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

by

Lindsay Alexandra Parnell

Candidate for a B.S. in Biology

Submitted to the Department of Biology in partial fulfillment of the

requirements for the completion of the Ethel Waddell Githii Honors Program

at SPELMAN COLLEGE

April 2012

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, and monitored the frequencies of loss of heterozygosity (LOH) in response to hydrogen peroxide concentration. We found that the increase of hydrogen peroxide-dependent genomic instability occurs before a drop in viability. This leadoff is inversely proportional to cells’ ability to maintain homeostasis despite substantial H2O2 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 H2O2 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 H2O2-induced oxidative stress. Overall, our results demonstrate strong associations between oxidative stress, genomic instability, and mitotic asymmetry within the context of aging.

**Acknowledgements**

I owe the completion of this thesis to my advisor, Hong Qin. I thank him for constant support, commitment, and encouragement during this process. I would also like to thank research technicians Erin Jackson, Jenney Rodrigues, and Nilin Gupta and my undergraduate research colleagues Meghan Parker, Megan Maghee, and Brittni Wilson. This project was supported by a grant from the National Science Foundation.

I would like to extend a special thanks to the Spelman College Biology Department and the Ethel Waddell Githii Honors program for enriching my learning experience. Lastly, I will forever be grateful for my parents and sisters who have offered their unwavering support and have provided a diverse lifestyle from which I have drawn much of my scientific inspiration. I hope that I have made you all proud and that I can use my knowledge and experience to make a positive contribution.

**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](#_ENREF_3" \o "Gompertz, 1825 #1151)). In essence, this is a statistical definition asserting that the probability of dying increases with age ([Defossez](#_ENREF_2" \o "Defossez, 1998 #1467) *[et al.](#_ENREF_2" \o "Defossez, 1998 #1467)* [1998](#_ENREF_2" \o "Defossez, 1998 #1467)). 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](#_ENREF_20" \o "Williams, 1957 #273)) and the disposable soma theory ([Kirkwood 1977](#_ENREF_7" \o "Kirkwood, 1977 #56)). From the evolutionary perspective, natural selection likely acts upon young individuals, and advantages at early life will inevitably lead to detrimental effects later in life ([Williams 1957](#_ENREF_20" \o "Williams, 1957 #273)). Huntington Disease, for example, is a genetic disease characterized by the decline of the central nervous system. Because of its late onset, individuals with the disease can pass deleterious alleles to progeny before their natural death (Conneally 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](#_ENREF_7" \o "Kirkwood, 1977 #56)). Hence, aging is a conserved fundamental biological phenomenon because of differential selection on individuals in age-structured populations during evolution (Charlesworth 1994).

Calorie restriction (CR) is a strong evidence to support that aging 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 universal effect of CR is that most species have only sporadic access to nutrients in 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](#_ENREF_19" \o "Willcox, 2004 #1476") *[et al.](#_ENREF_19" \o "Willcox, 2004 #1476")* [2004](#_ENREF_19" \o "Willcox, 2004 #1476")).The Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) is a longitudinal study conducted in humans to determine whether the 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 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](#_ENREF_16" \o "Stanfel, 2009 #797) *[et al.](#_ENREF_16" \o "Stanfel, 2009 #797)* [2009](#_ENREF_16" \o "Stanfel, 2009 #797)).

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 integrity, this can lead to detrimental consequences in the organism.

The free radical theory of aging is an accepted mechanistic explanation for aging in eukaryotic organisms ([Harman 1956](#_ENREF_5" \o "Harman, 1956 #1036)). 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 (Harman 1956; Harman 1972; [Yu](#_ENREF_21" \o "Yu, 2012 #1478) *[et al.](#_ENREF_21" \o "Yu, 2012 #1478)* [2012](#_ENREF_21" \o "Yu, 2012 #1478)).

Reactive oxygen species (ROS), a form of free-radicals, are natural by-products of the mitochondrial respiratory chain. H2O2, for example, is generated via oxidase-catalyzed reactions (Figure 1). The endogenous level of ROS also plays a role in signaling transduction and normal cell functions ([Blagosklonny 2008](#_ENREF_1)). Superoxide, hydroxyl radicals, H2O2 and singlet oxygen, can also oxidize lipids, proteins and nucleic acids (Moradas-Ferreira *et al.* 1996).Damage caused by ROS can accumulate over time, and has been mostly accepted as a mechanistic cause of aging (Harman 1956). Oxidative stress can alter metabolic homeostasis and cell growth ([Ristow and Schmeisser 2011](#_ENREF_14" \o "Ristow, 2011 #1034)).Cells naturally convert superoxide to H2O2 as a defense mechanism. Thus, low levels of H2O2 and ROS can be beneficial to the cell. Many ROS are required for cell communication and signaling ([Rahman 2007](#_ENREF_13" \o "Rahman, 2007 #1468); [Weinberger](#_ENREF_18" \o "Weinberger, 2010 #864) *[et al.](#_ENREF_18" \o "Weinberger, 2010 #864)* [2010](#_ENREF_18" \o "Weinberger, 2010 #864)) (Figure 2).

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. 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](#_ENREF_4); [McMurray and Gottschling 2003](#_ENREF_8" \o "McMurray, 2003 #244); [McMurray and Gottschling 2004](#_ENREF_9" \o "McMurray, 2004 #419)).

*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). Heterozygosity on the Methianine 15 locus (MET15+/-) is achieved by knocking out one copy of the wild-type allele using a kanamycin drug resistance marker. LOH can be monitored in *Saccharomyces cerevisiae* only when the heterozygous form of *MET15+/-* is converted into a homozygous recessive form (*MET15-/-*) following mitotic division. When this yeast strain is plated on medium containing lead, LOH occurs resulting in black sectors in cream-colored colonies. Thus, a *MET15-/-*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+/+*) and *MET15+/-* 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 was 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 3).

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](#_ENREF_17); [Weinberger *et al.* 2010](#_ENREF_18)).

*S. cerevisiae* can also be studied in both haploid and diploid states. 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) (Figure 3).

Apart from the technical benefits for using yeast to study aging, 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 humans (Wei *et al.* 2008).

H2O2-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).

Cellular signals either delay or promote growth depending on intracellular or external conditions. When nutrients like glucose are not readily available in the cell, quiescence occurs, in which metabolic activity is reduced in order to conserve cellular energy. Thus the progression of the cell’s life span is either slowed or halted. Conversely, elevated nutrient levels will speed up metabolic rates and promote cell-cycle progression. This can shorten CLS. Thus, LOH can be used as a sign of genomic alteration on the *MET15* locus.

To study the role of ROS on LOH, we altered the intracellular level of H2O2 through high exogenous H2O2 levels and monitor its effect on LOH, and examined the characteristics of theH2O2 dose-dependent changes of genome instability and viability in a collection of yeast natural isolates.

**Materials and Methods**

**Yeast strains and Culturing**

Strains with heterozygous Met 15 +/- were grown overnight at 30°C in 5 mLs of YPD using autoclaved glass tubes. Strains used with heterozygous Met15+/- were described previously ([Qin](#_ENREF_12" \o "Qin, 2008 #516) *[et al.](#_ENREF_12" \o "Qin, 2008 #516)* [2008](#_ENREF_12" \o "Qin, 2008 #516))(Table 1). Following incubation, a spectrophotometer was used to determine saturation of yeast in the glass tubes at an absorbance of 600 nm (A600). The yeast culture was diluted to A600 0.6 in fresh YPD in new autoclaved glass tubes with a final volume of 4 to 6 mLs. 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.

**H2O2 Treatment**

The protocol used models the H2O2 sensitivity test used in ([Yu](#_ENREF_21" \o "Yu, 2012 #1478) *[et al.](#_ENREF_21" \o "Yu, 2012 #1478)* [2012](#_ENREF_21" \o "Yu, 2012 #1478)). Stock solutions of 2X H2O2 containing 0.3%, 0.2%, 0.15%, 0.1%, 0,075%, 0.05%, 0.025%, 0.01%, and 0.005%, and 0% H2O2 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 ddH2O, 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 H2O2 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 ColonyDoc-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 A600, dilution, date, H2O2 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 statistical environment was used for data analysis.

**Results**

The interconnection between oxidative stress, genomic instability, mitotic asymmetry, and chronological life span in *Saccharomyces cerevisiae* was addressed using exogenous H2O2 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 H2O2 dose-response curves of LOH and viability with the viability change in normal aging.

**H2O2–dose dependent changes of viability and LOH**

The H2O2 dose-dependent change in viability and LOH are generally sigmoid. 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 H2O2-induced LOH than those occurred in chronological aging, suggesting that elevating intracellular H2O2 level can lead to break-down of mitotic asymmetry .

Qin et al. measured biological aging with a logistical model using the ratio Tg/Tc. Tg represents the midpoint of the genome instability, which is measured by LOH. Tc 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](#_ENREF_12" \o "Qin, 2008 #516) *[et al.](#_ENREF_12" \o "Qin, 2008 #516)* [2008](#_ENREF_12" \o "Qin, 2008 #516))(Figure 6). Thus, the biological survival curve will likely display a greater frequency of strains with Tg/Tc ratios at 1.0 or greater (Figure 7, 9).The logistical model for the hydrogen peroxide dose-response curve uses the ratio Cb/Cv. Cb represents the middle concentration of black colonies, which is a measure of genome instability. Cv represents the middle concentration of viability. With respect to dose-response curve, Cb usually comes before Cv in the strains used (Figure 8). The inverse of Cb/Cv thus represents the capability of cells to maintain viability after the increase of H2O2-induced LOH. For most natural isolates, this Cb/Cv ratio is lower than 1.0 (Figure 8, 9). This comparison suggested that endogenous level of H2O2 must be held low up to the dying off phase during chronological aging. A regression analysis suggested that strain sensitivity to H2O2 varies. There is a significant association between CLS and the Cb/Cv ratio with a p-value of 0.024, and a R-squared value of 0.54. A longer CLS corresponds to a smaller Cb/Cv. A value (Cb/Cv) less than 1.0 indicates that Cb comes before Cv, and thus a greater dose of H2O2 is required to kill the cell. Thus, strains with lower ratios are more tolerant to hydrogen peroxide with respect to viability. A Cb/Cv value greater than 1.0 indicates that Cb comes after Cv, and thus cells are more sensitive to hydrogen peroxide treatment. Strain M13 seems to be the most tolerant 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 H2O2 treatment (Figure 10).

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

A regression analysis revealed that there is a positive correlation between Cb/Cb and L0, 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, and the Cb/Cb. 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 Cb/Cv seem to have a lower L0 (Figure 11).

**Discussion**

In this study, we report that the biological survival curve and the hydrogen peroxide dose-response curve exhibit contrasting switching patterns. These results suggest that there is opposite timing of genomic instability with regards to viability. Results from the H2O2 dose-response curve are consistent with the biological survival curve in that cells with a higher tolerance to ROS have longer chronological life spans ([Qin](#_ENREF_12" \o "Qin, 2008 #516) *[et al.](#_ENREF_12" \o "Qin, 2008 #516)* [2008](#_ENREF_12" \o "Qin, 2008 #516)). However, daughter cells seem to have a lower mitotic asymmetry and longer chronological life span compared to mother cells. This positive correlation indicated that there is a trade-off between tolerance to H2O2–induced damage and mitotic asymmetry

H2O2-induced DNA damage likely contains double-strand breaks that asymmetric DNA repairs. This leads to lead to LOH. If this damaged DNA is detected, one allele is replaced the other allele on its homologous chromosome.

Comparison between aging and apoptotic transcriptome (Laun *et al.* 2005)

CLS screen of 550 mutants (Burtner *et al.* 2011)

Catalase activity in the star strains by catalase dose-depedent inhibitors (AZT?)

Gourlay and Aysough Nature review 2005 on actin, ROS, apoptosis and ageing.

(Breitenbach *et al.* 2005; Gourlay *et al.* 2004)

This will ultimately increase LOH in yeast. Alternatively, we propose that loss of viability will occur as increased ROS levels damage organelles, proteins, and lipids. LOH and viability drop have not been shown to be directly linked, it is clear that they are associated events because they are both caused by increasing ROS. Thus, our objective is to compare the H2O2 dose-response curve of LOH and viability with the viability change in normal aging (Figure 7, 8A). Biological aging has become a popular area of study because it has broad biomedical implications. This area of research has aesthetic implications; for many of us want to look and experience the benefits of youth for as long as possible. Ultimately, this area can open brand new realms for understanding phylogenic relationships among species, and contribute to the study of age-related diseases like Alzheimer’s disease, cancer, and atherosclerosis, and diabetes. Ultimately, these studies can introduce ways to extend human life span.

Future directions include studying the effect of CR or rapamycin on H2O2 induced LOH pattern. Rapamycin inhibits TOR1 and thus mimics the action of calorie restriction. If rapamycin is introduced to H2O2-treated cells. DR also increases respiration and boosts mitochondrial functions, decreases proton leakage and ROS production in the mitochondria (Lin et al. 2002; Barros et al.2004; Pamplona et al. 2004; Sanz et al. 2006), and attenuates the accumulation of oxidative damage (Reverter-Branchat et al. 2004).

CR in the CLS paradigm was found to increase cell’s resistance to heat and oxidative stresses, prevent protein oxidative damage, reduce thelevel 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.

Compromised cellular responses to DNA damage accelerate chronological aging by incurring cell wall fragility in Saccharomyces cerevisiae, Shanshan Yu • Xian-en Zhang • Guanjun Chen • Weifeng Liu. This paper seems to argue for a connection from DNA damage to CLS.

*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 resistance 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 H2O2 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*

The timing at which there is 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, H2O2dosage has more of an immediate effect on the robustness of the cell. Viability drops more rapidly in H2O2-treated cells because ROS levels are increased via the external elevation of H2O2 and the inhibition of superoxide dismutase (SOD) activity ([Weinberger *et al.* 2010](#_ENREF_18)) (Figure 1).

We also showed 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 (LIU et. al 2010).

Future directions include testing gene deletion mutants with H2O2. MSN2/4 has been shown to be vital to the pathway for extending CLS in yeast. This gene product functions by upregulating genes that enable the cell 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](#_ENREF_10)).Low concentration of H2O2 has been shown to increase CLS by increasing SOD activity and increase in CLS by H2O2 is further increased in stationary phase in yeast (MESQUITA *et. al*). In this study it has been shown that CR or inactivation can increase CLS by increasing H2O2. Conversely, the Weinberger model proposes that inhibition of SOD activity can result in the increase of ROS levels and reduce CLS in yeast ([Weinberger](#_ENREF_18" \o "Weinberger, 2010 #864) *[et al.](#_ENREF_18" \o "Weinberger, 2010 #864)* [2010](#_ENREF_18" \o "Weinberger, 2010 #864)). If the SOD gene is deleted, we should see similar Cv and Cb 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.

Future plans also involve treating strains with paraquat dichloride (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride) to induce superoxides directly. Superoxide levels when cells are treated with H2O2 and paraquat will also be measured directly using a fluorescent probe.

If the average human lifespan were compared in 1800 and 2012, one would see that a substantial difference. Increased life-expectancy can be attributed to the wide range of technological advancements and effective public health initiatives. Improved sanitation, new drugs and treatment methods, and many other factors have improved the quality of life in humans. We live to see age-related consequences because these advancements are continually being modified to delay death.

References

Blagosklonny, M. V., 2008 Aging: ROS or TOR. Cell Cycle **7:** 3344-3354.

DAS S.A., C GILHOOLY, J.K. GOLDEN, A.G. PITTAS, P.J FUSS, et. al., 2007Long-term effects of 2

energy-restricted diets differing in glycemicload on dietary adherence, body composition, and metabolism inCALERIE: a 1-y randomized controlled trial. Am J Clin Nutr **85:**1023-1030.

Breitenbach, M., P. Laun and M. Gimona, 2005 The actin cytoskeleton, RAS-cAMP signaling and mitochondrial ROS in yeast apoptosis. Trends Cell Biol **15:** 637-639.

Burtner, C. R., C. J. Murakami, B. Olsen, B. K. Kennedy and M. Kaeberlein, 2011 A genomic analysis of chronological longevity factors in budding yeast. Cell Cycle **10:** 1385-1396.

Charlesworth, B., 1994 *Evolution in Age-structured Populations*. Cambridge University Press, Cambridge.

Conneally, P. M., 1984 Huntington disease: genetics and epidemiology. Am J Hum Genet **36:** 506-526.

Defossez, P. A., P. U. Park and L. Guarente, 1998 Vicious circles: a mechanism for yeast aging. Curr Opin Microbiol **1:** 707-711.

Farrer, L. A., P. M. Conneally and P. L. Yu, 1984 The natural history of Huntington disease: possible role of "aging genes". Am J Med Genet **18:** 115-123.

Gompertz, B., 1825 On the Nature of the Function Expressive of the Law of Human Mortality, and on a New Mode of Determining the Value of Life Contingencies. Philosophical Transactions of the Royal Society of London **115:** 513-585.

Gourlay, C. W., L. N. Carpp, P. Timpson, S. J. Winder and K. R. Ayscough, 2004 A role for the actin cytoskeleton in cell death and aging in yeast. J Cell Biol **164:** 803-809.

Gravel, S., and S. P. Jackson, 2003 Increased genome instability in aging yeast. Cell **115:** 1-2.

Harman, D., 1956 Aging: a theory based on free radical and radiation chemistry. J Gerontol **11:** 298-300.

HARMAN, D., 1972 A biologic clock: the mitochondria?. Journal of the American Geriatrics Society **20** (4): 145–147.

Harrison, D. E., and J. R. Archer, 1989 Natural selection for extended longevity from food restriction. Growth Dev Aging **53:** 3.

Hiraoka, M., K. Watanabe, K. Umezu and H. Maki, 2000 Spontaneous loss of heterozygosity in diploid Saccharomyces cerevisiae cells. Genetics **156:** 1531-1548.

Holliday, R., 1989 Food, reproduction and longevity: is the extended lifespan of calorie-restricted animals an evolutionary adaptation? Bioessays **10:** 125-127.

Kirkwood, T. B., 1977 Evolution of ageing. Nature **270:** 301-304.

Koubova, J., and L. Guarente, 2003 How does calorie restriction work? Genes Dev **17:** 313-321.

Laun, P., L. Ramachandran, S. Jarolim, E. Herker, P. Liang *et al.*, 2005 A comparison of the aging and apoptotic transcriptome of Saccharomyces cerevisiae. FEMS Yeast Res **5:** 1261-1272.

LIU, B., L. LARSSON, A. CABALLERO, X. HAO, D.O. LING*et al.*, 2010 The Polarisome is required

for segregation and retrograde transport of protein aggregates. Cell **120:** 257-267.

McMurray, M. A., and D. E. Gottschling, 2003 An age-induced switch to a hyper-

recombinational state. Science **301:** 1908-1911.

McMurray, M. A., and D. E. Gottschling, 2004 Aging and genetic instability in yeast. Curr Opin Microbiol **7:** 673-679.

Medvedik, O., and D. A. Sinclair, 2007 Caloric restriction and life span determination of yeast cells. Methods Mol Biol **371:** 97-109.

MESQUITA, A., M WEINBERGER, A. SILVA, B. SAMPALO-MARQUES, B. ALMEIDA, *et. al,* 2010 Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H2O2 and superoxide dimutase activity. PNAS **107:** 15123–15128

Moradas-Ferreira, P., V. Costa, P. Piper and W. Mager, 1996 The molecular defences against reactive oxygen species in yeast. Mol Microbiol **19:** 651-658.

Qin, H., and M. Lu, 2006 Natural variation in replicative and chronological life spans of Saccharomyces cerevisiae. Exp Gerontol **41:** 448-456.

Qin, H., M. Lu and D. S. Goldfarb, 2008 Genomic instability is associated with natural life span variation in Saccharomyces cerevisiae. PLoS One **3:** e2670.

Rahman, K., 2007 Studies on free radicals, antioxidants, and co-factors. Clin Interv Aging **2:** 219-236.

Ristow, M., and S. Schmeisser, 2011 Extending life span by increasing oxidative stress. Free Radic Biol Med **51:** 327-336.

Ruckenstuhl, C., D. Carmona-Gutierrez and F. Madeo, 2010 The sweet taste of death: glucose triggers apoptosis during yeast chronological aging. Aging (Albany NY) **2:** 643-649.

Stanfel, M. N., L. S. Shamieh, M. Kaeberlein and B. K. Kennedy, 2009 The TOR pathway comes of age. Biochim Biophys Acta **1790:** 1067-1074.

Wei, M., P. Fabrizio, J. Hu, H. Ge, C. Cheng *et al.*, 2008 Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. PLoS Genet **4:** e13.

Weinberger, M., A. Mesquita, T. Caroll, L. Marks, H. Yang *et al.*, 2010 Growth signaling promotes chronological aging in budding yeast by inducing superoxide anions that inhibit quiescence. Aging (Albany NY) **2:** 709-726.

Willcox, B. J., K. Yano, R. Chen, D. C. Willcox, B. L. Rodriguez *et al.*, 2004 How much should we eat? The association between energy intake and mortality in a 36-year follow-up study of Japanese-American men. J Gerontol A Biol Sci Med Sci **59:** 789-795.

Williams, G. C., 1957 Pleiotropy, natural selection and the evolution of senescence. Evolution **11:** 398-411.

Yu, S., X. E. Zhang, G. Chen and W. Liu, 2012 Compromised cellular responses to DNA damage accelerate chronological aging by incurring cell wall fragility in Saccharomyces cerevisiae. Mol Biol Rep **39:** 3573-3583.

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

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

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

|  |  |
| --- | --- |
| Terms and variables | Explanation |
| CLS | Chronological Life span is a measure of that quantifies the number of times |
| RLS | Replicative Lifespan is a measure of life span in budding yeast that quantifies the amount 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 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. |
| MET15 locus | Locus at which LOH is detected via the knock-out of one allele using a kanamycin-resistance marker. |
| Cb | A variable in the H2O2 dose-response curve that represents the middle concentration black colonies on MLA plates. |
| Cv | A variable in the H2O2 dose-response curve that represents the H2O2 concentration at which cell viability decreases by half. |
| Tg | Based on the biological survival curve, Tg represents the time at which there is a 50% decrease in genomic integrity (Qin 2008). |
| Tc | Based on the biological survival curve, Tc represents the midpoint of CLS (Qin 2008). |
| L0 | The initial time at which mitotic asymmetry first occurs. |

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 H2O2via superoxide dismutase activity (SOD). Thus, natural levels of ROS and H2O2 can be beneficial to the cell. Damage cause by ROS can accumulate over time and is considered to be the mechanistic cause of aging.

Figure 2. H2O2 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 H2O2 production in low levels. Introducing H2O2 externally activates an opposite pathway. A rise in H2O2 inhibits SOD activity and increases ROS levels. Aging and effects associated with aging is a consequence of elevated intracellular ROS levels.

Figure 3.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 +/+* genotype. Only 50% of the LOH events are observed because *MET15+/-* and *MET15+/+* are indistinguishable. Fully black colonies are homozygous recessive at the *MET15* locus (*MET15-/-*) and represent LOH as a result of a LOH event that is most likely linked to mitotic recombination.

Figure 5.Increasing H2O2dosage to yeast strains causes a decline in viability (Cv) and an increase in percentage of mutants (Cb). *MET15+/-*yeast strains were treated with various concentrations of H2O2. Cells were plated on lead-containing medium. Excess H2S was generated as a result of alteration of *MET15* locus in response to induced oxidative stress. The interaction of H2S with Pb2+ 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 H2O2dosage. 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). 5A shows strain M1-2\* at 0.01% treatment. 5Bshows strain M1-2\* at 0.51% treatment.



Figure 7.Based on the biological survival curve in yeast, Tc is the midpoint of chronological life span and Tg is the midpoint of genome instability. In normal aging, a decrease in viability precedes an increase in genomic instability ([Qin *et al.* 2008](#_ENREF_12)).

  
**Figure 8.** At a Cv of 0.05, the initial concentration of colonies decreased by approximately one-half indicated by the blue curve. At a Cb of 0.02, the percentage of black colonies should have doubled from the initial quantity at a 0% H2O2 dosage.



Figure 9. There was a contrasting switching pattern of pattern of H2O2and Chronological Aging on LOH. The midpoints of the biological survival curve and the H2O2 dose-response curve were taken to normalize the data. A) The H2O2 dose-response curve suggests that most strains have Cb/Cv ratios that are less than one (represented by the black columns). This implies that Cv generally comes before Cb. Data from the biological survival curve ([Qin *et al.* 2008](#_ENREF_12)) suggests that strains have Tg/Tc ratios that are greater than (represented by the gray columns). This implies that Tc comes before Tg.



Figure 10. Genome tolerance (Cb) and viability tolerance (Cv) to H2O2 induction varies by strain backgrounds. Cells with a greater tolerance to H2O2 have a longer CLS. Strain YPS128 has the shortest life span and has the least tolerance to H2O2since it has a larger Cb/Cv ratio. M13 has the longest life span, which corresponds to a smaller Cb/Cv ratio.



**Figure 11**. **Trade-off between tolerance to oxidative stress and mitotic asymmetry.** Mitotic asymmetry (L0) is the ratio of the frequency of daughter cells by LOH frequency in mother cells. A smaller L0corresponds to better (lower) mitotic asymmetry in daughter cells compared to mother cells. A lower Cb/Cv 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 H2O2viability 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.