**An analysis of the effect of robustness on cellular aging in *Saccharomyces cerevisiae***

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**Abstract**

Cellular aging has been a highly debated topic for decades. It has been suggested that the evolution of prokaryotes and eukaryotes is the cause for the initiation of aging in asymmetric dividing cells. Yeast *Saccharomyces cerevisiae* is an effective model to study cellular aging. Yeast aging can be measured in two ways: Replicative life span (RLS) and Chronological life span (CLS). Robustness is the ability of a cell to maintain homeostasis throughout environmental changes and mutations, such as temperature, time, and cellular damage. In this study the relationship between RLS and robustness in the yeast *S. cerevisiae* was examined. We hypothesized that a gene’s role in robustness is associated with its role in aging. The factors used in this study were the number of protein interactions, evolutionary distance, morphological plasticity and fitness. We performed linear regressions to compare the protein robust factors to cellular aging. Multiple regressions were also performed to find the dependency among the various robustness proxies and cellular aging. All of the computational analyses were conducted in the R statistical language and environment. The results showed that fitness is the strongest factor related to cellular aging followed by morphological plasticity.

1. **Introduction**

The concept of aging and its definition has been a highly debated biological topic for several decades. Even with the advancement of technology and increased study of aging, aging still remains a very controversial biological subject today. For the purpose of this study we will define aging as the loss of function that is generally accompanied by decreasing fertility and increasing mortality with advancing age (Kirkwood & Austad, 2000). In our model organism, *Saccharomyces cerevisiae*, aging is defined as the increased number of cell divisions that occur prior to senescence. Additionally, aging in yeast has also been defined as the amount of time a population remains alive (Fabrizio & Longo, 2003). Aging is a necessary topic of study because it may help with the identification of what directly causes cellular aging in humans and other higher mammals.

In order to ethically study aging, the use of model organisms is necessary. Recent studies have revealed that some of the molecular mechanisms of aging existing in higher eukaryotes have also been unraveled in *Saccharomyces* cerevisiae (Qin, et.al. 2008). Because of such findings, the budding yeast *Saccharomyces cerevisiae*, also known as baker’s or brewer’s yeast, has proven to be an advantageous model for aging research. Yeasts are unicellular organisms which express asymmetrical division. Yeast cells are known to be very similar to higher organism’s, specifically human’s. Several human genes have been identified as having yeast counterparts. Some human genes have also been found to function effectively when placed in yeast cells (Fu, et.al. 1997). Similar to higher organisms, yeast cells contain compartmentalized organelles such as a nucleus and mitochondria. In yeast cells, the mitochondria serves as the energy power house that provides all necessary energy to carryout necessary task and functions.

In yeasts, aging is measured by the amount of divisions a cell undergoes before senescence or the point at which diploid cells can no longer divide. New yeast cells are formed by the budding of yeast cells (daughter cells) from original mother cells. According to previous studies, it has been found that the mortality rate of yeast cells increases exponentially as age increases (Gavrilov & Gavrilova, 2001). For the remainder of this article, the lifecycle of yeasts will be referred to as Replicative life span or RLS. Replicative life span is defined as the number of daughter cells created prior to senescence (Kaeberlin, et.al. 2007). The Replicative life span of yeasts is often held in contrast to the Chronological life span. Chronological life span is defined as the amount of time a yeast cell remains in an arrested non-dividing state (Kaeberlin, et.al. 2007). For this study we only examined RLS because more information about this yeast life span measurement was available.

There are several key factors that have been found to significantly affect RLS. Silent information regulator 2 also known as Sir2 was originally found in yeast and has been hypothesized as a key factor in an organism’s response to stress. Sir 2 has been suggested as being responsible for altering lifespan. It is believed that the removal of Sir2 will significantly shorten life span. Similarly, it is has also been suggested that an over expression of Sir2, will significantly increase life span (Kaeberlin, et.al. 2007). Another known factor affecting RLS is Calorie restriction. Calorie restriction is the diet or nutrient restrictions that are involved in achieving longevity (Kaeberlin, et.al. 2007).The proteins, Mdh1 and Aat1, have been identified as factors that affect calorie restriction.

Aging in yeast cells has also been related to senescence in mammalian fibroblast.

(Kennedy et. al. 1994) It has been found that cells arrest in the G1 phase of the cell cycle. Enlargement of these cells has shown a decreased division potential. In a study done by Lumpkin et. al, 1986 the injection of poly A+ RNA from senescent human diploid fibroblast in young cells inhibited DNA synthesis. This study bolstered the idea that senescence is a dominant occurrence. It also suggested that the number of genes that encode inhibitory proteins are expressed in senescent cells (Kennedy et.al, 1994).

There are many hypotheses that attempt to describe why aging occurs in yeast cells. One example is the free radical theory of aging. This study was produced by Denham Harman in 1956. Harman suggested that free radicals are the underlying cause of oxidative damage that results in aging and death. In his study, Harman noted parallels between the effects of aging and ionization radiation (Beckman, 1998). Free radicals accumulate in cells when the membrane integrity of the mitochondria is degraded. This degradation results in an influx of free radicals thus resulting in aging of the cell.

In the current study we observed the effect of robustness on cellular aging in the yeast *S. cerevisae* becauseprevious research has given evidence that phenotypic capacitors influence robustness. This suggested that protein robustness is a contributing factor for cellular aging. For this study, robustness was defined as the ability of a cell to maintain homeostasis throughout environmental changes and mutations, such as temperature, time, and cellular damage. It has been hypothesized that cells with a greater robustness will experience a longer life span. Specifically, our formulated hypothesis for this study is that the robustness of protein networks will be directly proportional to the RLS of *S. cerevisiae*. In this study, several robust factors were identified and suggested as key factors in aging amongst yeast cells. The robust factors of interest were the number of protein interactions, evolutionary distance, fitness and morphological plasticity. These factors were chosen for our study because of their data was the most easily accessible. This study examined each factor to determine the correlation of each to yeast Replicative life span.

**2. Materials & Methods**

*2.1. Data Source*

The RLS and CLS for 502 different *Saccharomyces cerevisiae* yeast gene deletion mutants were obtained from the Kaeberlin group. Fitness data was obtained from Steinmetz, et.al. 2002. Evolutionary Distance data was given by Dr. Hong Qin. The number of protein interactions data was obtained from Qin, et.al. 2003. The morphological plasticity data was obtained from the online *Saccharomyces cerevisiae* Morphological database (SCMD, http://scmd.gi.k.u-tokyo.ac.jp/). The variance of the morphological plasticity data set was calculated because it is proportional to the robustness of the cell. All of the obtained data was converted into tables in excel for easier use.

*2.2. Statistical Analysis*

A regression analysis was conducted between each of the robust factors to RLS and to each other. Two multiple regression analysis tests were performed. Those tests were between all of the robust factors and RLS and between morphological plasticity robustness, fitness robustness and RLS. Some of the data that was not normally distributed was logarithmically transformed.

The p-value and adjusted R-squared value for each regression analysis was calculated. For this study a p-value of less than 0.01 was considered significant. All of the regression analyses results were plotted with their regression line.

The false discovery rate (the R q-value package) was used to select the morphology mutants that were most informative to RLS.

*2.3. Implementation*

Computational Biology was used to conduct this study. All of the scripts used in this study were designed using the program Notepad++. Each analysis and numerical calculation was performed using the program R.2.10.1.

**3. Results**

*3.1. Experimental Design*

Five parameters were considered in this study: Replicative life span, fitness, morphological plasticity, number of protein interactions and evolutionary distance (Figure 1). Theoretically there were 10 different possible correlations among these five parameters. In figure 1 each of the correlations is represented by arrows and labeled using alphabetical letters. Two-headed arrows were used for the correlations in which the causal parameter of the correlation was unknown. For example, one hypothesis, represented by arrow a, is that the number of protein interactions directly affects the rate at which *S. cerevisiae* yeast cells evolve. A second example, represented by arrow e, is that *S. cerevisiae* yeast cells with a greater plasticity are subject to a longer Replicative life span. Amongst these correlations only a few of them are likely to be causal correlations which were tested using linear and multiple regression analyses.

*3.2. Correlation of growth mediums to RLS in S. cerevisiae yeast cells*

The fitness robust data contained results for the growth of *S. cerevisiae* in five different growth mediums. Those mediums were YPE, YPD, YDGE, YPG and YPL. To determine which growth medium was most informative to RLS and should be used for further experimentation regression analyses were conducted between all of the growth mediums and RLS. The strongest correlation was indicated by the largest R squared value.

Based on the p-values, we found that the growth measures in the YPG, YPE and YPL mediums were related to RLS (Table 1). To determine which of these three growth mediums was most informative to RLS we analyzed the calculated adjusted R-squared values. YPE gave the linear correlation with the best adjusted R-squared to RLS (Table 1). Therefore the fitness data for the growth medium YPE was used throughout the rest of the study.

*3.3. Causal Correlation of robust factors to RLS in S. cerevisiae yeast cells*

The correlations of the RLS robust factors were investigated by regression analysis. The strength of the correlations was based on the adjusted R-squared values.

Based on the p-values, we found that the robust factors, fitness and morphological plasticity, are correlated to RLS. Fitness robustness gave the linear correlation with the best R-squared to RLS (Figure 2A). This is an inverse correlation because the greater the fitness value the less robust the mutant is.

Morphological plasticity robustness gave a negative correlation to RLS (Figure 2B). This correlation can be explained based on the characteristics of the morphological plasticity robustness data. Because the standard deviation was calculated and used as the measure of robustness for morphological plasticity, our plot shows that as standard deviation increases RLS decreases, representing an inverse correlation.

Evolutionary Distance robustness and the number of protein interactions robustness gave no correlation to RLS (Figures 2C and 2D). Evolutionary Distance robustness to RLS gave a p-value of 0.3758 and an adjusted R-squared value of -0.0007506. The number of protein interactions robustness to RLS gave a p-value of 0.1759 and an adjusted R-squared value of 0.002081.

The observed correlations indicate that the robust factors morphological plasticity and fitness support the null hypothesis and the robust factors, evolutionary distance and the number of protein interactions, reject it.

The causal relationship was further evaluated by multiple regression analysis. The results of the multiple regression analysis between the robust factors and RLS showed that none of the factors serve as good predictors of Replicative life span in the yeast *S. cerevisiae*. Multiple regression analyses were conducted to verify all possible causal correlations between morphological plasticity robustness, RLS and fitness robustness. Every partial correlation between these 3 parameters remained significant when the remaining ones were controlled.

Here we concluded that, fitness and morphological plasticity are the only tested robust factors that affect RLS.

*3.4. Relationship of robust factors to each other*

To test all possible alternative hypotheses not directly related to RLS (represented by two headed arrows in Figure 1), we conducted a regression analysis between each of the robust factors and each other. The calculated p-values indicated that there were four alternative hypotheses correlations not directly related to RLS that were correct: morphological plasticity robustness is related to fitness robustness, the number of protein interactions robustness is related to evolutionary distance robustness, fitness robustness and morphological plasticity robustness (Table 2). With a log transformation of the data that was not normally distributed all of these correlations still remained present except for between the number of protein interactions robustness and fitness robustness. This test resulted in a marginal p-value of 0.07209.

*3.5. Informative morphological parameters on lifespan in S.cerevisiae yeast cells*

The false discovery rate measures the percentage of false positives in a test that have been identified as significant, based on the observed p-value. We determined the false discovery rate for the morphological parameters by conducting a q value test. A regression analysis was performed to obtain the p-values for each mutant. To determine the distribution the results were converted into a histogram. The p-values were not normally distributed (Figure 3). It was observed that a considerable amount of the p-values ranged between 0.0 and 0.1.

The q value for each p-value was calculated using the R q value package file. A 5% and 10% q value cut off test was performed. The 5% q value cut off test indicated that there were two morphological parameters that had a 5% chance of having a false positive. The 10% q value cut off test revealed that there were 96 morphological parameters that had a 10% chance of having a false positive. A 5% q value cut off was chosen because it yielded a smaller amount of morphological parameters that may not have a significant correlation. The twomorphological parameters that were suggested as having a 5% chance of being false positive were C125 and D203.

C125 is a cell wall that has a bud size to mother cell size ratio of 125 (SCMD, http://scmd.gi.k.u-tokyo.ac.jp/). Each time a new yeast cell buds, a scar is left on the wall of the mother cell that is equal to the size of that bud (Muller, 1971). The accumulation of these scars results in a decrease in the mother’s cell surface area (Muller, 1971). In order for a mother cell to function properly, a minimal surface area must be maintained (Muller, 1971). A small bud size yields small scars. If the mother cell is large, then a larger amount of yeast cell buds are needed to reach the minimal surface area, thus allowing the cell to have a longer lifespan. 125 is a very large ratio, therefore this morphological parameter is more likely to have a longer lifespan which results in a smaller chance of having a false positive.

D203 is a nucleus that has a multinucleated mother cell ratio of 203 (SCMD, http://scmd.gi.k.u-tokyo.ac.jp/).

*3.6. Summary*

The causal model explains the resulting correlations between the robust factors and RLS upon completion of the regression analyses tests (refer to 3.1). There were 6 correlations that resulted from the causal model in figure 1. Those correlations were that: the number of protein interactions robustness is correlated to the robust factors evolutionary distance, morphological plasticity and fitness; the robust factor morphological plasticity is correlated to RLS; the robust factor fitness is correlated to RLS; and that the robust factors fitness and morphological plasticity are correlated to each other (Figure 4).

To determine the goodness of each correlation the adjusted R-squared values were analyzed. Fitness robustness to RLS gave the linear correlation with the best R-squared value and fitness to the number of protein interactions gave the linear correlation with the worst R-squared value. Arrows were used to show the strength of the correlations in the resulting causal model. The thickness of the arrows directly correlates to the strength of the R-squared values (Figure 6).

**4. Discussion**

*4.1 Correlations*

Based on the computational methods used in this study, we were able to obtain substantial genetic evidence that fitness robustness and morphological plasticity robustness are directly related to RLS. Fitness robustness yielded a higher adjusted R-squared value than morphological plasticity robustness therefore we concluded that there was a stronger correlation between fitness robustness and RLS than between morphological plasticity robustness, although both were found to have an initial overall significant correlation.

Based on the calculated p-values, fitness robustness and morphological plasticity robustness were also found to be correlated although the causal parameter is presently unknown. This correlation suggest that the overall fitness or ability of the cell to survive and reproduce is related to the ability of the cell to adapt to various environmental factors that may require the cell to be altered with regard to shape. Based on this finding it can be hypothesized that cells that are able to adapt (change shape) as needed may have a greater ability to reproduce and avoid being destroyed by various factors.

Evolutionary distance robustness and morphological plasticity robustness were also found to have a correlation to the number of protein interactions robustness. Similarly, the causal parameter for these correlations is unknown. In addition to these correlations an indirect correlation between morphological plasticity robustness and evolutionary distance robustness was also observed. Presently the overall cause of this indirect correlation is unknown.

Based on the results of this study, we were able to conclude that in an *S. cerevisiae* yeast cell, the more robust factor increases the probability that it will have a longer Replicative life span. Amongst the four selected robust factors only two were found to directly affect cellular aging.

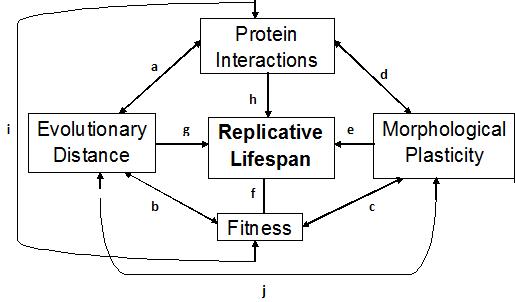
*4.2 Importance of the study and future experimentation*

This study underlines the advancing attempts to understand why aging occurs. The usage of the yeast *S.cerevisiae* as our model in this study, gave us the ability to compare our results to aging in higher organisms. It is hoped that with advances in technology and scientific understanding we will be able to know more about aging specifically as it relates to humans from studies such as this one.

Future studies should be conducted involving actual yeast cells that were grown and manipulated in the lab rather than using data obtained from other sources. This will allow direct observation of the different aging processes occurring within the yeast cells. The indirect correlation that was observed between morphological plasticity robustness and evolutionary distance robustness via protein interaction robustness should also be further examined. This correlation could provide very insightful information about the ways in which cells adapt to their environment and relate to other cells as aging occurs.

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**Appendix. Figure, table and plot results**

Figure 1: All of the theoretically 10 different possible correlations that could result from the 5 tested parameters. The hypothesized correlations are represented by arrows and alphabetical letters.

**Table 1: Regression results for growth mediums**

|  |  |  |
| --- | --- | --- |
| **Growth Medium** | **P-Value** | **R Squared** |
| YPD | 0.01499 | 0.02545 |
| YPDGE | 0.01034 | 0.02824 |
| YPG | 0.003657 | 0.03613 |
| YPE | 0.0001084 | 0.0632 |
| YPL | 0.004391 | 0.03474 |

**Relationship of Fitness Robustness in YPE to RLS**



p-value = 0.0001084

R-squared = 0.05913

Figure 2A: Fitness robustness in the growth medium YPE is directly related to RLS in the yeast

*S. cerevisiae*.



p-value = 1.349 e-05 R-squared = 0.03253

**The Relationship of Morphological Plasticity Robustness to RLS**

Figure 2B: Morphological Plasticity robustness is negatively related to RLS in the yeast

*S. cerevisiae*.

**The Relationship of Evolutionary** **Distance Robustness to RLS**



p-value = 0.3758

R-squared = -0.0007506

Figure 2C: Evolutionary Distance robustness is not related to RLS in the yeast *S. cerevisiae.*

**The Relationship of the Number of Protein Interactions Robustness to RLS**



p-value = 0.1759

R-squared = 0.002081

Figure 2D: The robustness of the number of protein interactions robustness is not related to

RLS in the yeast *S. cerevisiae.*

**Table 2: Regression results for the robust factors against each other**

|  |  |  |
| --- | --- | --- |
| **Robust Factor Test Parameters** | **P-Value** | **R Squared** |
| Evolutionary Distance vs Number of Protein Interactions | 1.363 x 10-09 | 0.01559 |
| Fitness vs Evolutionary Distance | 0.04481 | 0.004471 |
| Fitness vs Number of Protein Interactions | 0.00986 | 0.003768 |
| Morphological Plasticity vs Fitness | < 2.2 x 10-16 | 0.03732 |
| Morphological Plasticity vs Number of Protein interactions | 2.335 x 10-05 | 0.005051 |
| Morphological Plasticity vs Evolutionary Distance | 0.08989 | 0.0008607 |



P-Value

**Distribution of the P-Values for the Morphological Parameters**

Figure 3: Distribution of the morphological parameter p-values. The x-axis indicates the

p-value and the y-axis indicates the frequency of that p-value within the data set.

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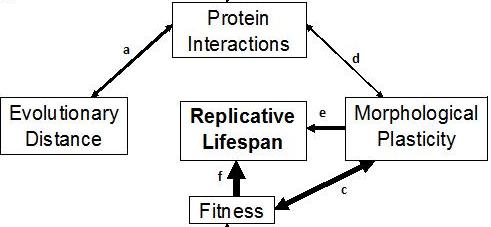


Figure 4: Results of the regression analyses tests. The arrows that support the null hypotheses have

been removed. The thickness of the arrows directly correlates to the goodness of each correlation

based on the R-squared values.

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