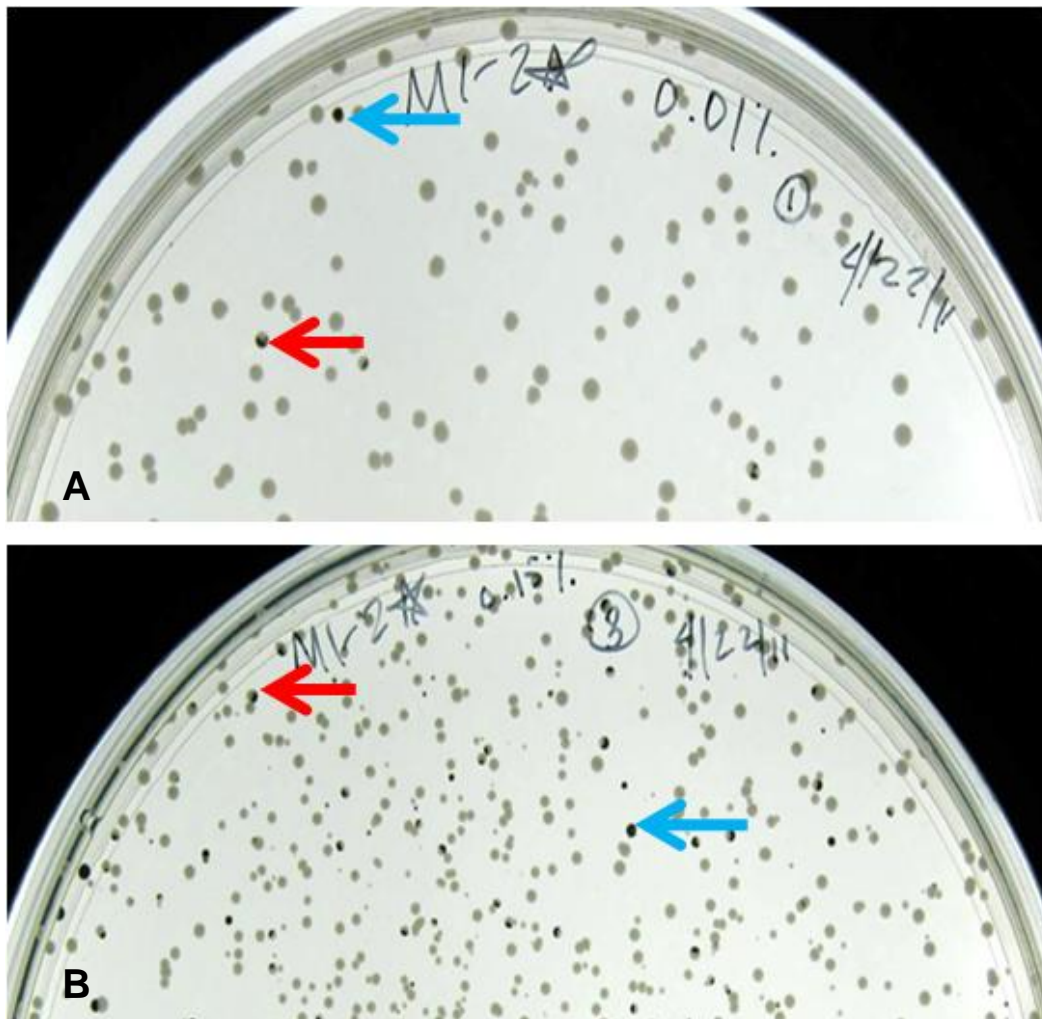
**Figure 3.**  $\text{H}_2\text{O}_2$  levels can be modified by a straightforward intervention method to increase ROS. ROS are a natural by-product derived from the breakdown of food. Oxygen from this metabolic process raises ROS levels. This activates SOD, which triggers  $\text{H}_2\text{O}_2$  production in low levels. Introducing  $\text{H}_2\text{O}_2$  externally activates an opposite pathway. A rise in  $\text{H}_2\text{O}_2$  inhibits SOD activity and increases ROS levels. Aging and effects associated with aging is a consequence of elevated intracellular ROS levels.



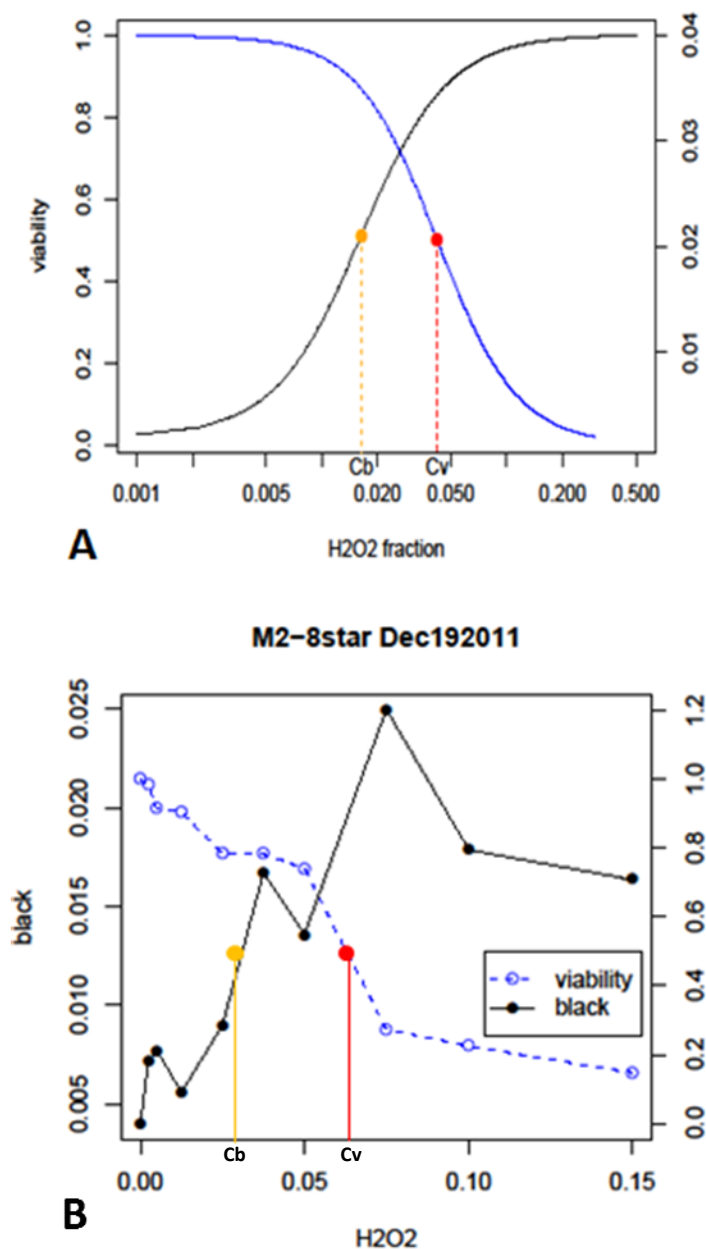
**Figure 4:** A Fisher FS20D water bath sonicator was used in this project.



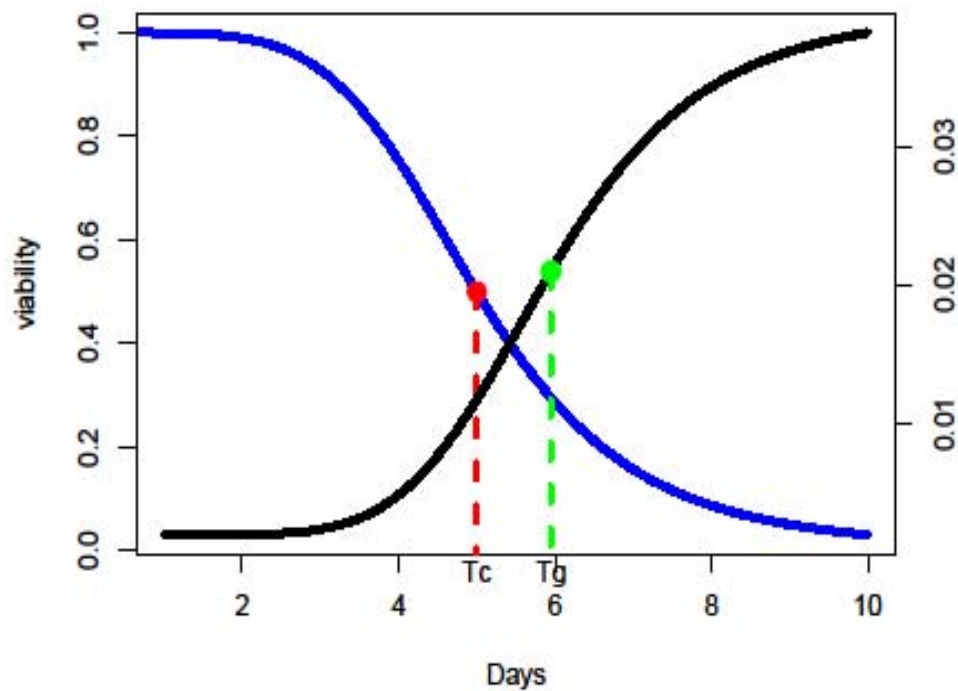
**Figure 5.** Increasing  $H_2O_2$  dosage to yeast strains causes a decline in viability ( $C_v$ ) and an increase in percentage of mutants ( $C_b$ ).  $MET15^{+/-}$  yeast strains were treated with various concentrations of  $H_2O_2$ . Cells were plated on lead-containing medium. Excess  $H_2S$  was generated as a result of alteration of  $MET15$  locus in response to induced oxidative stress. The interaction of  $H_2S$  with  $Pb^{2+}$  on MLA plates formed  $PbS$ , which gave colonies their black color. A drop in strain viability corresponds to fewer colonies grown on plates as a result of increase in  $H_2O_2$  dosage. There is an increase in the percentage of black colonies on each plate, despite a decrease in the total number of colonies.



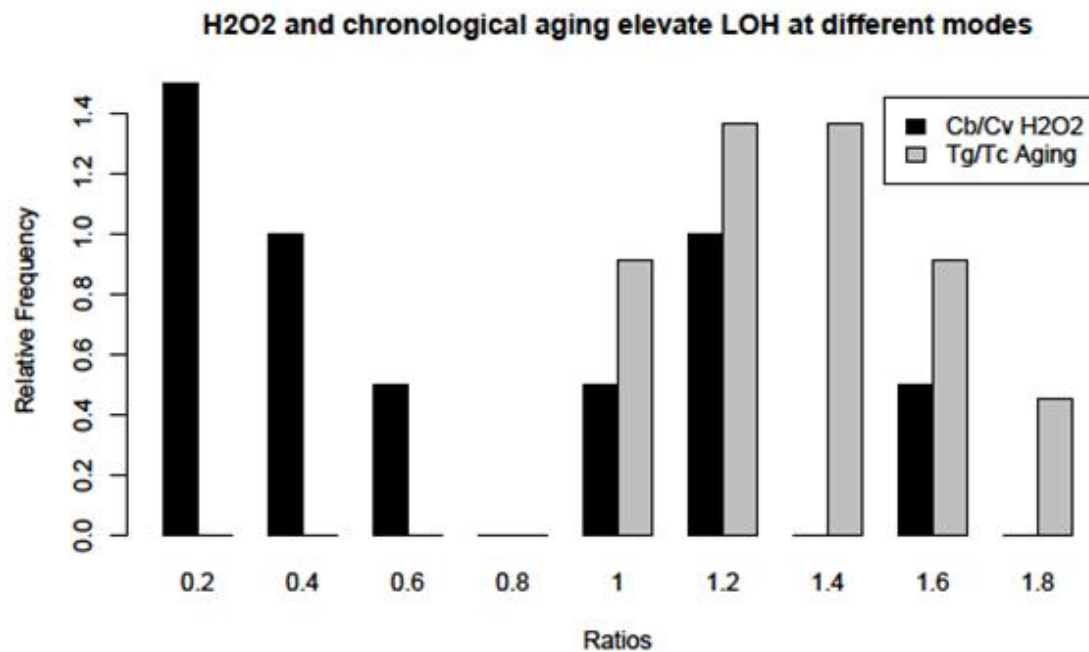
**Figure 6.** MLA plates show black and half-black colonies as a sign of LOH events, an indication of a loss in genomic integrity. Blue arrows point to fully black colonies, which result from LOH in mother cells. Red arrows point to half-black colonies following one or two mitotic events in a mother or daughter cell (QIN *et al.* 2008). **A** shows strain M1-2\* at 0.01% treatment of H<sub>2</sub>O<sub>2</sub>. **B** shows strain M1-2\* at 0.15% treatment of H<sub>2</sub>O<sub>2</sub>.



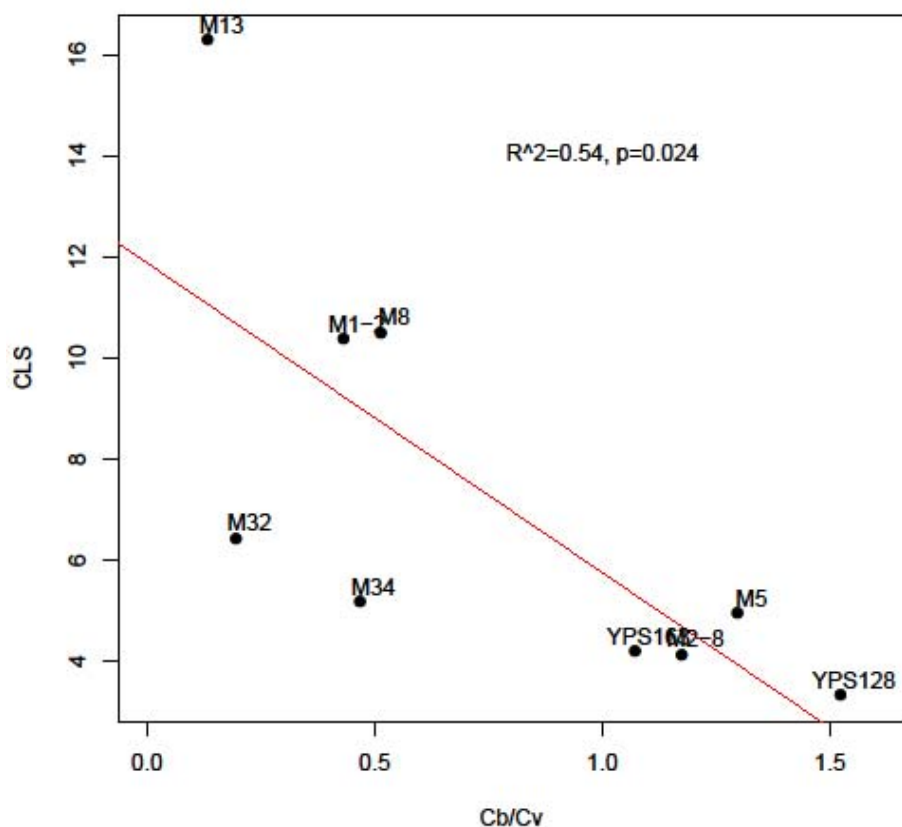
**Figure 7.** **A** shows the general sigmoidal trend of viability and genomic integrity based on  $H_2O_2$ -treated cells. **B** shows the  $H_2O_2$  dose-response curve of strain M2-8\*. At a  $C_b$  of approximately 0.025, the percentage of black colonies should have doubled from the initial quantity at a 0%  $H_2O_2$  dosage. At a  $C_v$  of 0.05, the initial concentration total colonies decreased by approximately one-half indicated by the blue curve.



**Figure 8.** Based on the biological survival curve in yeast,  $T_c$  is the midpoint of chronological life span and  $T_g$  is the midpoint of genome instability. In normal aging, a decrease in viability precedes an increase in genomic instability (QIN *et al.* 2008).

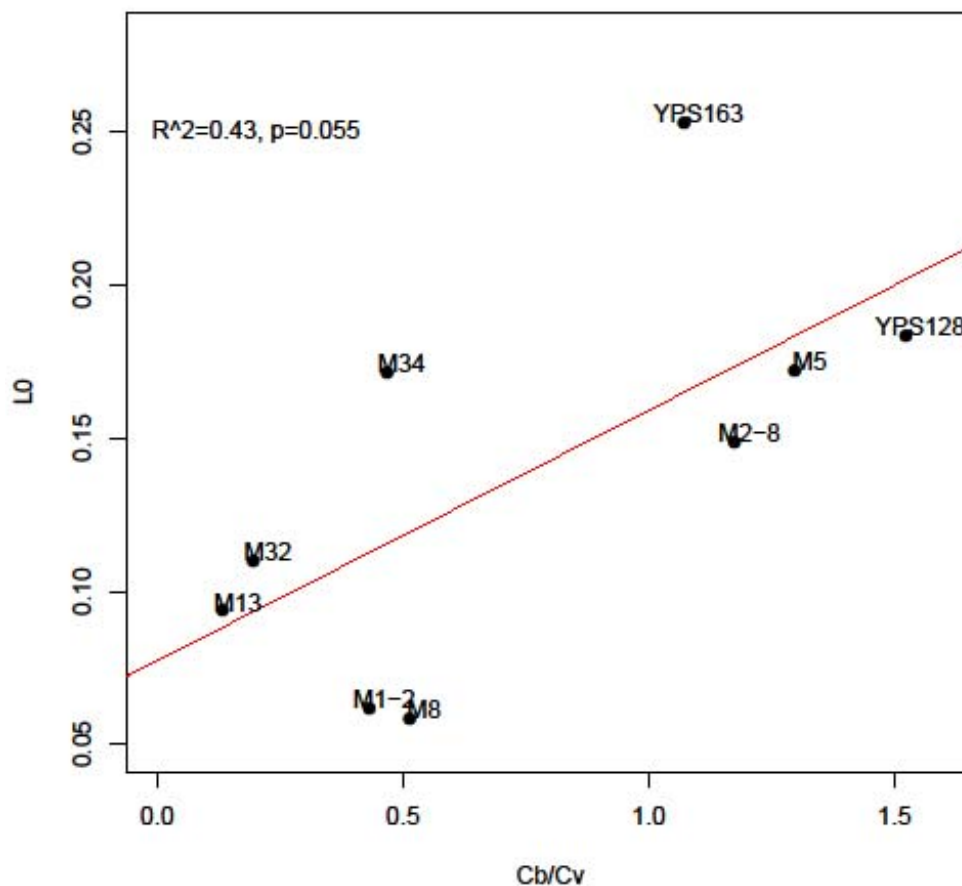


**Figure 9. There was a contrasting switching pattern of pattern of H<sub>2</sub>O<sub>2</sub> and Chronological Aging on LOH.** The midpoints of the biological survival curve and the H<sub>2</sub>O<sub>2</sub> dose-response curve were taken to normalize the data. A) The H<sub>2</sub>O<sub>2</sub> dose-response curve suggests that most strains have C<sub>b</sub>/C<sub>v</sub> ratios that are less than one (represented by the black columns). This implies that C<sub>v</sub> generally comes before C<sub>b</sub>. Data from the biological survival curve (QIN *et al.* 2008) suggests that strains have T<sub>g</sub>/T<sub>c</sub> ratios that are greater than (represented by the gray columns). This implies that T<sub>c</sub> comes before T<sub>g</sub>.



**Figure 10.** Genome tolerance ( $C_b$ ) and viability tolerance ( $C_v$ ) to  $H_2O_2$  induction varies by strain backgrounds. Cells with a greater tolerance to  $H_2O_2$  have a longer CLS. Strain YPS128 has the shortest life span and has the least tolerance to  $H_2O_2$  since it has a larger  $C_b/C_v$  ratio. M13 has the longest life span, which corresponds to a smaller  $C_b/C_v$  ratio.

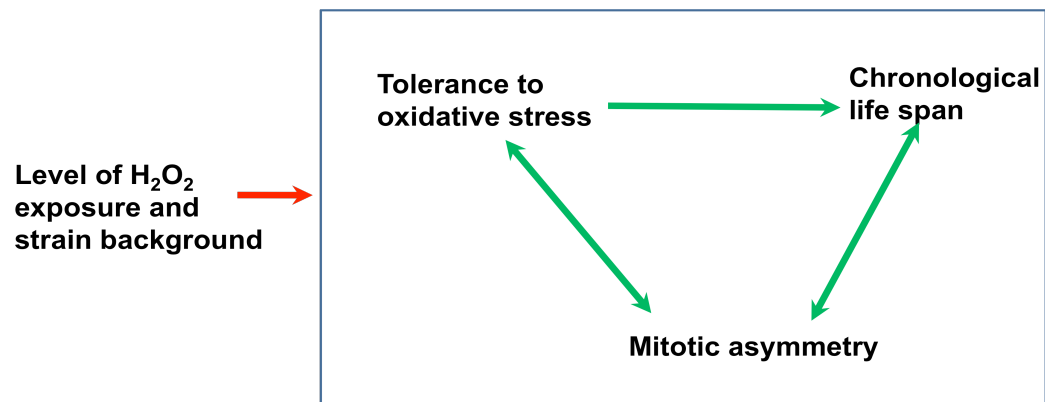




**Figure 11. Trade-off between tolerance to oxidative stress and mitotic asymmetry.**

Mitotic asymmetry ( $L_0$ ) is the ratio of the frequency of daughter cells by LOH frequency in mother cells. A smaller  $L_0$  corresponds to better (lower) mitotic asymmetry in daughter cells compared to mother cells. A lower  $C_b/C_v$  ratio corresponds to a longer life span.

The positive correlation suggests that cells with a better mitotic asymmetry have a longer life span and better  $H_2O_2$  viability tolerance.



**Figure 12.** Life span tolerance, length of CLS, and frequency of mitotic asymmetrical events are interrelated factors in budding yeast. A higher tolerance to oxidative stress is associated with a longer CLS. A greater tolerance to oxidative stress corresponds to a lower and better mitotic asymmetry. A better mitotic asymmetry corresponds to a longer CLS.