# Investigating the interconnection between cellular aging and network robustness

## Amanda Alexander

# Abstract

Cellular aging, a pleiotropic trait, is influenced by many components of gene networks.

We hypothesize that cellular aging is influenced by the configuration of gene and protein interaction networks for which robustness is a key factor in shaping the characteristics of the aging process.

Here, we aim to dissect the interconnection of network robustness and life-history traits in *Saccharomyces* *cerevisiae*. We evaluated the causal interactions of network connectivity, coefficient of variations of gene expression, evolutionary distance, fitness, morphological plasticity, and replicative life span using partial regressions.

{NEED to add the conclusions here}

The results of our study showed significant correlations between replicative lifespan and several robustness proxies. Specifically, replicative lifespan is negatively correlated to morphological plasticity and positively correlated to fitness robustness.

# Acknowledgements

# Introduction

The concept and definition of cellular aging has been a highly debated topic for several decades. Although many strides have been made towards understanding cellular aging, it is clear that the detailed mechanism of aging on a molecular level is far from understood. For the purpose of this study, aging will be defined as the loss of function that is generally accompanied by decreasing fertility and increasing mortality with advancing age.

Saccharomyces cerevisiae cells have proven to be an excellent model organism for the study of cellular aging. *S. cerevisiae* yeast cells are unicellular organisms that exhibit asymmetrical division and have the ability to adapt to severe environmental changes in order to maintain growth and function. Thus, they tend to live to different ages despite their genotypic similarities. It has been determined that these yeast cells share a similar complex internal cell structure to higher-level eukaryotes such as plants and animals, and thus exhibit similar molecular mechanisms of aging. Cellular aging in saccharomyces cerevisiae is most commonly measured in two ways: replicative lifespan, and chronological lifespan. Replicative lifespan is defined as the number of daughter cells created by mother cells before they senesce and cease to divide. Chronological lifespan measures how long a cell can survive in an arrested non-dividing state. In this study, cellular aging was measured based on replicative lifespan because this yeast lifespan measurement was more easily available to us.

Although several hundreds of genes in yeast have been found to effect cellular aging, none of these genes suggest a mechanism that is directly linked to aging. The factors that have previously shown direct effects on RLS include the silent information regulator 2 (Sir2) protein and calorie restriction (CR). Sir2 effects aging due to the “toxic” accumulation of extrachromosomal rDNA circles (ERCs) in the nucleus of a mother cell that can lead to the replicative aging of yeast (Kerberlein04PLoS). The deletion of Sir2 increases ERC formation and can thus significantly shortening lifespan. Conversely, it is hypothesized that an over expression of Sir2 will significantly increase life span. In addition to the Sir2 protein, calorie restriction has been found to have an effect on RLS. CR has been found to extend both RLS and CLS, and can be achieved by decreasing the glucose levels in the culture medium. The molecular mechanism for this phenomenon is unclear, but proteins, Mdh1 and Aat1, have been identified as factors that affect calorie restriction (citation?).

It has been found that genotypically homogeneous yeast cells from the same colony will live to different ages under identical environmental circumstances, suggesting that aging is a somewhat stochastic process. Despite this, there exist universal characteristics of aging at the demographic level (Strehler-Mildvan correlation), suggesting a common principle in the stochastic processes of aging. Several models of cellular aging have been hypothesized in attempts to accurately define cellular aging, for instance the two-parameter Gompertz model.

Where m is the mortality rate, m0 is the initial mortality rate, s is the survival fraction of a population (i.e. viability), t is time, and the Gompertz coefficient G, determines the acceleration rate of mortality rate over time and is therefore a parameter for aging. The Gompertz model ties to the Strehler-Mildvan correlation because it observes a negative correlation between G and the natural log of the initial mortality rate (this correlation was first observed in humans with the Strehler-Mildvan correlation). This correlation implies that there could exist an underlying model to determine cellular aging.

Previous research has provided evidence that cellular aging is an emergent property of gene networks, which allow for the communication of molecules inside the cell (citation). These gene networks are made up of a supply of DNA segments that interact with one another, but the level of gene expression varies depending on the type of cell and the environment. Based on earlier studies, it is evident that these gene networks allow the cell to adapt and survive, and thus depicts the robustness of the cell.

In this study, we investigate the interconnection between cellular robustness and cellular aging in the yeast Saccharomyces cerevisiae. Cellular robustness is defined as the ability of a cell to maintain homeostasis throughout genetic, environmental, or stochastic perturbations, such as temperature, time, and cellular damage. Previous research has hypothesized that cells with greater robustness experience a longer lifespan and that phenotypic capacitors influence robustness (citation?). Since cellular aging is defined as the deterioration of cellular functions, it follows that as a cell’s network robustness decreases it will be less able to adapt against external perturbations, causing a depletion of functionality of the protein activities (aging). Specifically, the Gompertz model predicts a positive correlation between cellular aging and cellular robustness. Thus, leading to the formulation of our hypothesis in this study that replicative lifespan in s. cerevisiae will be directly correlated to robustness and thus to several different proxies of robustness. The robustness proxies that we investigated in this study included: the number of protein interactions, the number of genetic interactions, evolutionary distance, fitness, and morphological plasticity. These robustness factors were selected because data in these areas was most easily accessible. This study examined each robustness proxy to determine the relationship to replicative lifespan using R statistical software. We examined the relationships between each individual robustness proxy and RLS, the robustness proxies to each other, and multiple combinations of the robustness proxies to RLS.

# Materials and Methods

## List of data sets

### Yeast deletion mutation with known effects on morphology is available at the Saccharomyces cerevisiae morphological database (SCMD, http://scmd.gi.k.u-tokyo.ac.jp/). SCMD provides a list of 501 morphological parameters in four groups: cell shapes, bud sized, nucleus locations, and actin localizations {Ohya, 2005 #534} and the analyzed data for morphological plasticity came from this database.

### The RLS data used in the study contained RLS for 564 genes measured by the Kaeberlein group {Managbanag, 2008 #563}.

### Fitness

#### Growth fitness measures in various conditions were obtained from Deutschbauer et. al. 2005, and Steinmetz et. al. 2002.

### Protein network and Genetic network

#### Several network datasets were used, including protein-protein interactions from DIP, BioGRID, and BIND {Xenarios, 2000 #1618;Bader, 2003 #2459;Stark, 2011 #2462} protein complexes {Warringer, 2003 #261;Warringer, 2003 #1969;Warringer, 2003 #261;Warringer, 2003 #1969}, and genetic interactions {Costanzo, 2010 #2074}.

### GFP CV, newman ????

### GEO CV of 3821 (glucose pulse) ????

### Evolutionary Distance???

#### Evolutionary distance data was given by Dr. Hong Qin

### The variances and coefficient of variation of the morphological plasticity data set was calculated because it is proportional to the robustness of the cell.

## Analysis Methods:

### Six different parameters were analyzed in this study: replicative lifespan, number of protein interactions, number of genetic interactions, fitness, morphological plasticity, and evolutionary distance

### R statistical software was used to perform linear and multiple regression analysis on the different variable factors that comprise robustness

### Firstly, regression analysis was performed between each fitness growth medium and RLS.

### Regression analysis was then conducted between replicative lifespan and each of the proxies of robustness.

### Multiple regression analysis was performed between various combinations of the robustness proxies evolutionary distance, number of protein interactions, number of genetic interactions, morphological plasticity, and fitness (YPE)

### Each analysis and numerical calculation was performed using R 2.15.1 and RStudio 0.97.332

# Current results

## Coefficient of Variation (CV) of the SCMD morphological plasticity data was calculated by dividing the calculated mean by the calculated standard deviation. Interestingly, after performing linear regression analysis, the CV did not show significant correlation to replicative lifespan (p=0.3542). Further, it was shown that there was significant correlation between SCMD mean and SCMD standard deviation (p<2.2e-16). It is possible that the correlation of these two parameters may have offset each other in the calculation of the CV, thus skewing the results of the linear regression. Due to this, the calculated standard deviation served as the robustness proxy for morphological plasticity and showed a significant correlation to replicative lifespan (p=1.349e-05).

## Regression Analysis

*Fitness vs. lifespan*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Comparison | Medium | Multiple R2 | p-value | Std. Dev. |
| RLS | YPD | 0.02545 | 0.01499 | 10.240 |
| RLS | YPDGE | 0.02824 | 0.01034 | 12.393 |
| RLS | YPG | 0.03613 | 0.003657 | 10.145 |
| RLS | YPE | 0.0632 | 0.0001084 | 12.246 |
| RLS | YPL | 0.03474 | 0.004391 | 11.620 |

YPE gave the largest R2 value and the lowest p-value, thus YPE has the most significant relationship with replicative lifespan.

## *Evolutionary distance vs. lifespan*

|  |  |  |  |
| --- | --- | --- | --- |
| Species 1 | Species 2 | Multiple R2 | p-value |
| SCE | SPA | 0.002773 | 0.3758 |
| SCE | SMIK | 0.3482 | 0.386 |
| SCE | SBAY | 0.3646 | 0.8462 |

\*\*Add full names of yeast species

SCE = S. cerevisiae, SPA = S. paradoxus, SMIK = S. mikatae, SBAY = S. bayanus

The large p-values (0.3758, 0.386, and 0.8462) show that there is no significant relationship between evolutionary distance and replicative lifespan in any of the yeast gene species.

RLS\_Del\_alpha ~ pDegree + YPE

|  |  |
| --- | --- |
|  | **Individual P-value** |
| pDegree | 0.522775 |
| YPE | 0.000459 |

|  |  |
| --- | --- |
| **Multiple R-squared** | **p-value** |
| 0.08851 | 0.0004161 |

RLS\_Del\_alpha ~ gDegree + YPE

|  |  |
| --- | --- |
|  | **Individual P-value** |
| gDegree | 0.995 |
| YPE | .030 |

|  |  |
| --- | --- |
| **Multiple R-squared** | **p-value** |
| 0.02567 | 0.06692 |

RLS\_Del\_alpha ~ gDegree + pDegree + YPE

|  |  |
| --- | --- |
|  | Individual P-value |
| gDegree | 0.8759 |
| pDegree | 0.2044 |
| YPE | 0.0993 |

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.04895 | 0.0601 |

gDegree ~ YPE

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.131 | 6.287e-08 |

pDegree ~ YPE

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.1021 | 2.046e-05 |

1/CV ~ RLS

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.01434 | 0.0687 |

Sqrt(1/CV) ~ RLS

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.01589 | 0.05521 |

RLS ~ sqrt(1/CV) + pDegree

|  |  |
| --- | --- |
|  | Individual P-value |
| Sqrt(1/CV) | 0.0193 |
| pDegree | 0.1643 |

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.05073 | 0.01262 |

RLS ~ scmd standard dev

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.03431 | 1.349e-05 |

RLS ~ sqrt(1/scmdstdev)

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.03178 | 2.844e-05 |

RLS ~ sqrt(scmd st dev)

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.03333 | 1.8e-05 |

RLS ~ scmdMean

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.01766 | 0.00188 |

Scmd st dev ~ scmdMean

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.5191 | <2.2e-16 |

Note: SCMD mean and stddev are highly correlated. We probably need a better way to use the morphology data set.

RLS ~ scmd CV

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.001581 | 0.3542 |

Multiple Regression Analysis

RLS ~YPE + Ka + pDegree + stddev

|  |  |
| --- | --- |
|  | Individual P-value |
| YPE | 0.9390 |
| Ka | 0.5707 |
| pDegree | 0.8377 |
| Stddev | 0.0723 |

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.06907 | 0.2389 |

RLS ~YPE + Ka + gDegree + stddev

|  |  |
| --- | --- |
|  | Individual P-value |
| YPE | 0.9811 |
| Ka | 0.6028 |
| gDegree | 0.7636 |
| Stddev | 0.0489 |

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.06088 | 0.2214 |

YPE ~ Ka + pDegree + stddev

|  |  |
| --- | --- |
|  | Individual P-value |
| Ka | 0.110256 |
| pDegree | 0.059991 |
| stddev | 0.000194 |

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.3525 | 2.318e-07 |

YPE ~ Ka + gDegree + stddev

|  |  |
| --- | --- |
|  | Individual P-value |
| Ka | 0.318 |
| gDegree | 0.378 |
| stddev | 3.48e-07 |

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.2921 | 6.359e-07 |

RLS ~ stddev + YPE (RLS held constant)

|  |  |
| --- | --- |
|  | Individual P-value |
| stddev | 0.00174 |
| YPE | 0.05157 |

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.09789 | 7.938e-06 |

Stddev ~ YPE + RLS (morphological plasticity held constant)

|  |  |
| --- | --- |
|  | Individual P-value |
| YPE | 3.85e-11 |
| RLS | 0.00174 |

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.243 | 1.65e-14 |

YPE ~ stddev + RLS (fitness held constant)

|  |  |
| --- | --- |
|  | Individual P-value |
| stddev | 3.85e-11 |
| RLS | 0.0516 |

|  |  |
| --- | --- |
| Multiple R-squared | p-value |
| 0.2227 | 3.366e-13 |

# Discussion

# Reference

# Tables & Figures

|  |  |  |  |
| --- | --- | --- | --- |
| Factors in Multiple Regression | | R2 value | p-value |
| RLS | * **Fitness** * **Evolutionary Distance** * **Number of Genetic Interactions** * **Morphological Plasticity** | **0.06088** | **0.2214** |
| RLS | * **Fitness** * **Evolutionary Distance** * **Number of Protein Interactions** * **Morphological Plasticity** | **0.06907** | **0.2389** |
| Fitness | * **Evolutionary Distance** * **Number of Protein Interactions** * **Morphological Plasticity** | **0.3525** | **2.318 x 10-7** |
| Fitness | * **Evolutionary Distance** * **Number of Genetic Interactions** * **Morphological Plasticity** | **0.2921** | **6.359 x 10-7** |
| RLS | * **Number of Protein Interactions** * **Fitness** | **0.08851** | **0.0004161** |
| RLS | * **Number of Genetic Interactions** * **Fitness** | **0.02567** | **0.06692** |
| RLS | * **Number of Genetic Interactions** * **Number of Protein Interactions** * **Fitness** | **0.04895** | **0.0601** |
| RLS | * **Morphological Plasticity** | **0.03431** | **1.349 x 10-5** |
| RLS | * **Morphological Plasticity** * **Fitness** | **0.09789** | **7.938 x 10-6** |
| Morphological Plasticity | * **Fitness** * **RLS** | **0.243** | **1.65 x 10-14** |
| Fitness | * **Morphological Plasticity** * **RLS** | **0.2227** | **3.366 x 10-13** |

|  |  |  |  |
| --- | --- | --- | --- |
| Factors in Multiple Regression | | R2 value | p-value |
| RLS | * **Morphological Plasticity** * **Fitness** | **0.09789** | **7.938 x 10-6** |
| Morphological Plasticity | * **Fitness** * **RLS** | **0.243** | **1.65 x 10-14** |
| Fitness | * **Morphological Plasticity** * **RLS** | **0.2227** | **3.366 x 10-13** |

Evolutionary distance = Ka

Protein Interactions = pDegree

Genetic Interactions = gDegree

Morphological Plasticity = scmdstddev

Fitness = YPE