ASPIRE Research Proposal

Investigating the Interconnection between Cellular Aging and Network Robustness

Student: Keyana Scott; Advisor: Hong Qin

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 is a unicellular organism and has proven to be an excellent model organism for the study of cellular aging. *S. cerevisiae* yeast cells asymmetrically divide 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 (RLS), and chronological lifespan (CLS). 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 yeasts lifespan measurement was more readily available to us.

Although several hundreds of genes in yeast have been found to affect 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 extra chromosomal rDNA circles (ERCs) in the nucleus of a mother cell that can lead to the replicative aging of yeast. The deletion of Sir2 increases ERC formation and can thus significantly shorten the 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.

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 largely stochastic process [8]. 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 [8]. Several models of cellular aging have been hypothesized in attempts to accurately define cellular aging, for instance the two-parameter Gompertz model.

$$\text{Mortality rate}: \quad \mu(t) = -\frac{1}{S(t)}\frac{dS(t)}{dt} \qquad \text{(Eq. 1)}$$

Where μ is the mortality rate, m_0 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 (Qin, manuscript in preparation). 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.

RESEARCH PLAN

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 (Qin, manuscript in preparation). 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.

1. Data Sources

RLS data for 564 different Saccharomyces cerevisiae yeast gene deletion mutants was obtained from the Kaeberlein group. Growth fitness measures in various conditions were obtained from Steinmetz, et.al. 2002 and Deutschbauer, et.al. 2005. Several network datasets were used including protein-protein interactions from DIP, BioGRID, and BIND, protein complexes, and genetic interactions. Evolutionary distance data was given by Dr. Hong Qin. 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 sizes, nucleus locations, and actin localizations and the analyzed data for morphological plasticity came from this database. The variance, standard deviation, and coefficient of variation of the morphological plasticity data set were calculated because they are proportional to the robustness of the cell.

2. Data Mining

Each analysis and numerical calculation will be performed using R and an open source software called R-studio. We will perform linear and multiple regression analysis on the different variable factors that comprise robustness and cellular aging. Multiple R-squared values and p-values were analyzed to determine significant correlations between replicative lifespan and several proxies of cellular robustness.

The mean, standard deviation, and coefficient of variation (CV) of the fitness data will be calculated to assess the fitness plasticity. This calculated coefficient of variation for fitness plasticity was incorporated into a column of the lifespan table and linear regression was performed to assess the correlation between replicative lifespan and 1/CV, and replicative lifespan and the square root of 1/CV. Multiple regression was also conducted between replicative lifespan and the square root of 1/CV and the number of protein interactions Weibull aging occurs in homogenous systems like machinery, and Gompertz aging occurs in heterogeneous systems like organisms.