Effect of the deletion of conserved aging genes on the formation of extrachromosomal ribosomal DNA circles

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I am a part of the Kaeberlein Lab which studies aging in nematodes and yeast. Extensive previous work by Dr. Matt Kaeberlein, in collaboration with the lab of Dr. Brian Kennedy, has lead to the discovery of 25 conserved aging genes in yeast and nematodes. From a basic biology standpoint, aging is commonly defined as a degenerative process that that results in increased mortality with time. Knockout of the conserved aging genes increases lifespan in both nematodes and yeast. This conservation of gene function in two very divergent species is indicative of the possibility that the human homologues to these genes will also have an effect on lifespan. However, there is still a great deal of ongoing debate and research concerning the actual molecular mechanisms by which these genes affect lifespan.

A proposed mechanism for aging in yeast is the accumulation of extrachromosomal ribosomal DNA circles (ERCs) in cell nuclei. ERCs form during the copying of regulatory DNA sequences as a result of genomic instability due to increased homologous recombination (Park *et al*). Once they form they act as plasmids and undergo replication and accumulate as the cell divides. A study done by Dr. Kaeberlein found that deletion of the gene *Sir2* shortens replicative lifespan and increases the rate of rDNA recombination and ERC formation. He also found that deletion of *Fob1* decreases the rate of rDNA recombination and increases the replicative life span of the yeast (Kaeberlein *et al*). These data strongly indicate there is a correlation between lifespan and the genomic stability at the rDNA that is causally correlated to ERC formation.

My project involves exploring the effects that the 25 conserved aging genes have on the formation of ERCs, in hopes to elucidate whether they modulate longevity in yeast by

controlling genomic stability. Though research has been done on the effects of *Fob1* and *Sir2*, the conserved aging genes have not yet been analyzed with respect to recombination frequency. This presents a great opportunity for novel research pertaining to the hypothesis that lifespan reduction is related to the formation of ERCs, which was predominantly my original motivation for becoming involved in the project. The results of this experiment could be of great use for scientists analyzing the molecular mechanisms of aging, and the genes which contribute.

To test this hypothesis, I am using the W303AR yeast strain. This strain has an ADE2 marker incorporated into its ribosomal DNA. The Ade2 protein modifies a red colored adenine precursor molecule in the adenine synthesis process. When a recombination event occurs that excises *ADE2* in W303AR, the red pigment builds up in the cell resulting in a visibly red yeast colony. In the first cell division after the plating of W303AR, if a recombination event occurs that causes the ADE2 marker to be lost from the genome in an ERC, the resulting colony will be half red and half white. By determining the proportion of half red cells on the plate, the frequency of homologous recombination can be determined.

The first step of the project is constructing deletion strains by knocking out the 25 conserved aging genes in the W303AR background. I began this part of the project in July, working closely with George Sutphin, a graduate student in the Kaeberlein Lab. I was instructed in PCR amplification, lithium acetate heat shock transformation, smash and grab DNA extraction, and PCR verification. I began to transform in late July, but quickly concluded that our protocol was unacceptably inefficient in the W303AR background, and I spent the next two months tweaking my methodology. However, after much struggle, I have now developed a modified protocol which reproducibly yields transformants, and I have successfully constructed 10 of the 25 desired deletion strains in the W303AR background. The second phase of the

project involves performing large scale recombination assays of each deleted strain. This involves counting the number of white and half-white half-red cells on large YPD plates (density around 1000colonies/plate). I will ultimately need upwards of 10,000 colonies per strain to achieve any meaningful statistical significance. As experimental controls, I will use a *Fob1* deletion (known to decrease recombination), and a *Sir2* deletion (known to increase recombination). By comparing the *Fob1* and *Sir2* assays to the other deletion assays I have prepared, I can determine whether the gene deletions are increasing or decreasing recombination frequency.

Preliminary assays I have performed in the last month indicate that at least three of the deletion strains I have already prepared may increase recombination. My mentor, Dr. Kaeberlein, and I were surprised with the preliminary results. Based on the current model, one would expect that for genes which deletion increases lifespan, deletion would also decrease the rate of ribosomal recombination. This new data may support the hypothesis that lifespan extension in these mutants is independent of ERC formation. However, I must emphasize that the data was very preliminary, utilizing only 3000 cells for statistical analysis. Dr. Kaeberlein has encouraged me to replicate these results by testing independently derived transformants. I have a substantial amount of work yet to do before I can make any true claims about these data, however preliminary data still suggest great potential for this project to contribute novel data to the study of aging mechanisms in yeast.

When I began my proposed project at the beginning of the summer, I was confident I would finish or at least be well on my way to definitive results by the time the quarter had ended. However, it did not take long for me to learn that good science does not happen in days or weeks. I had vastly underestimated the difficulties I would face, and the time it would take for

me to resolve them. For the past four months I have been flung round and round the relentless cycle of trial and error that is the scientific method, and though I have experienced setbacks, I have realized that a good scientist must not dwell on his or her failures, but rather must recognize and appreciate that there is meaningful knowledge in failure, often as much as in success. This project has become an ineffable part of my life, a daily lesson in humility and patience. I am continuing to learn a lot and I am prepared to tackle my project this year with the intellectual and scientific vigor it requires of me.

It is not at all unreasonable to claim that I have learned more about science in these past four months of research than I could possibly ever learn in a classroom. No class could have demonstrated the meticulous and precise manipulations of protocol a scientist must undergo to achieve quantifiable results. The process of seemingly endless trial and error I underwent to establish a competent transformation protocol in the W303AR strain could have never been taught in a two hour lab section. I will admit that the problems I have experienced have been immensely frustrating. However, I have gained a great appreciation for the scientific method, and the virtue of patience. The skills and knowledge I am gaining in the lab as an undergraduate will be invaluable assets moving forward in a world that demands erudition and precision. I appreciate that the chance to work on an independent research project is a rare educational opportunity that entails benefits which extend far beyond the classroom. Every day in the lab I am acquiring new analytical tools for solving complex problems that I will use for the rest of my life.

References

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