Western Washington University
Western CEDAR

WWU Honors College Senior Projects | WWU Graduate and Undergraduate Scholarship

Spring 2015

# The effect of ribosomal protein inhibition on lifespan in Drosophila melanogaster

Ella Lamont
*Western Washington University*

Follow this and additional works at: https://cedar.wwu.edu/wwu_honors

Part of the Biology Commons

## Recommended Citation

Lamont, Ella, "The effect of ribosomal protein inhibition on lifespan in Drosophila melanogaster" (2015). *WWU Honors College Senior Projects*. 530.
https://cedar.wwu.edu/wwu_honors/530

This Project is brought to you for free and open access by the WWU Graduate and Undergraduate Scholarship at Western CEDAR. It has been accepted for inclusion in WWU Honors College Senior Projects by an authorized administrator of Western CEDAR. For more information, please contact westerncedar@wwu.edu.

# The effect of ribosomal protein inhibition on lifespan in *Drosophila melanogaster*

Ella Lamont

Advisor: Dr. Sandra Schulze

Honors Senior Project

Western Washington University

## Introduction

We take aging for granted as an immutable law of nature, and while at the surface this process may appear straightforward, aging is in fact a **complex trait**. Aging is controlled by combinations of genetics and external stimuli, and is associated with a plethora of age related diseases (Christensen et al. 2009). Until recently these seemingly disparate diseases, such as Alzheimer's, cancer, and diabetes, have been studied separately and treated as distinct and isolated processes. However, as we understand more about the overall nature and characteristics of aging, it is becoming clear that these apparently distinct disorders are connected at the genetic level (Christensen et al. 2009). It is therefore necessary to study the genetics of aging in order to understand and treat its associated diseases and improve quality of life for the aging.

Humans share similar **genes** with many animals, including mice, roundworms, and fruit flies; genes which have been overwhelmingly unaltered throughout evolution, and are therefore considered "highly conserved" (Terzian et al. 2013). The fruit fly *Drosophila melanogaster* is a common model organism used in genetic experiments, as many biological and genetic pathways have been conserved between humans and fruit flies (Beckingham et al. 2005). Most fly genes have human gene homologs, including age related genes that cause degenerative human diseases such as Alzheimer's and Parkinson's (Beckingham et al. 2005). The study of aging in model organisms such as the fruit fly is therefore an excellent way to indirectly study the genetics of human aging.

The process of aging involves cell and organismal metabolism. A cell's metabolism can function in two states; one of growth and reproduction or one of cell maintenance and repair, and the lifespan of an entire organism will lengthen when the body's cells shift from a fast metabolic rate to the slower rate of **somatic** maintenance (Hansen et al. 2007). The period of reproduction

is the most energetically expensive stage of a cell's metabolism, and includes the most abundant protein production (Hansen et al. 2007). Lifespan is therefore extended when cells switch from this expensive period of reproduction to a period maintenance, a transition which can be initiated by slowing **translation** (Hansen et al. 2007). The best way to inhibit translation is to cause a disruption in the translational machinery: **ribosomes.** Experiments involving roundworms and yeast have proven that when the synthesis of ribosomal proteins is restricted, overall protein translation is inhibited, and lifespan is extended as the cell enters a longer period of maintenance (Steffen et al. 2008, Hansen et al. 2007).

There are multiple biological processes that inhibit ribosomal protein (RP) synthesis, including knockdown of the enzyme TOR and dietary restriction. TOR is an enzyme called a **kinase** which functions in a variety of cellular processes and growth pathways, including the regulation of translation and sensing of nutrients (like amino acids) (McCormick et al. 2011, Hansen et al. 2007). TOR is activated by an increase in the cell's nutrient levels; if nutrients remain low, the TOR pathway is not activated and there is a subsequent decrease in cellular processes, slowdown of metabolic rate, and extension of lifespan (McCormick et al. 2011, Vellai et al. 2003). This represents a genetic mechanism with which RP synthesis can be inhibited. Dietary restriction represents an environmental condition that can cause inhibition, as an organism's lifespan can be lengthened by reducing the amount of calories ingested, which is thought to initiate the down-regulation of the TOR pathway (Mair et al. 2008).

In addition to biological processes that inhibit RP synthesis, technical methods can also be used. RNA interference, or RNAi, is a tool that can be used to experimentally manipulate RP synthesis to extend lifespan. An RNAi is an inverted repeat of a specific single stranded **mRNA** that codes for a particular protein (Dietzl et al. 2007, Alic et al. 2012). The gene encoding RNAi

can be inserted into an organism's **genome** as a transgene, where it is transcribed into an RNAi strand (Bass 2013). This strand will bind to a protein complex that includes an endonuclease. The RNAi and connecting complex then binds to its complementary mRNA strand, allowing the endonuclease to cleave and degrade the now double stranded RNA and effectively inhibit the synthesis of the protein (Weiberg et al. 2013, Alic et al. 2012). This process takes advantage of a naturally occurring mechanism in the cell. RNA strands are naturally single stranded, and when double stranded RNA are present, cells will cleave them with endonucleases. The use of RNAi is therefore a way to manipulate this mechanism to target specific genes. When the RNAi system is directed to inhibit RP gene expression in the round worm *Caenorhabditis elegans*, thus affecting the protein generating machinery of the cell, the result is a reduction in translation of all other proteins, inhibition of growth pathways, and extension of lifespan (Hansen et al. 2007). An RNAi transgene can be inserted and expressed throughout all *D. melanogaster* tissues; however certain tissues are more directly involved in the biological processes of lifespan extension. One such tissue composes the fat bodies and salivary glands of fruit flies and is the nutrient sensing tissue of the organism. As nutrients have been shown to affect the biological processes of aging, RP synthesis can be inhibited in these specific tissues to determine if inhibition will extend lifespan. In order to only express RNAi in this specific tissue and not throughout the organism, a system known as UAS-GAL4 can be employed.

Induction of RNAi can be controlled using the UAS-GAL4 system (Duffy et al. 2000, Dietzl et al. 2007). This widely used genetic system requires two separate fly strains, known as the driver strain and responder strain, and is activated only in the progeny of these crossed strains. The driver strain contains a tissue specific promoter (part of a gene that controls when and where that gene is expressed) that stimulates the expression of the driver, a GAL4

**transcription factor**. GAL4 is a protein transcription factor, meaning its presence or absence can regulate the transcription of other genes, and it can be inserted into the fly genome with no harmful side effects (Duffy et al. 2000). The genome of the responder strain contains an upstream activating sequence (UAS) located upstream of the specific gene to be expressed, in this case a specific RNAi transgene (Duffy et al. 2000, Dietzl et al. 2007). When both the UAS responder sequence and the GAL4 promoter are located in the same genome, GAL4, itself being driven by a tissue specific promoter, binds to the UAS responder sequence upstream of the RNAi transgene and activates transcription of that gene (Duffy et al. 2000) (Figure 1). When a fly strain with the GAL4 driver is crossed to a strain with the UAS and responder gene, the offspring have a transcriptionally active responder gene (Duffy et al. 2000).

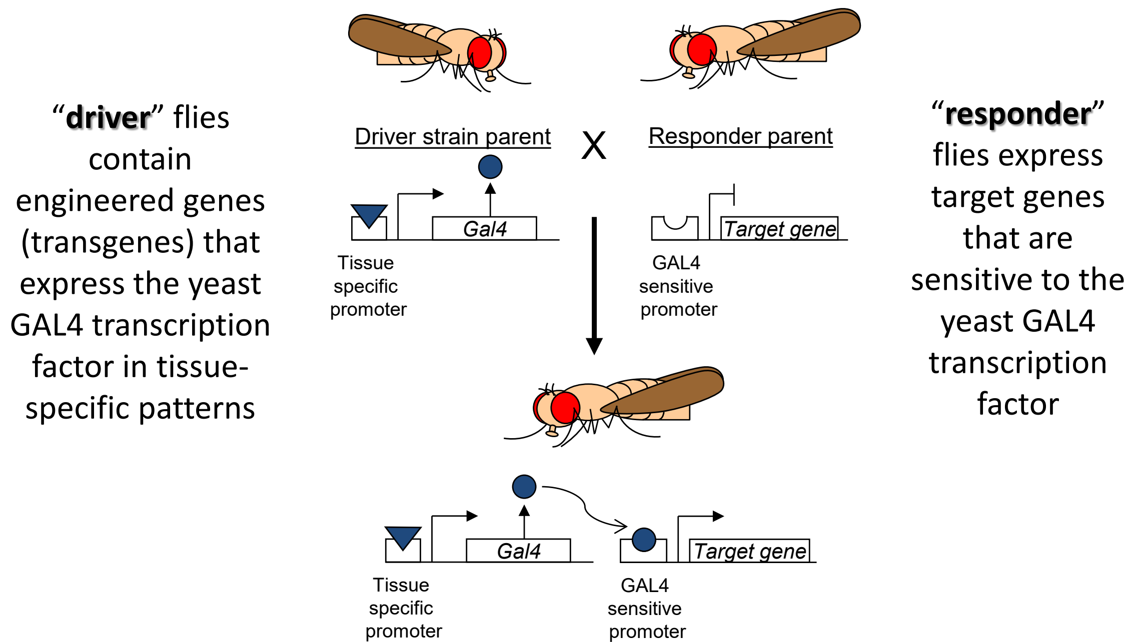


Figure 1. Driver and responder strains creating the UAS-GAL4 system in *D. melanogaster* and allowing the manipulation of gene expression. Transgenes are engineered genes injected into the genomes of model organisms. Image curtsey of Dr. Sandra Schulze.

In this experiment we used RNAi to specifically inhibit ribosomal proteins RPL10, RPS27, or RPS6 in the fat bodies and salivary glands. We chose these RPs because they have all had the effect of extending lifespan yeast and/or round worms when inhibited by a biological process. We used two negative controls; a control fly strain with the genetic background of both driver and responder strains but no GAL4 driver or RNAi transgene, and a control strain that contained a GAL4 driver and an RNAi transgene that specifically inhibited the GAL4 protein. We hypothesized that flies with RNAi transgenes that repress ribosomal proteins would live on average longer than the flies with the negative control genomes.

## Methods

In order to measure the difference in lifespan between flies with suppressed ribosomal proteins (RPs) and the negative control flies without, we ran a survival experiment. This experiment involved measuring the lifespan of flies with active RNAi transgenes inhibiting RP synthesis compared with negative controls. The length of time each fly in each experiment survived was then used in lifespan analyses.

### *Setting up Crosses*

We began this experiment with two types of fly strains; a driver strain containing a tissue specific promoter and GAL4 driver, and several responder strains of flies containing specific RNAi responder transgenes which inhibit the ribosomal proteins RPL10, RPS27, or RPS6. The tissue specific promotor used in the driver strain naturally promotes transcription of a larval serum protein (LSP) in fly fat body and salivary gland tissues (Massey et al. 1997). When inserted upstream of the GAL4 driver, this promotor (known as LSP) initiates expression of

GAL4 in the fat bodies and salivary glands. These strains were ordered from the Transgene RNAi Project at Harvard Medical School and had a uniform genetic background, with each transgene located on the third chromosome ("Targeted Transgenic RNAI" 2015).

To set up our experimental crosses, we mated males containing a specific responder RNAi transgene; RPL10, RPS27, or RPS6, with females containing a driver gene promoting GAL4 expression in the fat bodies and salivary glands. We therefore had three experimental treatments: progeny of LSP/RPL10, progeny of LSP/RPS27, and progeny of LSP/RPS6.

Our study contained two negative control fly strains. The first strain controlled for the genetic background of both driver and responder lines, a control necessary because aging is a complex trait. The background of the driver strain, containing no driver, was crossed with the background of the responder strain, containing a marker for the insertion site for the RNAi transgene but not the transgene itself. The progeny of this cross were used as the background negative control. The second strain controlled for any unknown side-effects of an actively functioning RNAi transgene. We crossed a fly strain containing a GAL4 driver with a strain containing an RNAi transgene specified to knock down GAL4. The progeny of this cross contained RNAi that actively knocked down GAL4 but supposedly had no other effect. We therefore had two negative control treatments: the background control, NONE/NONE, and the active RNAi control, LSP/GAL4.

Progeny of crosses between responder strain males and driver strain females were left to mate for 24 hours before the flies was collected (Figure 2). This experiment was divided into two sets (A and B); each set had two replicates of each treatment and was recorded and analyzed separately. We collected a total of 80 males and 80 females from the progeny of each cross and set up four total replicates for each treatment. Each replicate contained 20 male flies or 20

female flies of each cross, kept in separate vials. In total, we had 40 vials each containing 20 flies, divided into two sets of 20 vials. Each replicate was kept in a *Drosophila* vial stoppered with a cotton ball.

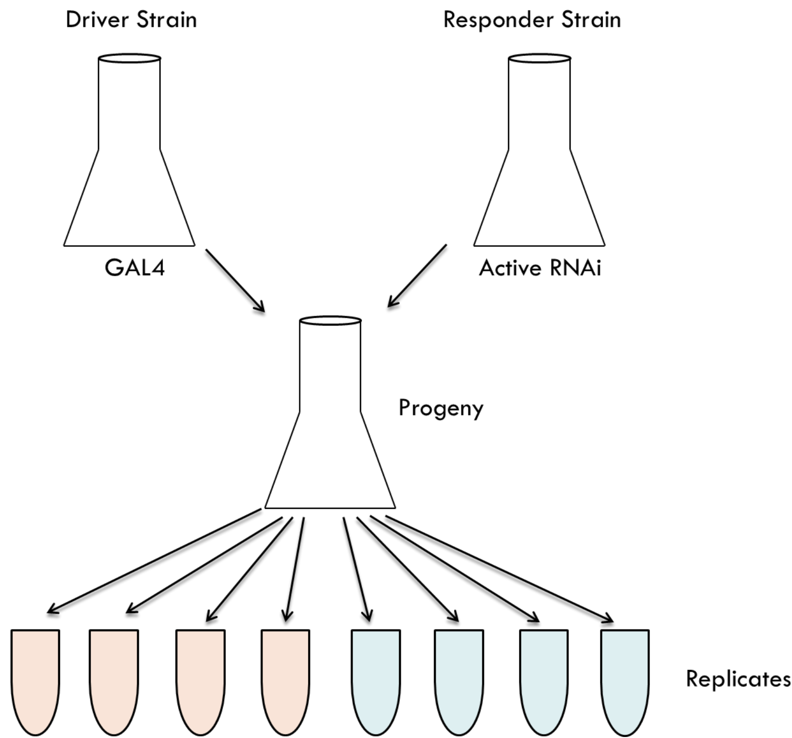


Figure 2. The generation of replicates. Progeny of driver and responder fly strains were left to mate for 24 hours before being collected and separated into four male fly vials and four female fly vials.

*Survival Experiment*

The main portion of this experiment involved measuring lifespan by recording the point in the experiment at which each and every fly died. To create a blind study, we first randomized the order of the vials in each tray, giving each vial a number instead of identifying them by genotype. All the vial trays were kept in a constant environment, room temperature was kept at about 19 to 20ºC and the trays were placed under a window to provide natural diurnal cycles. Each vial contained about 1 inch of standard *D. melanogaster* food containing sucrose, yeast,

corn meal, and mold inhibitor, and the flies in each vial were 'flipped' into a new vial containing fresh food every other day during the first half of the experiment and every third day during the second half. Continual fresh food was important because with time food can grow bacteria and mold which would kill the flies and influence the survival experiment.

Every time the flies were flipped the number of dead flies in each vial was counted and data was entered into the DLife computer program. This program was created by Dr. Scott Pletcher (of the University of Michigan) and is specifically designed to record *Drosophila* aging data (Linford et al. 2013). Dead flies that remained in the old vial after flipping were logged as "dead", dead flies that were carried into the new vial were logged as "carried", and flies that escaped or died by means unrelated to age were marked as "censored". This continued until the flies in all the vials had died. The duration of this survival experiment lasted 176 days.

*Data Analysis*

We visualized our data with means, medians, and Kaplan-Meier survival curves, and tested our hypothesis using log rank tests in DLife. The two sets of treatments were combined to determine means and medians and analyzed separately for survivorship curves and log rank tests. Kaplan-Meier survival curves and log rank tests are useful in survival analyses because they are able to make use of censored data. Data from flies that are censored (that either escaped or perished in a way unrelated to aging) still contribute to the analysis until the point at which they are removed (Goel et al. 2010).

Kaplan-Meier survival curves plot an organism's probability of surviving a given length of time by calculating the survival probability ($S_t$) during small time intervals (Equation 1), and plotting this probability against age (Goel et al. 2010). As male flies are known to live longer

than female files, we calculated survival curves of male and female flies for each treatment separately to prevent gender bias.

Equation 1. (Goel et al. 2010)

$$S_t = \frac{Initial\ number\ of\ living\ organisms - Number\ of\ dead\ organisms}{Initial\ number\ of\ living\ organisms}$$

Log rank tests are non-parametric, meaning they are used for data that is not normally distributed, and are used in survival analyses to test the null hypothesis that all organisms in a study have the same probability of death at any given point (Goel et al. 2010). This method compares a χ2 test statistic (Equation 2) with a χ2 distribution to determine the **p-value** of the test (Bewick et al. 2004). The χ2 test statistic is a commonly used tool to measure statistical significance. For this study, we performed a log rank test on the combinations of different fly strains, looking at two fly strains at a time. We ran tests on male and female flies for each treatment separately and considered any $p \leq 0.05$ as statistically significant. A p-value$\leq 0.05$ indicates that the chance that the two compared fly strains have the same probability of death is less than 5%.

Equation 2. (Bewick et al. 2004)

$$\chi 2 = \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2}$$

$O_1$=Total number of observed deaths in first fly strain

$O_2$=Total number of observed deaths in second fly strain

$E_1$=Total number of expected deaths in first fly strain

$E_2$=Total number of expected deaths in second fly strain

## Results

In this study we compared survivorship curves and survival probabilities of multiple genetically modified *Drosophila melanogaster* strains to determine whether ribosomal protein (RP) inhibition can significantly lengthen lifespan. The mean longest lived females were the LSP/RPS27 experimental strain and the mean shortest lived were the NONE/NONE control strain (Table 1a). The mean longest lived males were the LSP/GAL4 control strain and the mean shortest lived the LSP/RPL10 experimental strain (Table 1b). As expected, males lived longer than females overall.

Table 1a and b. The survival in days for all treatments of female and male flies. Errors indicated standard error and for each strain n=40.

Table 1a. Female flies

| *Treatment* | *Strain* | *Survival (Days)* | |
|---|---|---|---|
| | | *Mean* | *Median* |
| Ribosomal Protein Knock-down | LSP/RPL10 | 123.50±4.09 | 124.88±2.89 |
| | LSP/RPS27 | 129.17±0.14 | 133.92±3.96 |
| | LSP/RPS6 | 104.15±1.70 | 111.03±2.93 |
| Control strains | NONE/NONE | 97.13±5.38 | 101.34±1.56 |
| | LSP/GAL4 | 127.96±1.45 | 133.92±3.96 |

Table 1b. Male flies

| *Treatment* | *Strain* | *Survival (Days)* | |
|---|---|---|---|
| | | *Mean* | *Median* |
| Ribosomal Protein Knock-down | LSP/RPL10 | 121.62±1.50 | 124.88±2.89 |
| | LSP/RPS27 | 131.67±2.21 | 141.40±3.05 |
| | LSP/RPS6 | 123.51±8.12 | 130.83±3.05 |
| Control strains | NONE/NONE | 132.97±0.51 | 133.92±3.96 |
| | LSP/GAL4 | 140.94±1.78 | 148.73±0.02 |

Kaplan-Meier survival curves visualize the survivorship of our fly strains. The female survivorship graphs demonstrate that the background negative control strain had the lowest survivorship and the LSP/RPS27 the highest survivorship (Figure 3 and 4). Overall, the male survivorship graphs show a higher variability. In Set A the male LSP/RPL10 strain had the lowest survivorship, in Set B LSP/RPS6 had the lowest survivorship, and in both cases the LSP/GAL4 control strain had the highest survivorship (Figure 5 and 6).

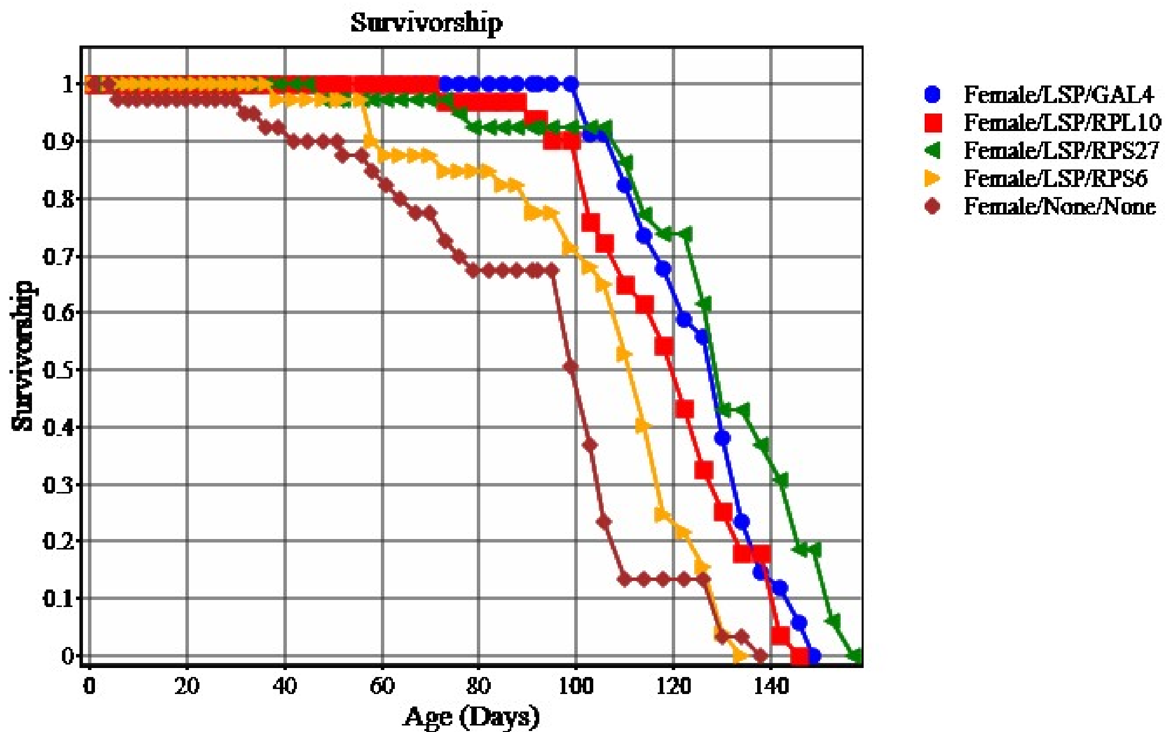


Figure 3. Kaplan-Meier survival curves for Set A female fly strains. For each strain, n=40.

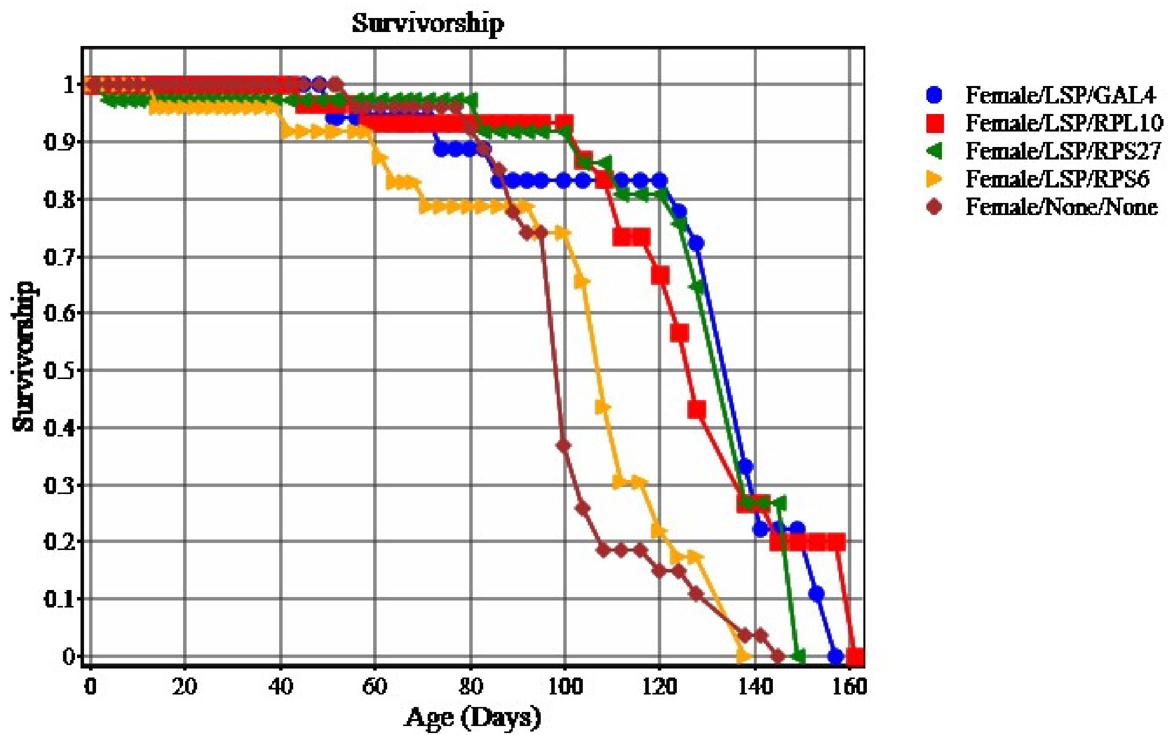


Figure 4. Kaplan-Meier survival curves for Set B female fly strains. For each strain, n=40.

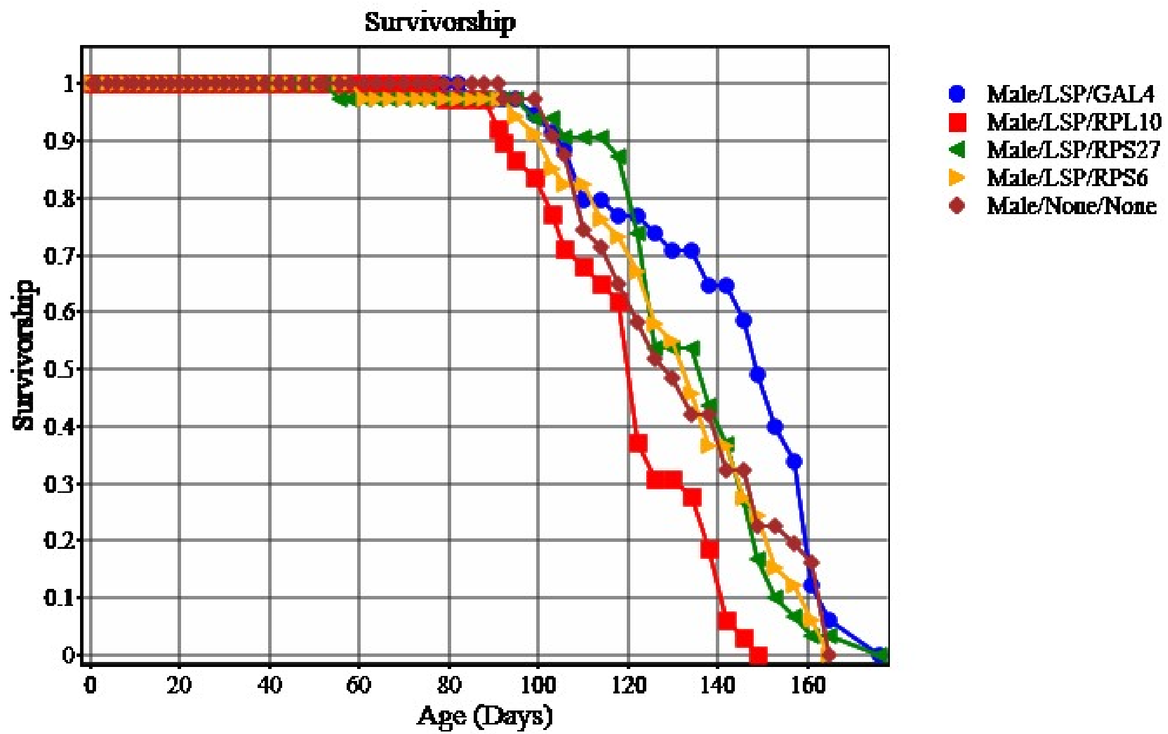


Figure 5. Kaplan-Meier survival curves for Set A male fly strains. For each strain, n=40.

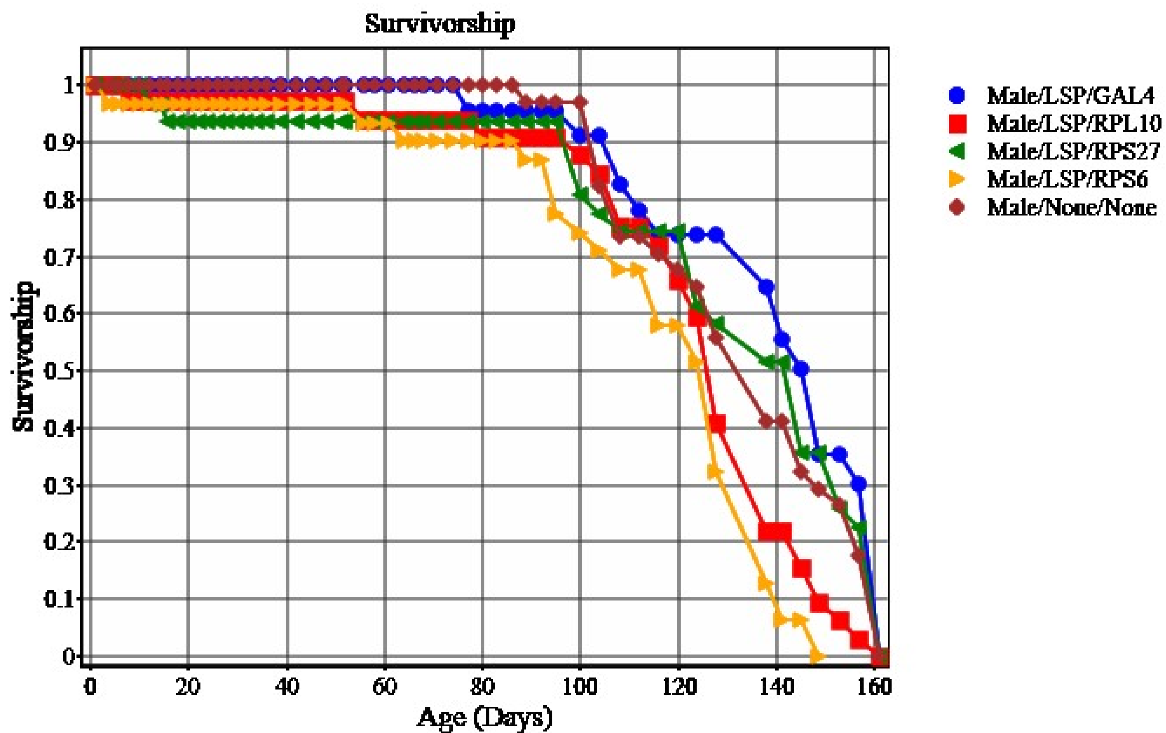


Figure 6. Kaplan-Meier survival curves for Set B male fly strains. For each strain, n=40.

While survivorship curves provide a visualization of survival times, log rank tests allow us to determine which strains have significantly different survivorships. By using both log rank tests and survivorship curves of female fly strains (which showed the most consistent data) we can see that, as expected, the background negative control strains generally had a shorter lifespan than the experimental strains (Table 2, Figures 3 and 4). Interestingly, however, the RNAi negative control strains were either longer lived or not significantly different from the experimental strains, and were significantly longer lived than the background negative controls (Table 2, Figures 3 and 4). Log rank tests between each pair of experimental strain also demonstrate significantly different survival times within the experimental strains (Table 2).

Table 2. The results of log rank tests for all combinations of female fly strains for both set A and set B replicates. The χ2 test statistic, **degrees of freedom**, and p-values for each test are given, and p-values in bold are statistically significant.

| | **Set A** | | | | **Set B** | | |
|---|---|---|---|---|---|---|---|
| *Cross* | *$\chi^2$* | *df* | *p-value* | | *$\chi^2$* | *df* | *p-value* |
| LSP/GAL4 vs LSP/RPL10 | 2.2 | 1 | 0.139 | | 0 | 1 | 0.852 |
| LSP/GAL4 vs LSP/RPS27 | 3.9 | 1 | **0.048** | | 0.4 | 1 | 0.551 |
| LSP/GAL4 vs LSP/RPS6 | 19.7 | 1 | **<0.001** | | 14.4 | 1 | **<0.001** |
| LSP/GAL4 vs NONE/NONE | 31.7 | 1 | **<0.001** | | 14.5 | 1 | **<0.001** |
| NONE/NONE vs LSP/RPL10 | 15.8 | 1 | **<0.001** | | 19 | 1 | **<0.001** |
| NONE/NONE vs LSP/RPS27 | 33.8 | 1 | **<0.001** | | 17.5 | 1 | **<0.001** |
| NONE/NONE vs LSP/RPS6 | 3.4 | 1 | 0.064 | | 1.3 | 1 | 0.249 |
| LSP/RPL10 vs LSP/RPS27 | 8.3 | 1 | **0.004** | | 0 | 1 | 0.892 |
| LSP/RPL10 vs LSP/RPS6 | 7.0 | 1 | **0.008** | | 12.1 | 1 | **<0.001** |
| LSP/RPL27 vs LSP/RPS6 | 23.9 | 1 | **<0.001** | | 12.8 | 1 | **<0.001** |

Log ranks tests performed on the survivorship of male fly strains revealed fewer differences in lifespan than observed within the female strains. The majority of experimental strains did not live significantly longer than the background negative controls (Table 4, Figures 5 and 6). However, with only one exception in Set B, the RNAi negative control strains lived significantly longer than the experimental strains (Table 3, Figures 5 and 6). Survivorships were not significantly different between control strains and showed varied significance between experimental strains (Table 3).

Table 3. The results of log rank tests for all combinations of male fly strains for both set A and set B replicates. The χ2 test statistic, degrees of freedom, and p-values for each test are given, and p-values in bold are statistically significant.

| | **Set A** | | | | **Set B** | | |
|---|---|---|---|---|---|---|---|
| *Cross* | *$\chi^2$* | *df* | *p-value* | | *$\chi^2$* | *df* | *p-value* |
| LSP/GAL4 vs LSP/RPL10 | 25.5 | 1 | **<0.001** | | 8.7 | 1 | **0.003** |
| LSP/GAL4 vs LSP/RPS27 | 5.7 | 1 | **0.017** | | 0.5 | 1 | 0.471 |
| LSP/GAL4 vs LSP/RPS6 | 5.3 | 1 | **0.021** | | 15.7 | 1 | **<0.001** |
| LSP/GAL4 vs NONE/NONE | 2.2 | 1 | 0.142 | | 1.4 | 1 | 0.239 |
| NONE/NONE vs LSP/RPL10 | 7.6 | 1 | **0.006** | | 3.8 | 1 | 0.052 |
| NONE/NONE vs LSP/RPS27 | 0.1 | 1 | 0.799 | | 0.1 | 1 | 0.723 |
| NONE/NONE vs LSP/RPS6 | 0.2 | 1 | 0.621 | | 10 | 1 | **0.002** |
| LSP/RPL10 vs LSP/RPS27 | 11.7 | 1 | **<0.001** | | 5.2 | 1 | **0.020** |
| LSP/RPL10 vs LSP/RPS6 | 8.8 | 1 | **0.003** | | 2.2 | 1 | 0.141 |
| LSP/RPL27 vs LSP/RPS6 | 0 | 1 | 0.974 | | 12.1 | 1 | **<0.001** |

## Discussion

This study could not demonstrate that ribosomal protein (RP) inhibition can significantly lengthen lifespan in *Drosophila melanogaster.* Most experimental strains did have a higher survivorship than the background negative control; however few experimental strains were significantly different from the activated RNAi negative control. We therefore cannot conclude that ribosomal protein inhibition can lengthen lifespan.

Many of the experimental strains showed significantly different lifespans from each other. This suggests that inhibition of different ribosomal proteins can have dissimilar effects on lifespan, and while some RP inhibitions might correspond with lengthened lifespan, others might be correlated with a lower survivorship. In the female strains, flies with inhibited RPL10 and RPS27 had a significantly longer survivorship than female flies with the background negative control, while flies with inhibited RPS6 did not live significantly longer than this negative control. A similar result is seen in the male fly strains; in which flies with inhibited RPL10 lived significantly longer than those with the background negative control stain, whereas flies with inhibited RPS27 or RPS6 did not. The survivorship of the male RPS27 and RPS6 strains were also not significantly different from each other in the Set A replicates. This discrepancy in survivorship between different RP inhibited strains suggests that the RPS27 and RPS6 were possibly knocked down too far by RNAi, and the lack of these ribosomal proteins caused functional problems that lowered survivorship. This result would need to be confirmed by direct measurement of RP mRNA levels in future studies.

While a survival analysis of the female strains did verify that the negative controls with the same genetic background had a shorter average life span than most strains with an inhibited ribosomal protein, this was not the case for the second control strain. A surprising finding was

that the second negative control, which contained an activated RNAi transgene, either lived significantly longer or were not significantly different from the experimental strains, and lived significantly longer than the background negative control strains in all replicates. Male fly survivorship was more varied, but survivorship curves of the male strains also shows that the lifespan of the active RNAi (LSP/GAL4) control was greater than almost all of the experimental strains and was slightly greater (although not significantly different) from the background (NONE/NONE) negative control. These results suggest that, although specific ribosomal protein inhibition may not be affecting lifespan, something else that is impacting both the experimental strains and the active RNAi control treatment is serving to lengthen lifespan.

This second negative control was intended to control for any unknown side-effects of an activated RNAi transgene. Since our results suggest this control strain had a survivorship similar to or greater than our experimental strains, something involved in activating the RNAi transgene is most likely the cause of increased survivorship. Ubiquitous expression of RNAi has been known to shorten fly lifespan (Alic et al. 2012), so it is unlikely that active RNAi is the cause of high survivorship. Instead, it is possible that expression of the RNAi transgene is upregulating transcription of a nearby gene. This can be caused when the insertion and subsequent expression of a transgene opens up the **chromatin** and allows more active transcription of nearby genes (Németh and Längst 2004). Molybdenum cofactor synthesis 1 (*Mocs1*) is a gene located directly downstream of the RNAi transgene in our responder strains (Dos Santos et al. 2015), and could have been upregulated as a consequence of the activated RNAi transgene (Figure 7).

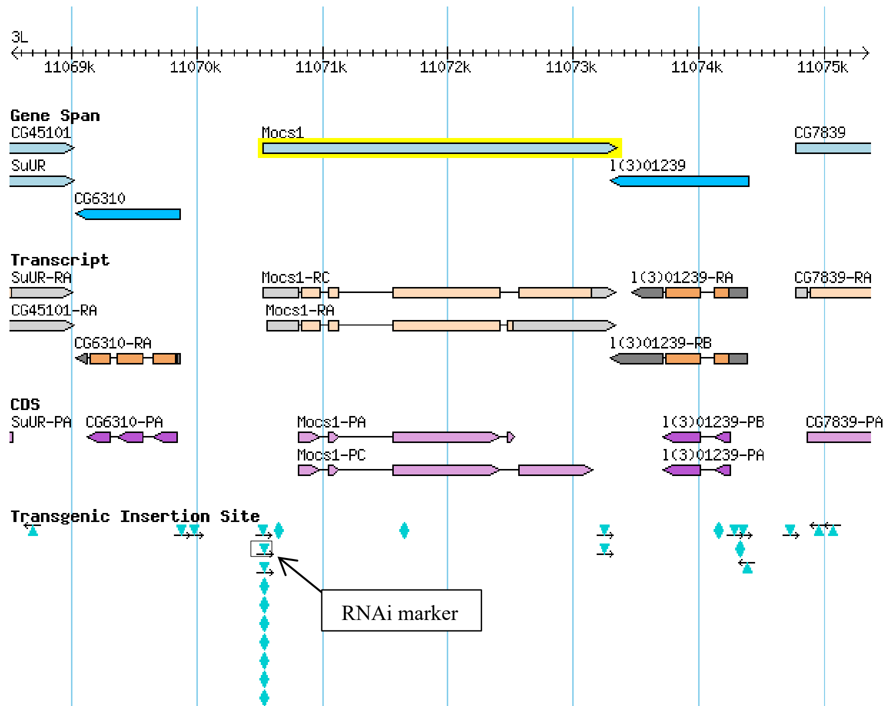


Figure 7. Genomic location of the *Mocs1* gene on the third chromosome in *D. melanogaster*. *Mocs1* is highlighted in yellow and transgene insertion sites are identified as turquoise triangles and diamonds (Dos Santos et al. 2015).

*Mocs1* is a **highly conserved gene** that codes for a protein necessary for a cell to utilize the trace element molybdenum (Mendel and Bittner 2006). Molybdenum (Mo) is a metal used in many enzymatic reactions, including carbon, nitrogen, and sulfur redox reactions (Mendel and Bittner 2006). Bacteria, plants, and animals all use Mo, which must be bound to a cofactor called moco to be used by enzymes (Mendel and Bittner 2006). Once bound to the moco cofactor, Mo is integrated in the active site of specific enzymes resulting in activation (Mendel and Bittner 2006). The *D. melanogaster Mocs1* gene, with **homologs** in bacteria, plants, and animals, is involved in the synthesis of the moco cofactor (Mendel 2013). *Mocs1* catalyzes the first process in moco production, converting the nucleotide **GTP** to the cofactor’s precursor, a “sulfur-free pternin compound” (Mendel and Bittner 2006). If Mo is unavailable, or for some reason the co-factor is not synthesized, important metabolic process are prevented (Mendel

2013), and an inhibition in enzymatic functions in which Mo is involved causes lethal neurological damage (Mendel and Bittner 2006).

A particularly important Mo enzyme is sulfite oxidase (SO), which oxidizes sulfite into sulfate (Mendel and Bittner 2006). Sulfite is a "toxic metabolite" created when amino acids containing sulfur are degraded, and sulfite accumulates as a byproduct of cell metabolism (Mendel 2013). SO removes sulfite from the cell, and if the moco cofactor is limited or unavailable there is a buildup of sulfite in animal cells, causing neurological harm and usually early death (Mendel and Bittner 2006). We hypothesize, however, that *Mocs1* transcription is being upregulated in our fly strains, consequently causing more moco cofactors to be produced, and activating more SO in the cell. Thus increasing SO will remove more sulfite, and possibly lengthen lifespan as toxic metabolites are more quickly removed. If this is correct, up-regulation of the *Mocs1* gene could indeed be the cause of high survivorship in both our experimental and RNAi negative control fly strains.

This study did not include a method to measure up-regulation of the *Mocs1* gene; however, sample flies were removed and frozen at various intervals during the survival experiment. The next step in testing our *Mocs1* up-regulation hypothesis will be to perform quantitative PCR on these specimens to determine whether the *Mocs1* gene has indeed been up-regulated in flies containing an active RNAi transgene. While this study did not to support our initial hypothesis that ribosomal protein inhibition can increase survivorship in *D. melanogaster*, our results suggest a link between the up-regulation of the *Mocs1* gene and a longer lifespan, bringing us a step closer to teasing apart the genetics of aging.

## Glossary

Chromatin: "a complex of nucleic acid and basic proteins (as histone) in eukaryotic cells that is usually dispersed in the interphase nucleus and condensed into chromosomes in mitosis and meiosis" (Merriam-Webster Online Dictionary 2015).

Complex Trait: A trait, such as aging, that is strongly influenced by variations in genes and environmental factors.

Degrees of Freedom: "The number of independent observations in a sample minus the number of population parameters that must be estimated from sample data" (Stat Trek 2015).

DNA: Deoxyribonucleic acid; a double stranded helix "that carries genetic information in the cells of plants and animals" (Merriam-Webster Online Dictionary 2015).

Gene: "A specific sequence of nucleotides in DNA or RNA that is located usually on a chromosome and that is the functional unit of inheritance controlling the transmission and expression of a protein or controlling the function of other genetic material" (Merriam-Webster Online Dictionary 2015).

Genome: "All the genetic information possessed by any organism" (Medicine Net 2015).

GTP: Guanosine triphosphate; “an energy-rich nucleotide analogous to ATP that is necessary for peptide bond formation during protein synthesis” (Merriam-Webster Online Dictionary 2015).

Highly conserved gene: “A gene that has remained essentially unchanged throughout evolution” (Medicine Net 2015).

Homologs: Genes that have been evolutionary conserved and are similar between organisms.

Kinase: “any of various enzymes that catalyze the transfer of phosphate groups from a high-energy phosphate-containing molecule (as ATP) to a substrate” (Merriam-Webster Online Dictionary 2015).

Phenotype: “The observable properties of an organism that are produced by the interaction of the genotype and the environment” (Merriam-Webster Online Dictionary 2015).

p-value: “measures the strength of evidence in support of a null hypothesis” (Stat Trek 2015.)

Ribosome: A structure in the cell’s cytoplasm that synthesizes proteins.

RNA: “Ribonucleic acid, which functions in cellular protein synthesis in all living cells” (Merriam-Webster Online Dictionary 2015).

Somatic Cell: “any of the cells of the body that compose the tissues, organs, and parts of that individual other than the germ cells” (Merriam-Webster Online Dictionary 2015).

Transcription Factor: A protein that “that binds to DNA and plays a role in the regulation of gene expression by promoting transcription” (Merriam-Webster Online Dictionary 2015).

Translation: “the process of forming a protein molecule at a ribosomal site of protein synthesis from information contained in messenger RNA “(Merriam-Webster Online Dictionary 2015).

## References


Alic N, M.P. Hoddinott, A. Foley, C. Slack, and M.D. Piper. "Detrimental Effects of RNAi: A Cautionary Note on Its Use in *Drosophila* Ageing Studies". PLoS ONE 7.9 (2012).

Bass, Brenda L., "RNA interference: The short answer". Nature 41 (2001): 428-429.

Beckingham, Kathleen M., J. Douglas Armstrong, Michael J. Texada, Ravi Munjaal, and Dean A. Baker. "Drosophila Melanogaster- the model organism of choice for the complex biology of multi-cellular organisms". Gravitational and Space Biology 18.2 (2005): 17-29.

Bewick, Viv, Liz Cheek, and Jonathan Ball. "Statistics review 12: Survival analysis". Critical Care 8.5 (2004): 389–394.

Christensen, K, G. Doblhammer, R. Rau, and J.W. Vaupel. "Ageing populations: the challenges ahead". Lancet 374 (2009): 1196–1208.

Dietzl, G., D. Chen, F. Schnorrer, K.-C. Su, Y. Barinova, M. Fellner, B. Gasser, K. Kinsey, S. Oppel, S. Scheiblauer, A. Couto, V. Marra, K. Keleman, and B.J. Dickson. "A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila". Nature, 448 (2007): 151–6.

Dos Santos, G, AJ Schroeder, JL Goodman, VB Strelets, MA Crosby, J Thurmond, DB Emmert, WM Gelbart; the FlyBase Consortium. FlyBase: introduction of the Drosophila melanogaster Release 6 reference genome assembly and large-scale migration of genome annotations. (2015).

Duffy, J.B. "GAL4 system in Drosophila: a fly geneticist's Swiss army knife". Genesis 34 (2000): 1–15.

Goel, Manish Kumar, Pardeep Khanna, and Jugal Kishore. "Understanding survival analysis: Kaplan-Meier estimate". International Journal of Ayurveda Research 1.4 (2010): 274–278.

Gray, Todd and Robert D. Nicholls. "Diverse splicing mechanisms fuse the evolutionarily conserved bicistronic MOCS1A and MOCS1B open reading frames". RNA 6 (2000):928–936.

Hansen, M., S. Taubert, D. Crawford, N. Libina, S.J. Lee, and C. Kenyon. "Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans". Aging Cell 6 (2007): 95–110.

Linford, N. J., C. Bilgir, and S. D. Pletcher. "Measurement of lifespan in Drosophila melanogaster". J Vis Exp 7 (2013).

Mair, W. and A. Dillin. “Aging and survival: the genetics of life span extension by dietary restriction”. Annual Review of Biochemistry 77 (2008): 727–54.

Massey, HC Jr., J. Kejzlarová-Lepesant, R.L. Willis, A.B. Castleberry, and H. Benes. “The Drosophila Lsp-1 beta gene. A structural and phylogenetic analysis”. Eur. J. Biochem. 245 (1997): 199-207.

McCormick, M.A., S.Y. Tsai, and B.K. Kennedy. “TOR and ageing: a complex pathway for a complex process”. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 366 (2011):17–27.

“Medicine Net”.MedicineNet Inc (2015).

Mendel, Ralf R and Florian Bittner. “Cell Biology of Molybdenum”. Biochimica et Biophysica Acta 1763 (2006): 621–635.

Mendel, Ralf R. “The Molybdenum Cofactor”. The Journal of Biological Chemistry 288 (2013):13165-13172.

“Merriam-Webster Online Dictionary”. Merrian-Webster Incorporated (2015).

Németh, Attila and Gernot Längst. “Chromatin higher order structure: Opening up chromatin for transcription”. Briefings in Functional Genomics & Proteomics 2.4 (2004): 334-343.

"StatTrek". Stattrek.com (2015).

Steffen, K.K., V.L. MacKay, E.O. Kerr, M. Tsuchiya, D. Hu, L.A. Fox, N. Dang, E.D. Johnston, J.A. Oakes, B.N. Tchao, D.N. Pak, S. Fields, B.K. Kennedy, and M. Kaeberlein. "Yeast life span extension by depletion of 60s ribosomal subunits is mediated by Gcn4". Cell 133 (2008): 292–302.

Terzian, T and N. Box, "Genetics of ribosomal proteins: curiouser and curiouser." PLoS Genetics 9 (2013).

"Targeted Transgenic RNA." The Transgenic RNAi Project. Harvard Medical School (2015). Web. 19 May 2015.

Vellai, T., K. Takacs-Vellai, Y. Zhang, A.L. Kovacs, L. Orosz, and F. Müller. "Genetics: influence of TOR kinase on lifespan in C elegans". Nature 426 (2003).

Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, Huang HD, and Jin H. "Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways". Science 342 (2013): 118-23