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An Analysis of the effects of Mocs1 upregulation on lifespan in Drosophila melanogaster

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Abstract

Molybdenum-cofactor biosynthesis protein 1 catalyzes the first step in the synthesis of molybdenum cofactor (Moco), which is conserved across taxa and present both in prokaryotes and in eukaryotes.[1] The effects of *Mocs1*-upregulation on *Drosophila* lifespan were determined using the GAL4-UAS system to drive *Mocs1* expression. Driven females lived significantly longer than controls ($p=5.7x10^{-6}$, $p=9.0x10^{-4}$) while males did not show increased lifespan ($p=0.49$, $p=1.8x10^{-5}$). The data suggest a link between *Mocs1* and lifespan which has not been previously explored.

Introduction

The biochemical basis of aging is currently a matter of considerable interest in the scientific community. The ability to more completely comprehend the underlying inter- and intra-cellular interactions which lead to organismal aging and death will enable more sophisticated treatments to be developed to reverse or mitigate these effects, eventually providing the practicing medical professional with a greatly enhanced toolkit to evaluate individual treatment options for patients. To this end, a great deal of research has been conducted to increase scientific understanding of aging. This is difficult due to the fact that the process of aging is mediated through a host of genetic and environmental factors: aging, in short, is a complex trait.

Since it is not feasible (or ethical) to perform controlled breeding experiments using human subjects, a number of model organisms are used in studies of aging. Ideally, these organisms should be small, should have short generation times, should have many genes that are homologous to human genes, and should have genomes which are easily manipulable. Model

organisms which have been utilized in aging experiments include nematode worms (*C. elegans*)[2], yeast (*S. cerevisiae*)[3], mice (*M. musculus*)[4], and fruit flies (*Drosophila melanogaster*)[5]. For the remainder of this paper, we have chosen to emphasize the molecular basis of aging in *Drosophila*; the basic pathways discussed here will generally apply to all organisms, but the exact details may differ slightly.

One pathway which has been shown to be relevant to the aging process is the TOR (Target Of Rapamycin) pathway, which has also been implicated in the process of nutrient sensing.[6] (In mammals, this is called the mTOR pathway, where "m" is for "mammalian", or sometimes "mechanistic", target of rapamycin. However, in *D. melanogaster*, the species of interest in this paper, and in many other nonmammalian organisms, there is just a single gene called TOR.) TOR proteins, which are multi-domain serine-threonine kinases, play key roles in cell cycle determination and in nutrient sensing and have a broad array of effects on downstream proteins.[6] Expression of TOR is implicated in increased cell growth and protein production; downstream proteins include eukaryotic translation initiation factor 4E (eIF4E), which is a translation factor assisting ribosome-mRNA binding, ribosomal protein S6 kinase (S6K), and other proteins (see Figure 1).[6] Data suggest that TOR is implicated both in protein synthesis and ribosome assembly.[6,7] When nutrients are abundant, TOR is activated, and when they are scarce, TOR is naturally downregulated, leading to decreased protein (and probably ribosome) synthesis.[7] TOR knockdown, for example by the antibiotic rapamycin, is well-known to increase lifespan in a number of model organisms.[7] Interestingly, dietary restriction (DR) also has a positive effect on organism lifespan, apparently in part due to natural downregulation of TOR as a response to lack of nutrients.[7]

The TOR pathway interacts substantially with the insulin/IGF-1 (insulin-like growth factor 1) pathway - IIS pathway. Like TOR, the IIS pathway is nutrient-sensitive; plentiful nutrients lead to its upregulation while scarcity downregulates it.[8] Additionally, increased IIS expression is associated with faster wound repair in *Drosophila*.[9] There is significant crosstalk between the TOR and IIS pathways.[9] Like TOR, downregulation of the IIS pathway is implicated in lifespan extension.[9] IIS expression causes downregulation of FOXO (Forkhead Box O) transcription factors (see Figure 1).[10] FOXO induces expression of antioxidant proteins as well as increasing proteasomal activity leading to more efficient recycling of defunct cellular components; through these means, expression of FOXO contributes to increased lifespan.[10,11] In summary, it is thought that the life-extending effects of dietary restriction may be mediated in part through the insulin/ILS pathway as well as through TOR.

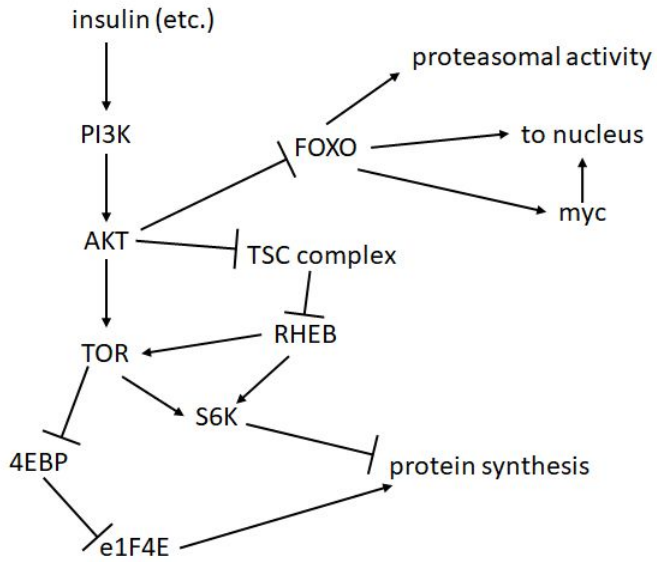

Figure 1. ILS/TOR pathway. Adapted from a figure by Sandra Schulze (unpublished) and modified by data from Bjornsti and Houghton 2004.[12] This is only a very small selection of the enzymes involved in nutrient signalling, designed to demonstrate the complexity of this pathway. For a fuller exploration and a more complete figure, I recommend the paper *Ageing in Drosophila: The role of the insulin/Igf and TOR signalling network* by Partridge et al.[6] (Since the Bjornsti paper from which parts of this figure were derived is discussing the TOR pathway in other organisms as well, it is possible that some minor details may differ in *Drosophila*.)

In addition to the pathways outlined above, there are many other interactions which have been implicated in aging. Recently, a number of general categories contributing to the phenomenon of aging have been outlined by Lopez-Otin et al: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.[8] (See Figure 2, reprinted from Lopez-Otin et al). The TOR and insulin/ILS-1 pathways both fall largely under the heading of "deregulated nutrient sensing" in Lopez-Otin et al's list. An exhaustive description of the current understanding of aging is well beyond the scope of this introduction, but it is hoped that the general background provided above will give the reader a glimpse into a few of the basic mechanistic processes underlying aging.

Figure 2. Factors causing the aging phenotype. Reprinted from Lopez-Otin 2013.

One gene which is not particularly well-characterized but which we have discovered may play a role in lifespan extension is *Mocs1* (molybdenum cofactor synthesis 1), also known as *lxd* (low xanthine dehydrogenase). There is a human ortholog to this gene, named *MOCS1*; therefore, the conclusions obtained for *Mocs1* in *Drosophila* could conceivably have applications in *H. sapiens* as well. If *Mocs1* does in fact have a connection to lifespan, then, given the key regulatory roles of the TOR and ILS pathways, it would be conceivable that there is an as-yet-undiscovered connection between Mocs1 and TOR and/or ILS; this possibility is discussed under *Dietary Effects* on page 18.

There are two protein isoforms of the gene *Mocs1* derived by alternative splicing, Mocs1a and Mocs1b.[13] Together, they are responsible for catalyzing the first reaction in a pathway leading to the synthesis of the molybdenum cofactor known as Moco, which is necessary for the survival of all multicellular eukaryotic organisms (see Figure 3).[14]

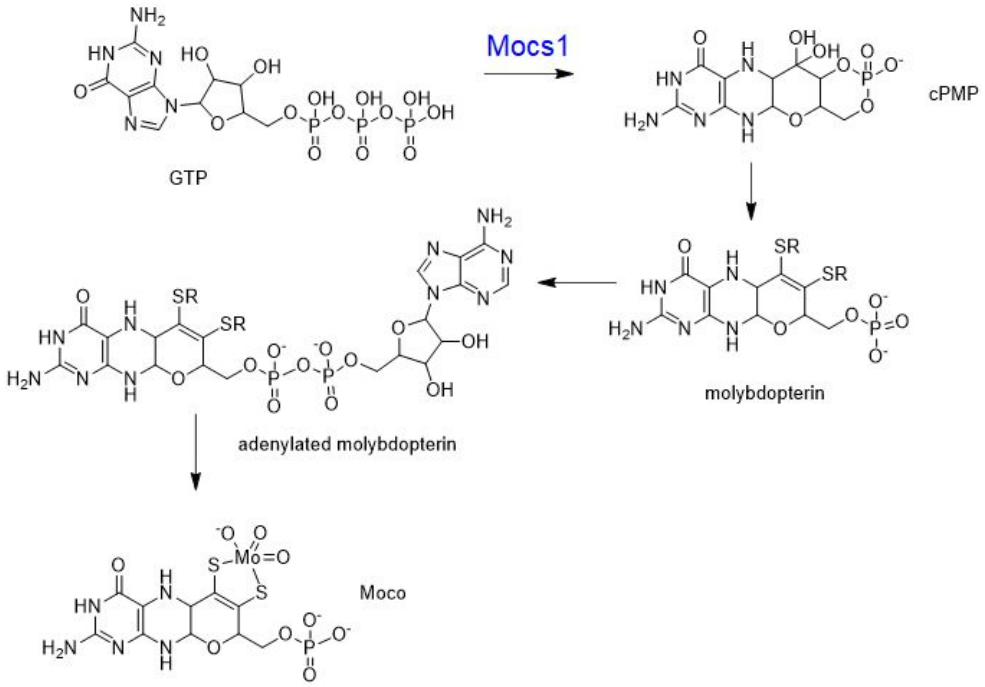

Figure 3. Moco synthetic pathway. Adapted from Mendel 2013 and Schwarz 2005.[14,15]

In eukaryotes, there are several enzymes which require Moco for proper functionality. Among them is the enzyme sulfite oxidase, which oxidizes sulfite (SO_3^{2-}), which is a toxin produced by breakdown of sulfur-containing amino acids, to sulfate (SO_4^{2-}), which is harmless.[15] Given the deleterious effects of endogenous sulfites, we have hypothesized that upregulation of *Mocs1* may lead to increased lifespan, an effect mediated by increased synthesis of Moco, which could in turn increase sulfite oxidase activity, removing toxins more quickly.

The data in the literature suggesting that *Mocs1* expression may increase lifespan, are, however, currently limited to a QTL analysis (which only demonstrates the existence of a correlation between the general region of the *Drosophila* genome containing *Mocs1* and lifespan; Tahoe et al)[16], and unpublished data from the Schulze lab at Western Washington University.[17] Interestingly, the Schulze lab data implicating *Mocs1* comes from an earlier experiment testing the effects of ribosomal protein downregulation on *Drosophila* lifespan via knockdown with RNAi (see *Methods and Materials* and *Results* sections). Downregulation of ribosomal protein expression has been demonstrated in both *C. elegans* and yeast to cause life extension.[18,19] *Mocs1* emerged as a gene of interest due to a failed negative control in this earlier experiment (see Discussion section below). This led to the hypothesis that unintentional *Mocs1* upregulation had caused increased lifespan.

As a first step to testing the role of *Mocs1* in regulating lifespan, we performed an experiment to determine whether upregulation of *Mocs1* is causally related to lifespan extension in Drosophila.

Methods and Materials.*

GAL4-UAS system and implementation

One important tool that is often used in *Drosophila* genetics research is called the GAL4-UAS system, in which targeted gene expression using transgenes (engineered genes integrated into the fly genome) can be manipulated experimentally.[20] GAL4 is a transcription factor naturally occurring in yeast which binds to a yeast-specific upstream activating sequence (UAS) and increases expression of genes immediately downstream. A transgene bearing the UAS upstream of a gene of interest but not producing GAL4 is known as a responder whereas a transgene producing GAL4 but not possessing a target sequence for GAL4 to bind to is called a driver. There are many drivers now available in *Drosophila* that express GAL4 tissue- or stage-specifically (for example, only in the eye, or only during the pupal stage).[21] Since neither GAL4 nor its target UAS are naturally present in *Drosophila*, a fly expressing only a driver or only a responder will not express the gene of interest. If, however, flies from driver and responder strains are crossed (mated), then the progeny flies will contain the UAS while also producing GAL4 (in whichever tissues or developmental stages are dictated by the driver), leading to targeted gene expression (see Figure 4).

*Note: much of the following protocol was not performed by myself personally (because I joined the lab after much of the prep work was completed); my work consists of assisting with the fly lifespan experiment itself, described below, and the PCR mapping.

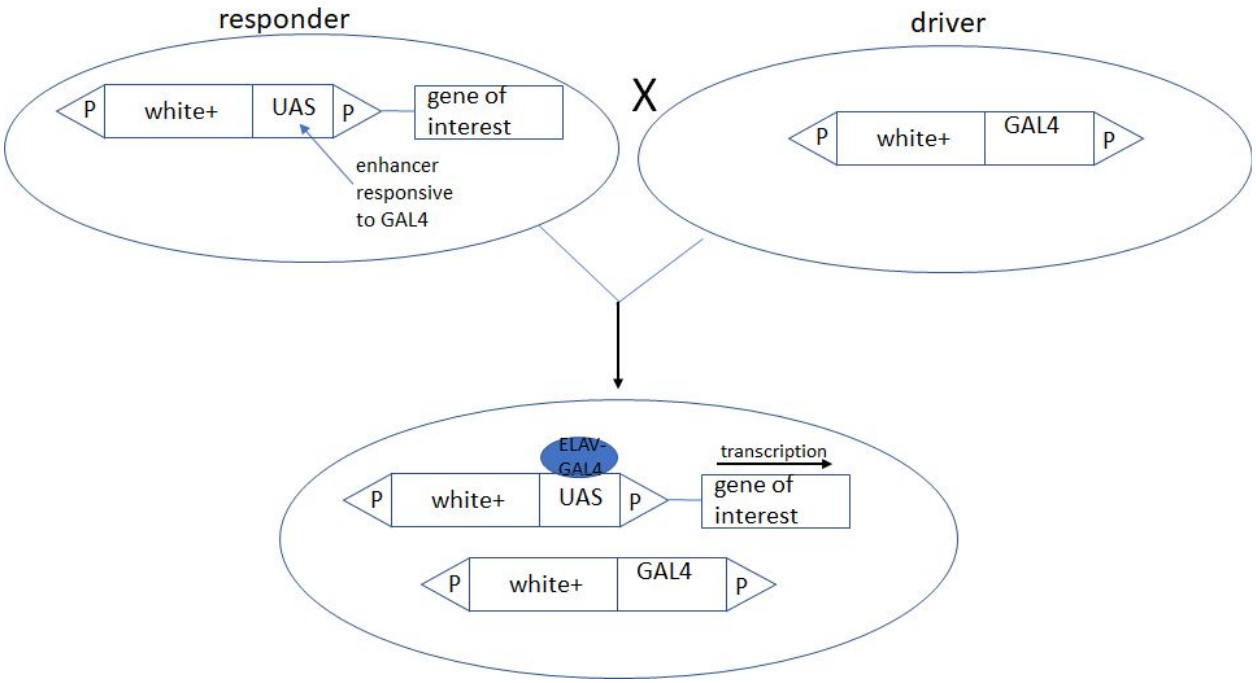

Figure 4. The principle behind drivers and responders.

Isogenizing genetic background

When performing experiments in Drosophila (or any genetics experiments in general), it is important that the strains being studied have close to the same genetic background so that any experimental variance between the strains may be attributed to the influence of the genes under consideration and not to confounding effects from other regions in the genome. The most reliable method of generating a uniform genetic background is to repeatedly backcross a given genotype to an isogenic strain (a strain homozygous at every locus). This method is only feasible if there is a clear phenotypic difference between the flies containing the allele of interest and those which do not. To isogenize the driver and responder strains in this experiment, an eye color reporter gene was used: the genetic background of these fly strains, as well as the isogenic strain, had the gene for eye color knocked out (they contained a specific mutant eye color allele called w[1118]), but in the driver and responder strains, there was an added gene (w+) immediately

adjacent to the desired genetic construct (GAL4 and the UAS, respectively), coding for red eye color. Therefore, after each cross between the driver (or responder) strain and the isogenic strain, the offspring flies with red eyes would be selected by visual inspection with a microscope to backcross with the isogenic strain again (Figure 5). The red eye allele was closely linked (by design) to the desired genetic construct in the driver and responder strains and thus unlikely to be separated from them by homologous recombination.

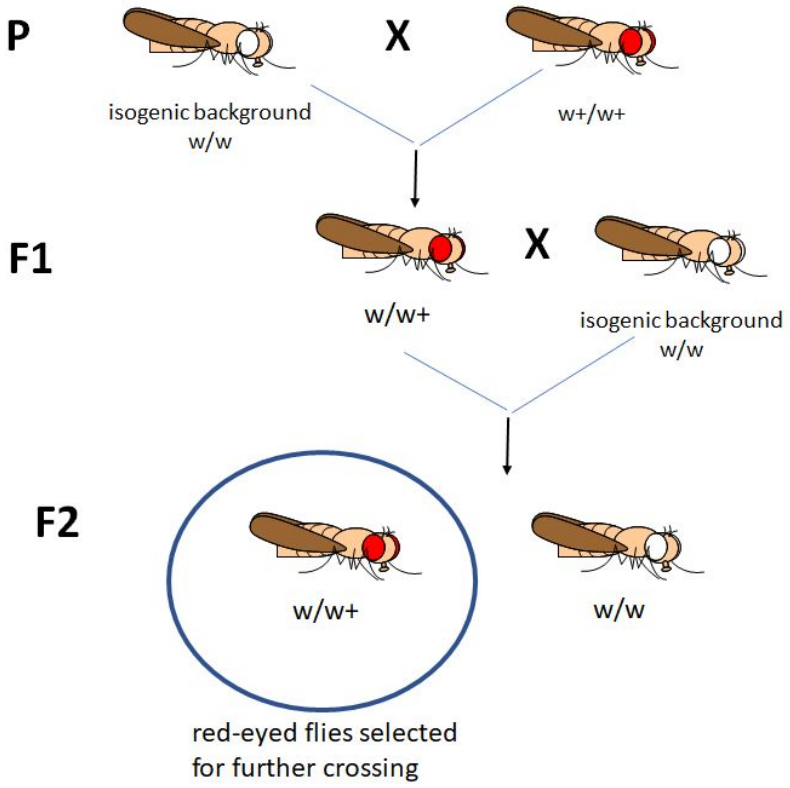

Figure 5. Schematic showing isogenization process.

After *n* generations of backcrossing, flies will share $1-(1/2)^n$ of their genome with the background strain. (For example, after 10 generations of backcrossing, the offspring flies will share $1-(1/2)^{10} = 1023/1024$ of their genome with the background isogenic strain. The 1/1024 which is not shared is, presumably, largely confined to the region around the genetic constructs

of interest.) All strains used in this experiment have been isogenized to this w[1118] genetic background by ten generations of backcrossing. Driver functionality was confirmed cytologically (by other Schulze lab personnel) using a GFP responder to verify that repeated rounds of backcrossing had not separated the w+ reporter from the GAL4 driver itself. (A list of the specific genotypes used in this experiment is provided in Appendix A of this report.)

Diet

In this experiment, the diet of the flies consisted of a recipe containing 4% w/v cornmeal, 2% w/v yeast, 1% w/v sugar, 0.6% w/v fly agar, and 0.0017% v/v tegosept as a mold inhibitor (see Appendix B for full preparation description).

Fly strains and mating protocol

Two fly strains were purchased from the Bloomington Drosophila Stock Center. One strain, EY00759, has an enhancer trap integrated immediately upstream of the *Mocs1* gene (see figure). (Enhancer traps were so named as they enable identification, or "trapping", of genes near the site of integration. They consist of a UAS binding site upstream of an enhancer/promoter sequence.) The driver strain used in this experiment is ELAV, designed to express GAL4 predominantly in the central and peripheral nervous system. (For a full description of the genotypes used in this experiment, see Appendix A.)

The parental strains -- the ELAV driver and EY00759 (enhancer trap) responder -- were mated to generate the flies used in the experiment as follows.

The parental strains were raised in bottles (190 mL) for several generations under uncrowded conditions. Three months prior to the beginning of the lifespan experiment described in this paper, three crosses were set up in separate mating bottles. In one bottle, 120 virgin male

and female flies from the driver strain were mated; in the second, 120 virgin flies from the responder strain were mated; and in the third, 60 virgin males from the responder strain and 60 virgin females from the driver strain were mated (see Figure 6).

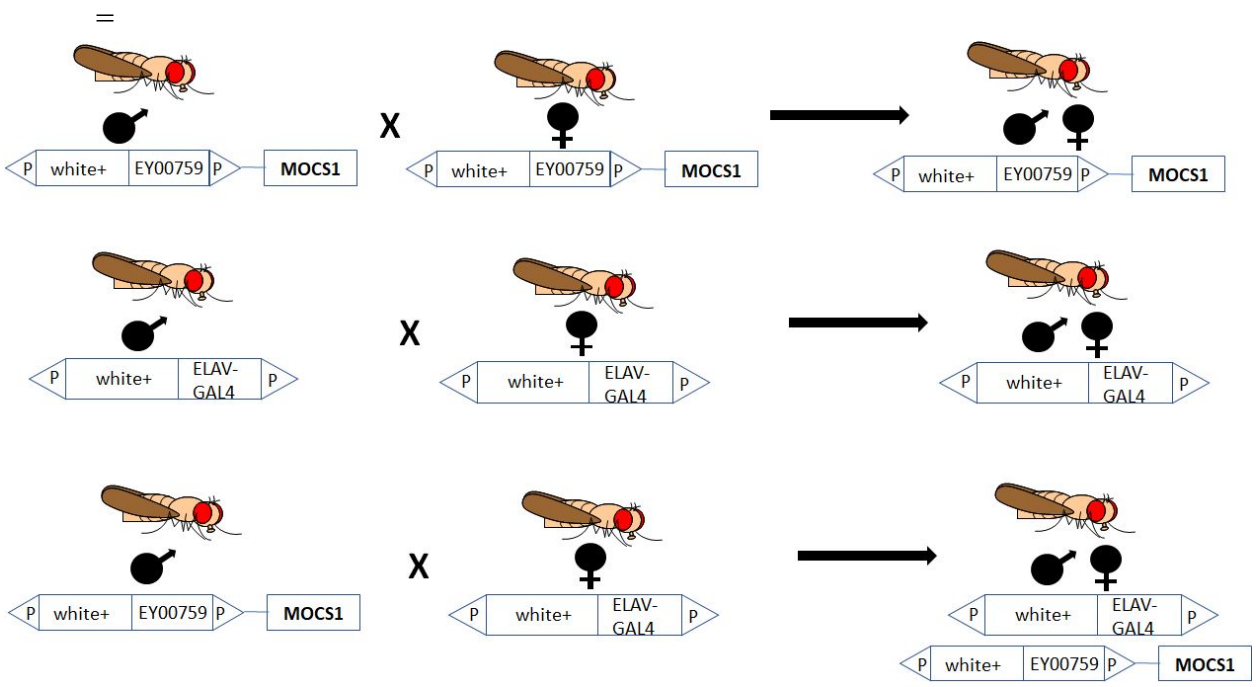

Figure 6. Fly crosses.

Every two days, the flies were transferred into fresh mating jars. The eggs from the initial mating were discarded (because in experiments such as this one, the first batch of eggs will consist of a nonsynchronous mix of fertilized and unfertilized eggs, which is undesirable). The fly larvae in the eggs were allowed to mature, and when the mature adults eclosed, they were themselves allowed to mate.

Experimental setup and DLife software

Subsequently, sixty male and sixty female flies from each strain -- homozygous ELAV, homozygous EY00759, and transheterozygous ELAVxEY00759 -- were randomly assigned to sex-separated 2 cm x 9.5 cm vials of twenty flies each (see Figure 7) using a program called

DLife (see next paragraph). Each vial had previously been filled with approximately 1 linear cm of the food preparation addressed above. A cotton plug was placed in the top of each vial to prevent flies from escaping. The fly vials were placed under a lamp which illumined them from 6:30 am to 6:30 pm, simulating a 24-hour day-night cycle. The laboratory temperature was approximately 21°C.

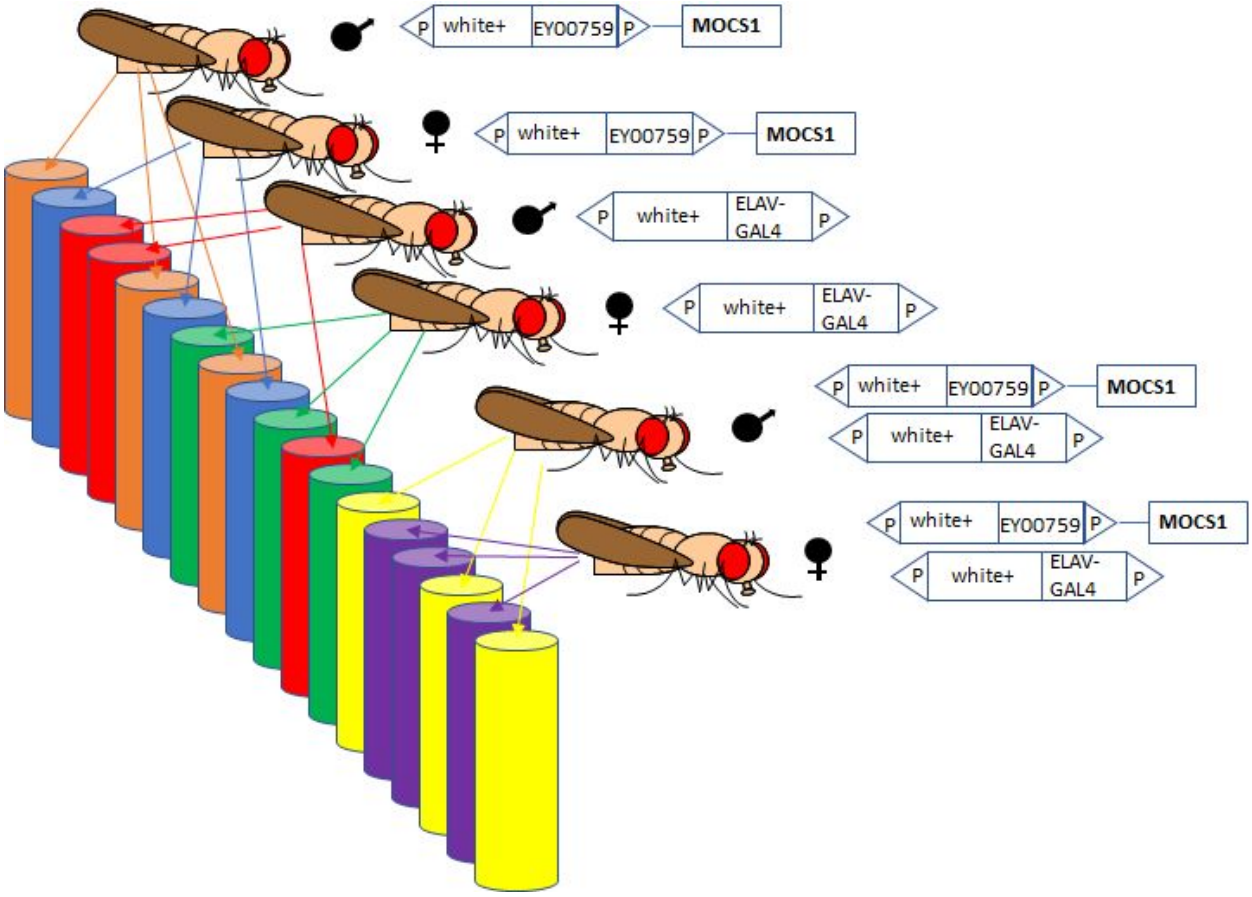

Figure 7. Schematic showing assortment of flies to vials.

Assignment of fly strains to vials was performed using DLife, a program created by Dr. Scott Pletcher, which was expressly designed for lifespan experiments in *Drosophila*.[22] For the remainder of the experiment, DLife was used to keep track of fly deaths for each strain. Whenever a fly was lost from the experiment due to causes other than a natural death (e.g.

escape from the experiment), it was recorded as "censored", meaning that DLife was notified that that fly would not provide further data for the experiment. (The lifespan data it had provided up until being censored, however, could still be utilized in the statistical analysis.) The experiment was run blind, as survival data were entered into DLife by vial, not by strain, eliminating the need for the researcher to know which vials belonged to each strain as the experiment progressed.

Every 2-3 days, the flies were transferred into a new set of tubes to diminish risk of bacterial or mold infections and to prevent the female flies from getting stuck in their own eggs. Each time the flies were transferred between vials, the number of deaths which had occurred in each vial since the previous measurement was recorded on paper and entered into DLife.

After all of the flies had died, an array of statistical analyses were applied to the data using the program Survival, encoded in R, to determine whether there was a statistically significant difference among the lifespans of the three strains (see *Results* and *Discussion* sections).

PCR mapping

Genomic DNA (extracted from 10 flies using ArchivePure DNA Cell/Tissue Kit #2300810) from the RNAi line BL41553 (from previous experiment; see *Introduction* and *Results*) was isolated and diluted 1:20 (roughly 200 ng/μL), then subsequently amplified via genomic end-point PCR using an upstream primer for the inverted repeat sequence flanking the transgene insert and a downstream primer in the *Mocs1* gene. Genomic DNA from the EY00759 line was amplified via genomic end-point PCR using the same methodology. (For primer sequences, see Appendix C.) The recommended concentrations of buffers, primers, and NTPs

were used. The cycling parameters were as follows: 95°C/2 min, then 30 cycles of (95°C/30 sec, 60°C/30 sec, 70°C/2 min), followed by a final eight minute extension at 70°C. The resulting PCR products were analyzed via gel electrophoresis using a 1.8% agarose gel followed by imaging under UV light using an AlphaImager machine (see Figure 8).

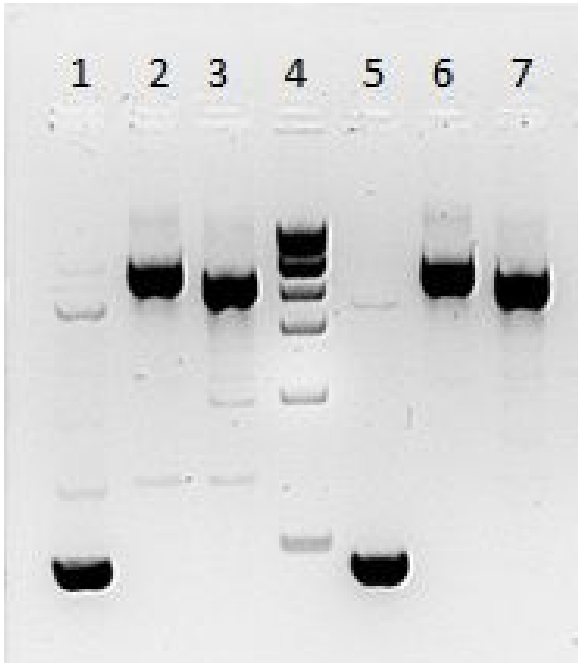

Figure 8. PCR gel. Lanes 1-3 have RNAi control BL41553 while lanes 5-7 have responder EY00759. Lane 4 contains a 1 kb ladder (New England Biolabs). Lanes 1 and 5 use primers P-out-31 and MOCSR02; lanes 2 and 6 use primers P-out-31 and MOCSR03; lanes 3 and 7 use primers P-out-31 and MOCSR04.

Results

Once the experiment had ended, the fly survival rates were plotted (see Figure 9, Figure 10, Table 1). The data were analyzed using a Kaplan-Meier estimator to approximate the survival function for each of the six cohorts (male drivers only, male responders only, males with upregulated *Mocs1*, female drivers only, female responders only, females with upregulated *Mocs1*), and the cohorts were compared using a log-rank chi-square test to determine whether differential lifespan was associated with the presence/absence of the test condition (upregulation

of *Mocs1*). Male and female cohorts were analyzed separately, as recommended by He and Jasper.[5]

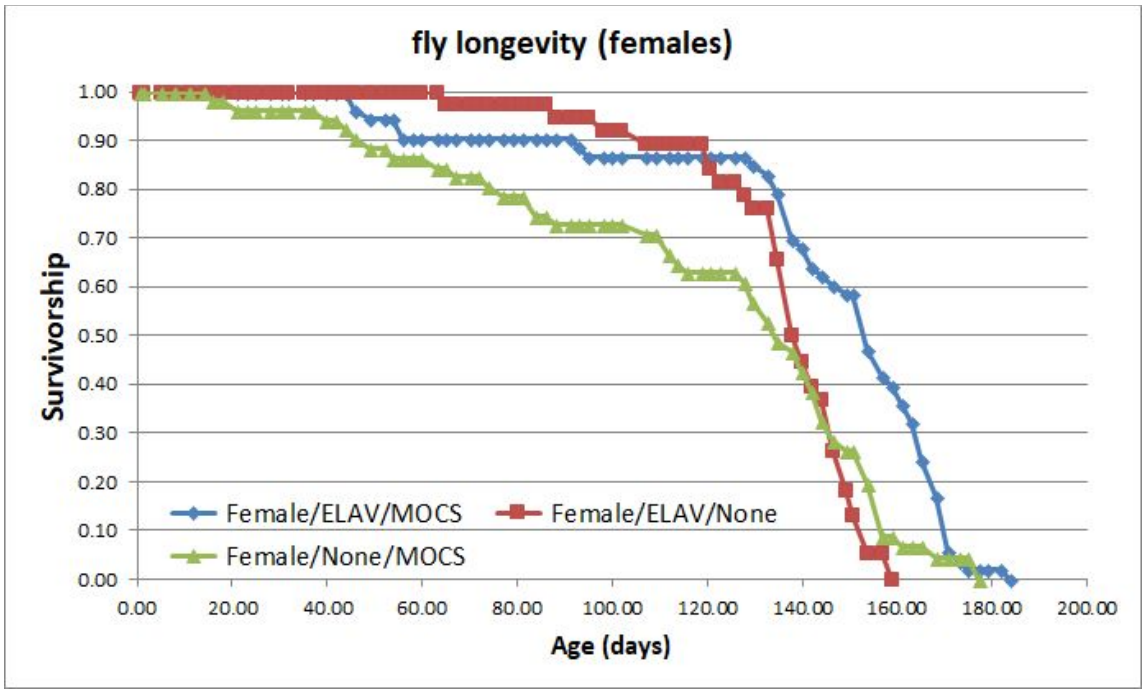

Figure 9. Female fly lifespan. Driver flies in red, responder flies in green, and *Mocs1*-upregulated flies in blue. Figure created in DLife.[22]

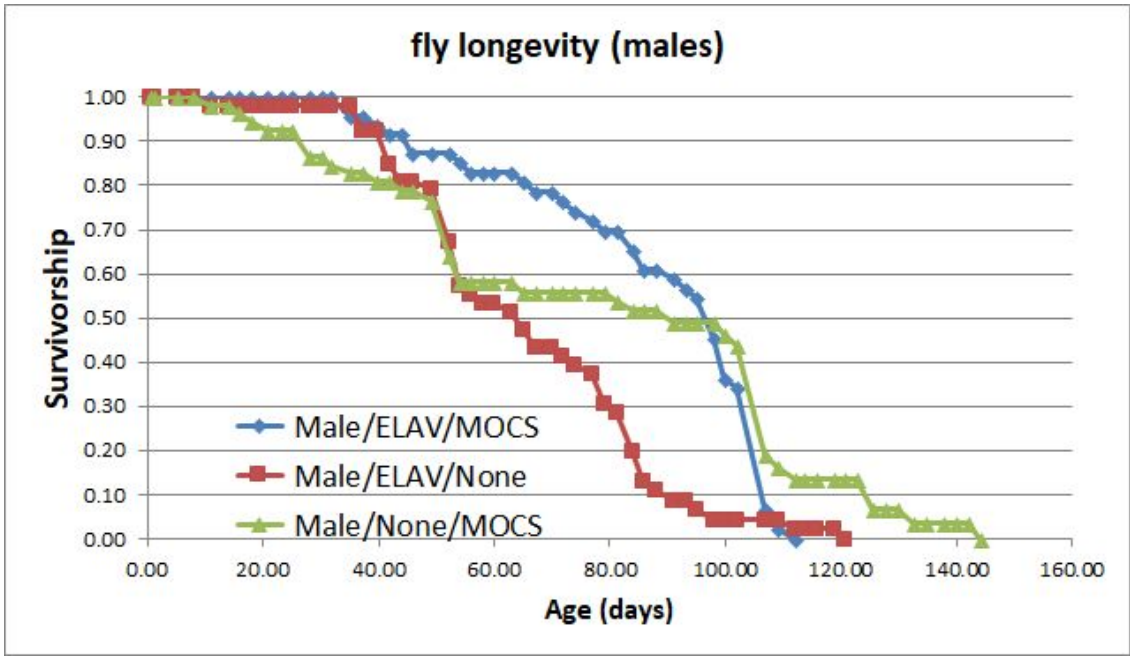

Figure 10. Male fly lifespan. Driver flies in red, responder flies in green, and *Mocs1*-upregulated flies in blue. Figure created in DLife.[22]

There is a significant difference in lifespan between females expressing only the driver or only the responder and *Mocs1*-upregulated females ($p=5.7x10^{-6}$ and $p=9.0x10^{-4}$). There is a significant difference in lifespan between driver males and *Mocs1*-upregulated males ($p=1.8x10^{-5}$) but no difference between responder males and *Mocs1*-upregulated males ($p=0.49$). Females outlived males for all treatment options. See Table 1.

Table 1. Mean and median lifespans for the fly cohorts, and the probability that the difference in lifespan compared to *Mocs1*-upregulated flies of the same sex is due solely to chance (p-value calculated using a log-rank chi-square test). p-values < 0.05 were colored green while p-values > 0.05 were colored red.

cohort	mean lifespan (days)	median lifespan (days)	p-value compared to *Mocs1*-upregulated flies of the same sex
driver females	136	139	$5.7x10^{-6}$
responder females	120	135	$9.0x10^{-4}$
Mocs1-upregulated females	144	153.7	N/A
driver males	66	65	$1.8x10^{-5}$
responder males	80	91	0.49
Mocs1-upregulated males	87	98	N/A

Discussion

Previous data implicating Mocs1

In this experiment, ELAV was chosen as the driver for several reasons: firstly, it has been widely used in Drosophila GAL4-UAS studies of aging; secondly, the nervous system has been implicated in *Drosophila* as a tissue in which biochemical pathways related to aging have been

linked to nutrient sensing;[23] and lastly, ELAV is the driver used in the previous aging experiment in the Schulze lab in which extended lifespan was observed (see *Introduction*).[17]

In that earlier experiment, the GAL4-UAS system was used to target knockdown of ribosomal transgenes; all of the responder transgenes had been integrated directly upstream of *Mocs1*. The RNAi stocks in this experiment had been purchased from TRiP, the Transgenic RNAi project, at Harvard Medical School, and all of their responder transgenes were integrated directly upstream of *Mocs1* because this site had been determined to be conducive to gene expression. In addition, integrating all responder transgenes in a single location obviated the effects of random transposon integration. A GAL4-sensitive negative control in that experiment that did not target any ribosomal proteins, P{VALIUM20-GAL4.1}, exhibited similar lifespan extension to the experimental group.[17] As that responder transposon was immediately upstream of the *Mocs1* gene, it was hypothesized, based on these data, that unintentional *Mocs1* upregulation had caused increased lifespan (see Figure 11). The EY00759 enhancer trap responder in this experiment is located in the same location as the TRiP (RNAi) responder transgenes in the previous experiment, as verified by PCR in *Methods and Materials*.

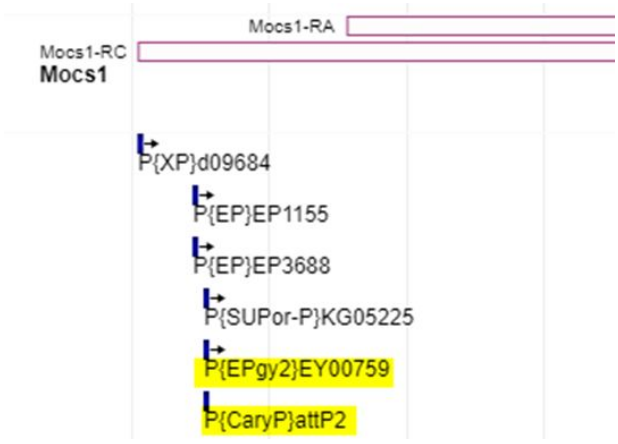

Figure 11. Map of *Mocs1* start site, EY00759 insertion site (highlighted), and P{VALIUM20-GAL4.1}, which inserts at P{CaryP}attP2 (highlighted). Screenshot from FlyBase, JBrowse viewer.

For this experiment, the ELAV driver and EY00759 responder strains were crossed, producing a ELAVxEY00759 strain with theoretically upregulated *Mocs1* expression; the lifespan of these three strains was compared using DLife. The ELAV and EY00759 parental strains served as controls; expression of GAL4 in ELAV was not expected to have any effect on lifespan since no UAS responding to GAL4 was present in this strain, and likewise, the presence of a UAS responding to GAL4 in EY00759 was not expected to have an effect on lifespan in the absence of GAL4 itself. The ELAVxEY00759 progeny strain was expected to display increased lifespan due to upregulation of *Mocs1* because both GAL4 and its corresponding UAS (upstream of *Mocs1*) were present. The flies in the lifespan experiment were mated (as noted in *Methods and Materials*); this is significant because mating status in *Drosophila* itself affects lifespan.[24]

Dietary effects

The relative proportions of ingredients in *Drosophila* diet may dramatically affect the outcome of a lifespan experiment.[5] As noted in the *Introduction*, dietary restriction has long been noted to result in increased lifespan across multiple taxa. The diet in this experiment constitutes moderate-to-strong dietary restriction (see Figure 12), which will itself increase the lifespan of all flies compared with a diet containing a higher percentage of sugar. The effects of caloric restriction and *Mocs1* upregulation appear to be additive rather than epistatic in our data; thus, it appears that the lifespan effects of caloric restriction (largely the TOR and ILS pathways) are mediated through a different pathway than the effect of *Mocs1* upregulation.

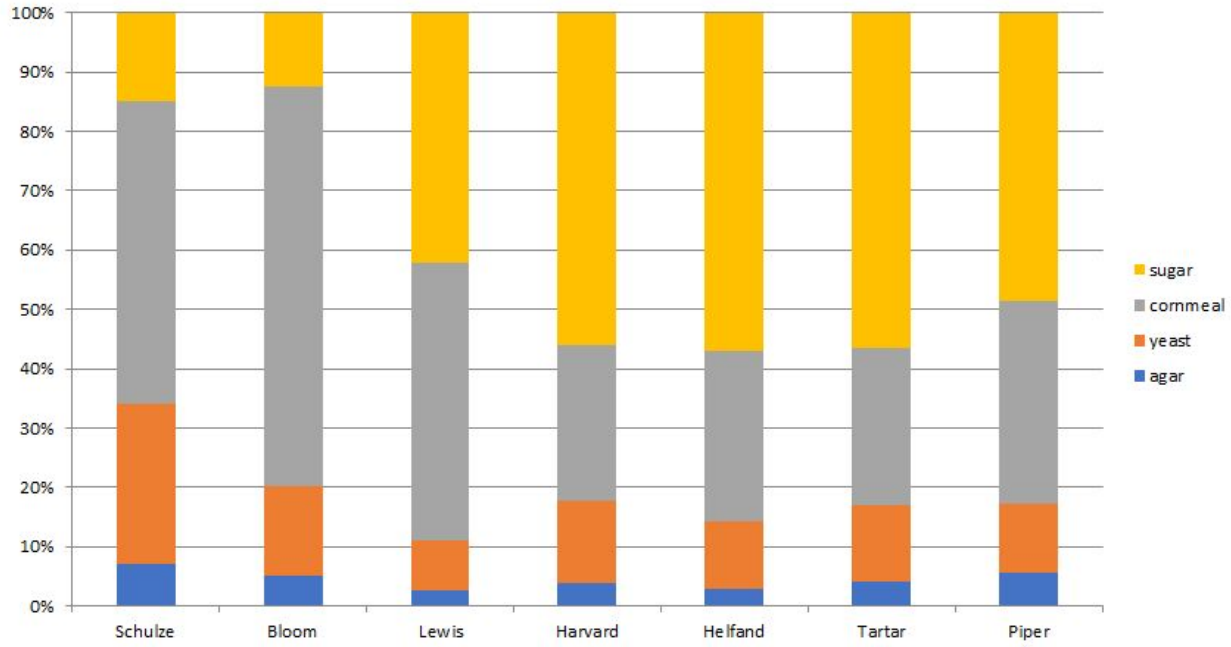

Figure 12. A comparison of nutrition content (dry weight) from a number of fly recipes. Figure courtesy of Dr. Sandra Schulze, from an unpublished work.

As noted in *Methods and Materials*, fly survival data were entered into DLife by vial rather than strain. In this way, the experiment was blind, because, except for the initial setup, there was no need for the researcher to know which strain was in a given vial, eliminating the bias which could conceivably result from knowing a particular vial's strain.

Mocs1 potentially lengthens lifespan in females, but not in males

The data indicate that *Mocs1*-upregulation has a significant effect on lifespan in females, while the results are more ambiguous in males. It is possible that this sex difference is an artifact of the experimental protocol: male *Drosophila* tend to be more active, so transferring them between vials proved more difficult, leading to artificially high death rates among all treatment groups. Alternatively, it is conceivable that the observed effects are indicative of an underlying sex-specific mechanism.[5]

It is worth noting that in this experiment, it was not conclusively demonstrated that *Mocs1* was in fact being upregulated as budgetary constraints did not allow qPCR to be performed, which could have verified the increase in *Mocs1* expression levels as compared to an undriven control. Given the promising nature of the results, it would be helpful, in a replication of this experiment, to carry out this procedure to verify *Mocs1* upregulation.

Along with this, it is possible that the differential lifespan of the strains in this experiment was mediated through a gene other than *Mocs1*. It is possible, for example, that genes other than *Mocs1* were upregulated due to the presence of the EY00759 promoter. EY00759 causes unwinding of nearby DNA, and it is theoretically possible that a different nearby gene was mediating the effect of increased lifespan. One way to rigorously rule out this possibility would be to perform RNA-seq on all three of the strains to check for differential expression of RNA transcripts. This also was outside of the resources at hand.

In summary, expression of the gene *Mocs1* was upregulated in male and female *Drosophila*, and their survival was analyzed over the subsequent months. It was demonstrated that *Mocs1*-upregulation correlates with increased lifespan in female flies as compared to undriven controls ($p=5.7\times10^{-6}$, $p=9.0\times10^{-4}$), but the same results did not apply to males ($p=0.49$, $p=1.8\times10^{-5}$). As *Drosophila Mocs1* has a human ortholog (*MOCS1*), continued study of the effects of upregulation of this gene could yield further insights into the study of human aging.

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Appendix A. Fly genotypes.

Background to which all other strains were isogenized: w[1118] line isogenic for chromosomes 1, 2, and 3, tested for normal learning, memory and circadian rhythms

Responder genotype: w[1118]; P{EPgy2}EY00759

Driver genotype: w[1118]; P{GAL4-elav.L}3

Appendix B. Fly food preparation description.

Ingredients.

- 1.5 liters water
- 9 grams agar
- 60 grams cornmeal
- 30 grams yeast
- 15 grams sugar
- 25 mL tegosept

The agar was sprinkled into the water and microwaved on high for about ten minutes with a break after five to stir the mixture. The remaining dry ingredients were mixed and sprinkled into the colloid while stirring. The mixture was microwaved on high for about five minutes with a pause after three minutes to stir. The mixture was microwaved on low for five minutes, then removed and stirred at room temperature for ten minutes. Tegosept was added. The mixture was added to fly vials (about 1.3 linear centimeters per vial).

Food preparation instructions adapted from an unpublished description in the Schulze lab.

Appendix C. Primer Sequences for PCR.

P-out-31 (upstream primer):

CGA CGG GAC CAC CTT ATG TTA TTT CAT CAT G

Downstream primers (in Mocs1 gene):

MOCSR03: CACGGAGGACAAGCTGATGT

MOCSR02: CCAATGGCTGAACACTTGCC

MOCSR04: TTGGCTGTAATTTCGGGCCT