**HHMI Student Proposal**

**Study the Aging Dynamics of the E. coli Gene Networks**

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**Introduction**

**1.1 Statement of the research problem, goals and objectives.**

For years people have wondered the cause of aging, when it starts, and what the aging markers are. Many have explored different theories on aging, none unraveling the true causes of it [5]. By understanding and examining new theories of aging, there is possibility to enhance successful aging. As an attempt to understand aging, we will explore a single cell and how it relates to its different organelles. Thus, giving a better understanding of aging and its effects on the body. Mitochondrial aging is known to be a major cause of human aging and disease. Mitochondrial theory of aging, a variant of free radical theory of aging, proposes that accumulation of damage to mitochondria and mitochondrial DNA (mtDNA) leads to aging of humans and animals [4]. Our overall goal is to use the aging dynamics of E coli gene networks to improve our understanding of the mitochondrial aging. Specifically, our first objective is to simulate the aging of the E coli gene network. Our second objective is to identify the critical component in E coli gene networks that can significantly increase the network lifespan.

**1.2 Endosymbiotic theory, aging of mitochondria and E. coli**

The mitochondrion has a proteobacterial origin based on the endosymbiotic theory. This theory states that the mitochondrion is a result of years of evolution created by endocytosis of bacteria. The bacteria are not digested; it became symbiotic instead. Endocytosis requires a cell to engulf another cell without passing through the membrane, which will create a double membrane. This process results in the outer membrane trapping in the foreign material causing an intracellular vesicle to form. Ultimately creating organelles such as the mitochondria.

Mitochondrial aging is a main cause of human aging, because the aerobic respiration that occurs inside of mitochondria produces reactive oxygen species (ROS) as byproducts. ROS are highly reactive molecules with unpaired electrons that can react with intracellular macromolecules and prevent them from performing normal functions. Aging development is often characterized by harmful ROS overproduction [1-2]. At the center of the free radical theory of aging lies the very important role of the mitochondria.

The free radical theory of aging has been a major theory of aging for more than 50 years. Dr. Harman in 1956 proposed that the collection of free radicals causes the damage of biomolecules by these reactive species resulting in cell senescence and organismal aging [3]. The free radical theory states that highly reactive molecules with unpaired electrons, termed as free radicals, cause oxidative damages to macromolecules within cells; thereby hindering normal biological functions. Mitochondria produce more reactive oxygen species such as superoxide radical and hydroxyl radical. These oxygen species with unpaired electrons are highly reactive and can damage the mitochondria’s DNA and proteins. The damaged mitochondria will then produce more ROS. This vicious cycle gradually leads to catastrophic consequences and is a major cause of aging.

*Escherichia coli* is a model organism in the phylum proteobacteria, and therefore is informative on mitochondrial aging. *E. coli* is a great model for mitochondria because they reproduce in the same manner. *E coli* is an organism with asymmetric division, no juvenile phase, and no identified separation between germline and soma; making it still susceptible to aging. Ituses asymmetric division that will exhibit no distinction between the parent and offspring. This division requires an old pole from the parent cell and builds a new pole with the occurring offspring. A Juvenile phase requires a cell to go through a time of growth or differentiation from the parent cell. *E. coli* does not demonstrate this phase and allows for immediate rejuvenation of the offspring.

**1.3 Gene network model of cellular aging**

Aging is defined as the increasing chance of failure with time. Recently, Qin proposed a gene network model of cellular aging. The basic idea of this network model of cellular aging is to use the random failure of gene interactions to model the declining of cellular activities. When an essential gene loses all of its gene interactions, it is equivalent to the deletion of an essential genes and thereby leads to cell death. We will apply this general framework to study the aging of *E coli* gene networks.

­­­To test our modeling work, we will compare the aging process of the E coli gene network with experimental data. We will implement this modeling work in the open source language R environment.

**1.4 Significance of Study**

By understanding how E. Coli gene networks interact within its system we will be able to model

how the mitochondrial gene networks interact as well. The relationship between the mitochondria, organelle, and the overall cell will give us different insights on how aging relates to single cells and the entire body. The mitochondria plays a major role in the production of energy for the cell. If the damages in the mitochondria affect the operation of the overall cell and leads to cell deterioration, cellular aging, we are now able to explore how to strengthen single organelles to control aging. We plan to bring a clear understanding of the importance of the mitochondria and its relation to the existence of the cell. It is important to know that the mitochondria has its own nucleus and the connection between the cells nucleus and the mitochondrias nucleus will also give a better understanding of their connection.

**1.5 Hypothesis**

One of the major causes of human aging, mitochondrial aging, can be understood through the modeling of E. Coli gene networks.

1. **Methodology**

Objective 1. Input the network and genome data into our computing platform R.

The gene networks of E coli is availabe at the Database of Interacting Proteins (DIP) (<http://dip.doe-mbi.ucla.edu/dip/Download.cgi?SM=7&TX=562>). There are 12263 pairwise gene interactions in this network data set.

E.Coli gene network database is also available at EcoCYC

The list of essential genes in E coli is availabe at EcoWiki ( <http://ecoliwiki.net/colipedia/index.php/Essential_genes> )

Objective 2. Establish the simulation framework for studying the aging of the E coli gene network.

The general principle of network aging simulation is to use the failure of essential gene to model cellular death. The biological activities of gene interactions will be modeled using exponential decaying function. When an essential gene loses all of its gene interactions, an event of cell death occurs. We will simulate the aging process of 1000 gene networks, i.e., cells, to obtain a survival curve of the population. We will implement the simulation process in R.

Objective 3. Identify the critical genes that can extend the lifespan of *E coli* gene networks.

We will systematically generate over-expression mutant for selected candidate genes in the E coli genome. We will compare the simulated lifespan of these over-expression mutants to the wildtype network and identify the genes that can significantly extend the average lifespan.

Our selection of candidate genes will be based on literature report and number of gene interactions per gene.

**2.5 Future Implications and Research**

After identifying the genes that can significantly extend the average lifespan, we can then test these specific genes and determine what role they play in aging by using experimental data. The human homolog of the E. Coli gene could then be examined to study its association with human aging-related diseases.

1. **Possible Timeline**

|  |  |
| --- | --- |
| Week | Task |
| 1 | Collect gene networks of E coli available at the Database of Interacting Proteins. Input these interactions into computing platform R Studio while reviewing relevant literature provided by Dr. Qin. |
| 2 | Continue input of the network and genome data into R Studio, which will allow for simulation of aging in the E.Coli gene network. |
| 3 | Continue simulation of aging in the E. Coli gene network. |
| 4 | Analyze survival curve of the population |
| 5 | Observe critical genes that can extend the lifespan of E.Coli gene networks. |
| 6 | Simulate overexpression of selected mutant genes from E. Coli genome |
| 7 | Continue simulation of overexpression of mutant genes and compare these mutants to the wildtype network. |
| 8 | Analyze simulation of overexpression of mutant genes with the wildtype and determine which gene can significantly increase lifespan from this data. |
| 9 | Summarize the study and prepare future research plans. |
| 10 | Prepare poster presentation and oral presentation for Research Day 2015. |
| 11 | Revise report and poster based on Research Day feedback. |

1. **Budget**

|  |  |  |
| --- | --- | --- |
| ***Description*** | ***Quantity*** | ***Price*** |
| Travel to meetings |  | $1500 |
| PeerJ Membership for publications |  | $350 |
| Endnote software |  | $300 |
| Consultant Fee |  | $500 |
| Stipend |  | $4000 |
| Total |  |  |

**\*The stipend will be divided between two student PIs.\***

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Mitochondrial theory of aging matures--roles of mtDNA mutation and oxidative stress in human aging.

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[Kunlin Jin](http://www.ncbi.nlm.nih.gov/pubmed/?term=Jin%20K%5Bauth%5D)