***Reading of: Comparative analysis of gene expression and regulation of replicative aging associated gene in S. cerevisiae***

***The Model***

Because many diseases of high morbidity and mortality such as cardiovascular disease, diabetes, nervous disorders etc have been found to be linked to aging, the authors of this paper argued that it is important to understand the mechanisms behind aging. In order to study the mechanisms associated with aging, models with 1) short life cycles and high proliferation rates that are 2) easily genetically mutated that have 3) well studied genomes and functional characterization and 4) established methods for high throughput studies were used. The organisms with these characteristics that is most commonly studied is *Saccharomyces cerevisiae,* which has many genes that have orthologs in the human genome. Its replicative lifespan is used to understand the lifespan of dividing cells of higher organisms while the chronological lifespan is used to study non-dividing cells of eukaryotes.

***Purpose of the Study***

The purpose of this study is to identify the genes whose modification can modulate lifespan in order to elucidate how gene expression and their regulatory controls modulate with age.

***Methods***

Data Set

In order carry out this study the data set for replicative lifespan was obtained from Kaeberlein *et al.*  which consisted of a list of 564 deletion strains that have been compared with the wild type. These strains were separated into three main groups on the basis of number of generations. These groups are long lived (LL) > 36 generations, short lived < 20 generations, and the middle group (MG) which consists of strains with a mean lifespan <26, not long lived (NLL) and those strains in which there is no significant difference.

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| --- | --- |
| Group | Number of Genes |
| LL | 44 genes |
| SL | 114 genes |
| MG | 406 genes |

Extending data set

The data set is extended by adding unique protein interactors (to all three groups using protein-protein interaction data from Krogran *et al.* Finding unique protein interactors help in identifying functions that are specific to each group of genes and prevents issues associated with ambiguous functions.

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| Group | Number of Genes | Number of genes in Krogan *et al.* Data set | Number of Unique Interactors |
| LL | 44 genes | 30 | 167 |
| SL | 114 genes | 74 | 159 |
| MG | 406 genes | N/A | 830 |

Statistical Method

* The p- value was calculated using the Wilcoxon test or the Mann-U Whitney test using the ‘R’ statistical package.
* A box plot was generated ( using ‘R’) to identify the middle 50% of the data, the median and the extreme points.

Comparative analysis of dynamic properties

This method was used to compare the static expression of long lived and short lived genes by examining the mRNA copy number, mRNA half-life, ribosome occupancy, protein half-life, protein abundance and noise. This data was assessed whether the long lived and short lived groups exhibited a different trend for each of the expression dynamic properties, the Wilcoxon rank-sum test ( assesses whether comparisons of two sample come from the same distribution or not) or Mannu- Whitney U test were used to calculate significance.

*Summary of Properties included in the analysis*

|  |  |
| --- | --- |
| Property | Why is it considered? |
| mRNA copy number | Enable the extent of transcription and translation rate of gene and transcripts to be studied |
| mRNA abundance | best described by the mRNA copy number per cell. |
| Ribosome occupancy | a measure of transcript engaged in translation |
| Half-life | a measure of protein stability, which is an estimate of the duration for which it occurs in the cell \* Excluding half-lives obtained by extrapolation |
| Protein abundance | gives the number of proteins molecules per cell |

Time Course Expression Analysis

This analysis was performed to analyze how the expression of genes associates with LL and SL groups changed with time during growth of yeast. It shows relative expression of each gene at different times in comparison with its own expression at t=0.

Epigenetic regulation

This analysis was performed to evaluate the differences in regulation of long lived and short lived genes at the epigenetic level to compare different histone modifications of the two groups.

***Main Findings***

*“Similar dynamic expression…”*

* Genes in the long and short lived groups show distinct effects on the life span of yeast.
* Similar mRNA abundance and ribosome occupancy in suggests that both long and short lived genes occur in similar concentrations at the protein level.
* Genes associated with LL and SL genes have no differences in stability.
* Gene ontology enrichment analysis show that long lived genes are enriched for several processes that form the core of the cell for normal functioning

*“…differing time course gene expression…”*

* The expression of long lived genes is low in the stationary phase. This may be responsible for the replicative aging in yeast.

*“LL and SL genes have common transcription factors(TFs)”*

* 88 TFs in common in the two groups, suggesting that genes associated with both groups are regulated by the same set of TFs. Thus, differences in gene expression cannot be explained on this basis.
* Some TFs increase with time and some TFs decrease with time. Expression of other TFs remain the same.

*“LL genes show higher epigenetic modifications”*

* Examination of differences in acetylation and methylation has shown a correlation of different histone acetylation and methylation with gene expression.
* LL and SL genes have no change in acetylation patterns in the extended data set
* LL genes show higher trimethylation at H3K79 positions in comparison to SL genes

*“Time course expression of methlylases and demethylasese” differ*

* The expression level of methylases specific to H3K36 and H3K79, Set1, Set2, and Dot1 respectively decrease with time while the expression of demethylases, Jhd1, Jhd2, and Rph1 increases with time.

*“LL and SL groups of replicative aging associated gene have different patterns of gene expression and their regulation can be distinguished only at the epigenetic level.”*

***Problems***

One of the issues that I noticed in the paper was pinpointing actual differences between LL and SL genes. Most of the time, the null hypothesis had to be accepted and many of the differences they sought to elucidate turned out to similarities.

***New research directions***

One direction that this research team might take is to determine if low expression of long lives genes during stationary phase is truly responsible for replicative aging in yeast by up-regulating the expression of long lives genes in yeast during the stationary phase using transformed vectors. Alternatively, they could look into the role of histone modifiers in yeast aging.