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IDENTIFICATION AND CHARACTERIZATION OF A MUTATION THAT ENHANCES THE MITOCHONDRIAL UPR RESULTING FROM LOSS OF METHIONINE SYNTHASE IN C. ELEGANS

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by

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April 23, 2021

ABSTRACT

IDENTIFICATION AND CHARACTERIZATION OF A MUTATION THAT ENHANCES THE MITOCHONDRIAL UPR RESULTING FROM LOSS OF METHIONINE SYNTHASE IN *C. ELEGANS*

Heba Zakaria, B.S. Biological Chemistry

The University of Texas at Arlington, 2021

Faculty Mentor: Mark Pellegrino

Mitochondria have vital cellular functions, notably, the generation of energy dysfunctional mitochondria can activate the mitochondrial unfolded protein response (UPR^{mt}), a conserved transcriptional response that regulates the expression of several mito-protective genes. Research has shown that UPR^{mt} activation promotes lifespan extension and increases resistance to infection. We recently discovered that loss of methionine synthase gene *metr-1* activates the UPR^{mt}. We performed a forward genetics screen to isolate modulators of the UPR^{mt} caused by the *metr-1* mutant. This was performed to determine how the loss of methionine synthase impacts the UPR^{mt} and longevity. Interestingly, one mutant was isolated from this screen (named *osa51*), which enhanced the UPR^{mt} in the *metr-1* mutant background. The goal is to characterize this new mutant

and attempt to identify the causative gene. Lifespan and developmental analyses yielded data showing the *osa51* mutant strain had a significantly shorter lifespan and developed slower than the wild-type strain.

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CHAPTER 1

INTRODUCTION

1.1 Mitochondrial Structure and Function

Mitochondria are organelles present in the cells of almost all eukaryotic organisms (Kühlbrandt, 2015). This organelle is notoriously known as the powerhouse of the cell as it produces a vast amount of adenosine triphosphate (ATP), an energy-rich compound that drives vital cellular functions. Although mitochondria are tasked with ATP synthesis through the process of cellular respiration, they have many other vital functions such as the production of NADH and GTP (tricarboxylic acid cycle (TCA cycle)), amino acids, heme groups, iron-sulfur clusters, and phospholipids (membrane biogenesis). Mitochondria are also involved in calcium signaling, stress responses, and cellular signaling.

Mitochondria evolved as a consequence of endosymbiosis whereby an ancient proteobacterial cell merged with a primitive eukaryotic cell. As such, mitochondria resemble α-proteobacteria including similarities in their respiratory chain complexes (Kühlbrandt, 2015). Because of this endosymbiotic origin, mitochondria possess their own genome, protein translation machinery, ribosomes, and tRNAs, and related protein factors. Despite having their own genome, the mitochondrial proteome is encoded predominantly by genes present in the nucleus (approximately 99%). These nuclear-encoded mitochondrial proteins are translated into the cytoplasm of the cell and imported into the organelle by protein translocases.

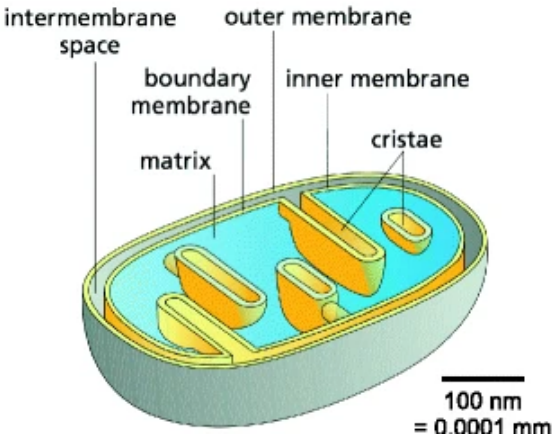

Figure 1.1: Transverse cross-section of mitochondria and labeled structures

Mitochondria form a dynamic tubular network, constantly adapting through division and fusion with the help of dynamin GTPases (Kühlbrandt, 2015). These organelles are separated from the cytoplasm by two membranes: an outer membrane and an inner membrane. The outer membrane is characteristically porous, allowing ions and small uncharged molecules to enter and leave the organelle. Proteins and other large substances are imported by translocases. Due to its unrestricted nature, a membrane potential does not exist across the outer membrane. Contrastingly, the highly regulated inner membrane allows ions and molecules to diffuse with the help of specific transport proteins, resulting in the formation of an essential electrochemical membrane potential (180mV). The inner mitochondrial membrane forms invaginations called cristae, which house the oxidative phosphorylation complexes called the electron transport chain and ATP synthase. Protein complexes responsible for driving ATP production via oxidative phosphorylation, reside in the inner membrane of the organelle. The interior mitochondrial matrix houses, among other factors, components of the Krebs cycle, which will generate high energy electron donors, NADH, and $FADH_2$, which will donate high energy electron donors to the complexes of the electron transport chain that mediate oxidative phosphorylation. DNA replication, transcription, protein translation, and many other

enzymatic reactions occur within the matrix. DNA in the mitochondria is compacted into nucleoids by a mitochondrial transcription factor (TFAM). Ribosomes present in the mitochondria are membrane-bound and produce hydrophobic membrane protein subunits. The intermembrane space is between the outer and inner membranes. Outer and inner membrane protein translocases (TOM and TIM) form a super complex that spans the intermembrane space.

1.1.1 The consequences of mitochondrial dysfunction

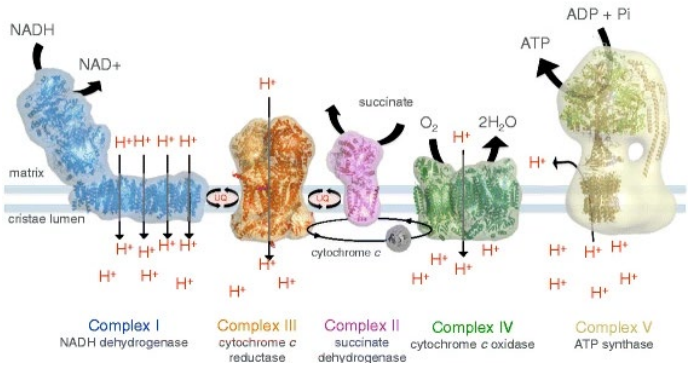

Figure 1.2: Complexes of ETC located in the inner mitochondrial membrane.

Since mitochondria are responsible for so many essential biological processes, mitochondrial dysfunction can result in severe disruption of organismal homeostasis (Gibson et al., 2010). Mitochondria that are damaged or dysfunctional are either repaired by complementation by an undamaged member of the mitochondrial network by fusion. Alternatively, mitochondrial fission divides the organelle into smaller pieces that can be degraded by mitophagy.

Impairment of oxidative metabolism and the accumulation of abnormal proteins have been associated with age-related neurodegenerative disease (Gibson et al., 2010). A metabolic disorder can result in decreased resistance and immunity to mild injury or disease. It can also cause an increase in reactive oxygen species (ROS), which greatly impact cell signaling by affecting transcription and post-transcriptional modifications.

Research has shown that oxidative changes in α-ketoglutarate dehydrogenase complex (KGDHC), for example, were linked to a deficiency in neuronal energy metabolism and increased production of ROS in Alzheimer's Disease (AD). Also, Huntington's Disease, a cognitive movement disorder, has been linked to complex II + III abnormalities. Disorders resulting from mitochondrial dysfunction are caused by oxidative stress, energy dysfunction, and abnormal concentration of calcium in the cytosol.

1.1.2 Mitohormesis: the mitochondrial basis of aging

Mitohormesis is the process by which low concentrations of ROS produced by the mitochondria serve as signaling molecules that initiate cellular cascades resulting in the protection of cells from harm (Dutta, 2021). When ROS was first discovered, it was regarded as a damaging molecule that could harm protein, lipid, and DNA integrity. However, more recent research has discovered that the presence of ROS in small concentrations can be beneficial to cells.

External and internal stresses can disrupt mitochondrial function resulting in the subsequent activation of cytoplasmic signaling pathways that promote recovery (Dutta, 2021). Stresses can include exposure to environmental toxins, disruption of the mitochondrial electron transport chain (ETC), or the accumulation of misfolded mitochondrial proteins. Transcriptional changes in nuclear DNA occur in response to this stress leading to cytoprotective mechanisms such as increased mitochondrial biogenesis, antioxidant defense mechanisms, detoxification of xenobiotics, and expression of mitochondrial protein chaperones. These cytoprotective mechanisms work in unison to slow aging, increase lifespan, and delay or reduce the onset of age-related disorders.

1.1.3 Different types of mitochondrial recovery programs

Several mechanisms are employed to restore mitochondrial function during stress including defense against excessive reactive oxygen species (ROS) production, the mitochondrial unfolded protein response (UPR^{mt}), mitochondrial dynamics, elimination by autophagy, and mitochondrial biogenesis (Valera-Alberni & Canto, 2018).

If the removal of metabolic byproducts produced from oxidative phosphorylation is not regulated properly, this may result in harm to the cell. ROS are produced mainly by the ETC and include oxygen anions forms like superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl free radical (OH^-) (Valera-Alberni & Canto, 2018). Increased production or accumulation of ROS due to inadequate initiation of an antioxidant response causes oxidative stress within the cell, increasing mitochondrial dysfunction and damage to nucleic acids, proteins, and lipids.

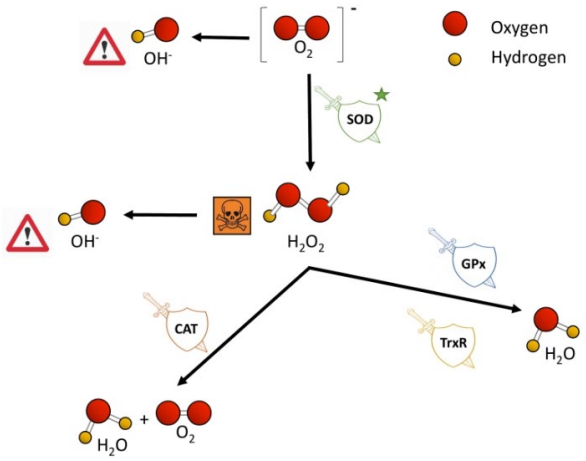

Figure 1.3: Antioxidation reactions catalyzed by different enzymes

Enzymatic and non-enzymatic mechanisms can be used for antioxidation (Valera-Alberni & Canto, 2018). Superoxide dismutases (SOD), catalases (CAT), thioredoxin reductases (TrxR), or glutathione peroxidases (GPx) can be used to enzymatically catalyze the conversion of ROS to harmless molecular forms. NF-κB, AP1, and MAPK pathways

cooperate in response to ROS. If insufficient, the NRF2/KEAP cascade is initiated and activates antioxidant defenses to reduce oxidative damage.

Proper folding of proteins is vital for proper mitochondrial function, which is regulated by the presence of molecular chaperones and proteases (Valera-Alberni & Canto, 2018). Both the endoplasmic reticulum (ER) and mitochondria respond to proteotoxic stress through the engagement of an unfolded protein response (UPR). The UPR encompasses a collection of signaling pathways that provide an environment fostering proper protein folding.

The mitochondrial UPR (UPR^{mt}) is a specific response to mitochondrial dysfunction involving the upregulation of nuclear genes that encode mitochondrial chaperones and proteases (Valera-Alberni & Canto, 2018). Furthermore, response to misfolded proteins in mitochondria and the ER were found to be organelle-specific. The UPR^{mt} pathway can be activated by mtDNA depletion, compromised mitochondrial protein quality, or OXPHOS dysregulations.

In *C. elegans*, regulators of the UPR^{mt} include the mitochondrial matrix protease CLPP, the ATP-binding cassette (ABC) transporter HAF-1, and the basic leucine zipper (bZIP) transcription factor ATFS-1 (Valera-Alberni & Canto, 2018). CLPP degrades misfolded proteins in the matrix of mitochondria, resulting in the release of peptides through the inner mitochondrial membrane ABC transporter HAF-1. The release of peptides to the cytosol is thought the activate ATFS-1. Intriguingly, ATFS-1 contains a mitochondrial targeting sequence (MTS) that imports the protein into healthy mitochondria. Mitochondrial protein import efficiency is reduced during stress, allowing ATFS-1 to accumulate in the cytosol and be transported in the nucleus to transcribe a

network of genes to repair mitochondria. Ubiquitin-like protein UBL-5 forms a complex with transcription factor DVE-1. ATFS-1 and DVE-1/UBL-5 cooperatively regulate the transcription of mitochondrial chaperones (HSP60 and mtHSP70). Recent research has found that chromatin remodeling occurs during mitochondrial dysfunction to regulate the UPR^{mt}. Histone demethylases (JMJD-3.1 and JMJD-1.2) and DVE-1/UBL-5 complex stabilize chromatin's transcriptionally active state, allowing ATFS-1 to access target gene promoters.

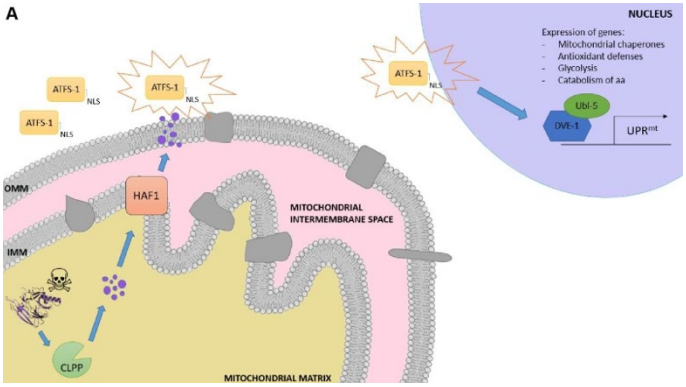

Figure 1.4: Schematic of molecules involved in the UPR^{mt} pathway

Mitochondrial dynamics can be best defined as cycles of fission and fusion cycles of mitochondria (Valera-Alberni & Canto, 2018). These cycles can be triggered by nutrient overload (fission of mitochondrial network) and nutrient starvation (fusion of mitochondrial network). To guarantee that mitochondria are equally distributed among daughter cells, the mitochondrial network goes through fission cycles before the beginning of mitosis. If mitochondrial dynamics are disrupted, health can be compromised and diseases like diabetes, obesity, heart disease, Alzheimer's disease, Parkinson's disease, and age-related diseases can occur. Having a dynamic network of mitochondria is favorable for the removal of defective mitochondria or when optimal matrix metabolites in one mitochondrion can be used to substitute a deficiency in another.

Mitophagy is the selective degradation of mitochondria by autophagy in an attempt to maintain or restore cellular homeostasis (Valera-Alberni & Canto, 2018). If any aspect of the autophagic machinery fails, dysfunctional mitochondria are not removed and can result in increased ROS production and susceptibility to cellular death.

Mitochondrial fission occurs before mitophagy since autophagosomes (APs) are more efficient at engulfing smaller mitochondria (Valera-Alberni & Canto, 2018). Acid hydrolase containing lysosomes fuse with APs and result in the degradation of the engulfed mitochondria.

1.2 Lifespan Regulation

The lifespan of an organism is regulated by genetic and environmental factors. For example, nutrient levels have been shown to influence the aging pattern of various animals. Specifically, low levels of the amino acid methionine increase animal lifespan, although the mechanism of this extension is not clearly understood. In addition, synergism between the genetic background of an animal and their diet can alter aging rates. The following will elaborate on each.

1.2.1 Methionine restriction and lifespan

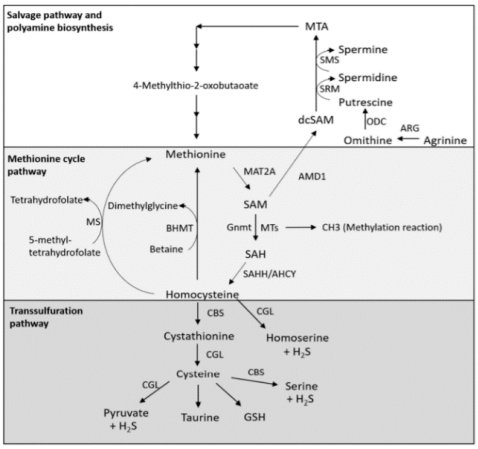

Figure 1.5: Schematic of the Salvage pathway, Methionine cycle pathway, and Transsulfuration pathway

Methionine is an essential sulfur-containing amino acid (AA) that is obtained from food and gut microbes (Kitada et al., 2021). Methionine is metabolized to S-adenosyl-methionine (SAM), which can activate the mechanistic target of rapamycin complex 1 (mTORC1), which suppresses the turnover of organelles by autophagy. Methionine is also vital for normal growth and development due to its role in initiating protein synthesis. Methionine restriction (MetR) prevents its conversion to SAM, which hinders the activation of rapamycin complex 1, resulting in autophagy. MetR also decreases oxidative stress by inhibiting the production of ROS in mitochondria and increases the production of hydrogen sulfide (H_2S), which is vital to lifespan extension by calorie restriction (CR). Methionine metabolism-related pathways are composed of three sub-pathways: the methionine cycle pathway, the transsulfuration pathway, and the salvage pathway.

In the methionine cycle pathway, methionine is catabolized to SAM (methyl donor) by methionine adenosyltransferase 2A (MAT2A) (Kitada et al., 2021). Glycine N-methyltransferase (GnmT) converts SAM to S-adenosyl-homocysteine (SAH), SAH hydrolase (SAHH) hydrolyzes SAH to adenosine and 1-homocysteine. The conversion of homocysteine to methionine-by-methionine synthase (MS) requires the presence of methyl donors (5-methyltetrahydrofolate or betaine homocysteine methyltransferase (BHMT)).

In the Transsulfuration Pathway, Cystathionine-β-synthase (CBS) condenses homocysteine and serine to produce cystathionine (Kitada et al., 2021). Cystathionine-γ-lyase (CGL) hydrolyzes cystathionine to cysteine, which is used to produce glutathione (GSH) and taurine. CGL catalyzes the conversion of cysteine to pyruvate and H_2S, and CBS catalyzes the conversion of cysteine to serine and H_2S.

In the methionine salvage pathway (5'-methylthioadenosine (MTA) cycle), SAM regenerates methionine, and polyamines (spermidine) are produced (Kitada et al., 2021). SAM is decarboxylated to dcSAM (amino-propyl group donor) by SAM decarboxylase 1 (AMD1). Arginase (ARG) converts arginine to ornithine, which is decarboxylated to putrescine by ornithine decarboxylase (ODC). Spermidine synthase (SRM) and spermine synthase (SMS) convert putrescine to spermidine and spermine, respectively. dcSAM is converted to MTA after donating the aminopropyl group to SMS. MTA is eventually converted back to methionine.

1.2.2 Gene-diet interactions and lifespan

Calorie restriction (CR), without malnutrition, increases lifespan and improves metabolic health by decreasing cellular oxidative stress (Amin et al., 2020). More current research suggests that specific diets can affect aging depending on the genetic background of the animal. For example, restriction of proteins in the diet can increase lifespan and suppress age-related diseases (Kitada et al., 2021). The restriction of methionine increased lifespan and metabolic health making it a potential target for stress and age-related diseases.

A recent study from our lab identified a link between mitochondrial function and diet that determines animal lifespan. Using a forward genetics approach in the model organism *Caenorhabditis elegans* (see below), a reduction of function allele in the mitochondrial ribosome gene *mrpl-2* was identified (Amin et al., 2020). The *mrpl-2* mutant activated the UPR^{mt} and had an increase in lifespan and host survival during infection. However, the activation of this stress response in this *mrpl-2* mutant and its increase in longevity were dependent on the animal's diet. Here, low levels of vitamin B12 in the diet

combined with the mrpl-2 mutation activate the UPR^{mt} and increase the lifespan of the animal. Vitamin B12 acts as a co-factor for two metabolic enzymes: methionine synthase and methylmalonyl-CoA mutase. Using genetics, it was found that reduced activity of methionine synthase due to low vitamin B12 in the diet was the cause of the UPR^{mt} activation and increase lifespan in *mrpl-2* mutant animals. Interestingly, loss of the methionine synthase gene, *metr-1*, alone was able to activate the UPR^{mt} and increase lifespan. However, the mechanism between this association is not known. This study showed the importance of diet and its synergy with genetics.

1.3 *C. elegans*: A Genetic Model Organism

C. elegans is a species of nematode that is used as a model organism to study human diseases (Yokoyama, 2020). Adult worms are approximately 1mm in length and are composed of 959 somatic cells. They appear transparent and are composed of epidermal, intestinal, neuronal, and muscle tissues making them physiologically similar to humans. Due to their ability to recognize and react to stimuli, they are considered to have a level of complex behavior. The *C. elegans* life cycle is about two to three weeks depending on temperature. The cycle begins with an egg followed by four larval stages and adult maturity. *C. elegans* display two sexes, XO males and XX hermaphrodites. Hermaphrodites can self-fertilize and lay hundreds of progenies.

1.3.1 Advantages of using C. elegans

C. elegans is a powerful model organism that is used in the laboratory for multiple purposes (Yokoyama, 2020). Having a model organism that is self-fertile is very useful because they reproduce in high numbers very quickly without the need to find a mate to reproduce. *C. elegans* are grown on Nematode Growth Medium (NGM) plate are fed *E.*

coli bacteria. Their small size allows large numbers of worms to be maintained without the need for extensive resources. *C. elegans* can survive in a starved state for months. They can also be frozen at -80°C for longer amounts of time.

1.3.2 Forward genetics

Forward genetics allows scientists to collect objective information about how mutations relate to disease (Forward genetics, 2020). One of the biggest advantages is that knowledge about the gene being studied is not required. Since human diseases can be the result of mutations, forward genetics can be used to identify responsible genes.

Chemicals like ethyl methanesulfonate (EMS) and other forms of mutagenesis like radiation are used to create random point mutations in the genome (Singh, 2021). Typically, the allele with the strongest phenotype is selected for analysis and further genetic mapping to identify the causative gene of interest.

CHAPTER 2

METHODOLOGY

To understand how methionine synthase dysfunction activates the UPR^{mt}, a forward genetics screen was conducted to isolate mutant animals that no longer activated the UPR^{mt} in the absence of methionine synthase. Although several mutants that suppressed the UPR^{mt} were isolated, curiously one mutant (called *osa51*) was identified that enhanced the UPR^{mt} in the absence of methionine synthase.

The goal of this project was to conduct a preliminary characterization of this mutant, *osa51* using multiple approaches. First, the isolated mutant worm was validated by backcrossing the mutant with a wild-type genetic background four times. This was performed because hundreds of mutations were also created in the genome as part of the forward genetics screen. Because we hypothesize that only one of these mutated genes enhances the UPR^{mt} pathway, we wished to remove as many unlinked mutations as possible through recombination.

Development analysis, lifespan analysis, UPR^{mt} activation quantification, and morphological differences were examined using the isolated, backcrossed mutant.

2.1 EMS Mutagenesis

Forward genetics was performed by first obtaining approximately two thousand SJ4100 animals from NGM plates. SJ4100 is a transgenic strain of *C. elegans* that contains the *hsp-6*::GFP transcriptional reporter that fluoresces green in the presence of mitochondrial stress as a readout of the UPR^{mt}. NGM plates were washed with 2 mL of S-

basal and resuspended with 2mL 60 μM 2X ethyl methyl sulfonate (EMS) solution. Worm suspension was placed on a rocker for four hours and then washed with S-basal three times. Worms were resuspended in 0.5 mL S-basal and placed onto NGM plates seeded with *E. coli* OP50 bacteria. After plates were incubated for 24 hours, fifty adult worms were transferred to seeded NGM plates. Worms were allowed to grow for seven days, and the first filial (F1) generation of animals was inspected for those that showed a difference in expression of the *hsp-6*::GFP UPR^{mt} reporter in the methionine synthase mutant (*metr-1(ok521))* background.

2.2 *osa51* Backcrossing

Approximately eight *osa51; metr-1(ok521)* L4 hermaphrodites and sixteen wild-type L4 males were placed on a mating plate. The mating plate was made by seeding an NGM plate with a 5 μL drop of *E.coli* OP50 bacteria at the center. The mating plate was incubated for 24 hours, and each adult hermaphrodite was singled out onto different OP50 seeded plates.

Worms were maintained until the F1 generation reached the L4 stage. F1 worms were observed under fluorescence, and the plate with the brightest fluorescent L4 hermaphrodite worms was chosen. Approximately fifteen L4 hermaphrodite worms exhibiting bright green fluorescence were singled out, each on a separate plate.

Worms were maintained until the F2 generation reached the L4 stage. F2 worms were observed under fluorescence, and the plate with the brightest fluorescent L4 hermaphrodite worms was chosen. Approximately fifteen L4 hermaphrodite worms exhibiting only bright green fluorescence were singled out and placed on a plate. Hermaphrodites were transferred to a mating plate along with sixteen wild-type L4 males.

The process was repeated three more times, and the final batch of hermaphrodite worms obtained from the fourth completed backcross was placed on separate *E. coli* OP50 plates. The plate with the brightest fluorescent adult worm and progeny was chosen for further analysis.

2.3 Fluorescence Intensity Quantification and Imaging

The Zeiss Observer Z1 upright microscope was used for fluorescence imaging. A drop of 2.5 mM sodium azide in S-basal and worms were added to agarose pad-lined glass microscope slides, causing the worms to be anesthetized. Worms were arranged in a head-to-tail fashion, visualized under the microscope, and imaged. For quantification of fluorescence intensity, ImageJ software was used to select the outlines of each worm, subtract any background fluorescence, and divide by worm size. This generated a fluorescence intensity value that was used to compare the fluorescence of each genetic background.

2.4 Developmental Analysis

To assess animal development, each genetic background was synchronized at the L1 stage. The developmental stage of each strain was quantified each day for 2 days. Approximately 50 animals were used for this assay. The developmental stage of the worms was determined based on the stage of vulva development, an often-used proxy for age. Pictures of all three strains were taken using Zen imaging software at 18.2x magnification three days after synchronizing the worms.

2.5 Lifespan Analysis

One hundred L4 animals were maintained on *E. coli* OP50 strain for the entirety of the assay. Worms were transferred every 1-2 days until progeny were no longer being produced. The number of dead, live, and censored worms were counted every day until all worms were either dead or censored. Animals were considered dead if touch by platinum wire did not result in a response. If worms escaped the plate or ruptured during the process of transfer, the worms were considered censored. To yield results with significant statistical power, three plates with 100 worms each, per strain, were used. The log-rank test was performed to determine statistical significance when compared to the wild-type strain. In general, results were considered significant if p-values <0.05.

2.6 Quantification of Transparent Tails

Day 7 adults were used for developmental assays and to quantify differences in morphology, specifically the tail of the worm. Pictures of all three strains were taken using the Zen microscope imaging software at 92.6x magnification.

CHAPTER 3

RESULTS

3.1 *osa51* enhances UPR^{mt} activation in *metr-1(ok521)* animals

The magnitude of fluorescence from the *hsp-6*::GFP transgene reflects the level of UPR^{mt} activation. We observed that the *osa51* mutation significantly enhanced *hsp-6*::GFP expression in the *metr-1(ok521)* mutant background. This suggests that the *osa51* mutation is resulting in an increase in UPR^{mt} activity following the loss of the methionine synthase gene *metr-1*.

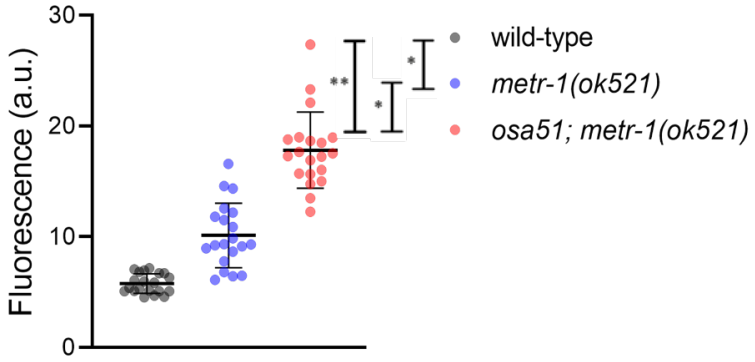

Figure 3.1: *hsp-6*::GFP Fluorescence Intensity Quantification Analysis data for wild-type, *metr-1(ok521)*, and *osa51; metr-1(ok521)* strains

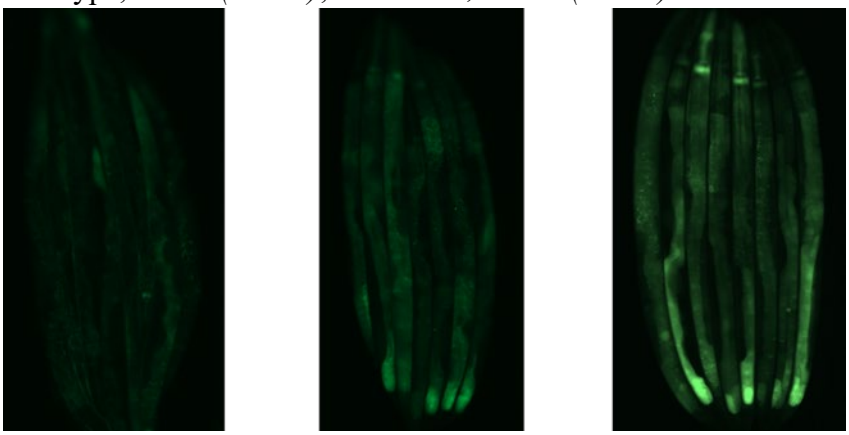

Figure 3.2: Fluorescence images of wild-type (A), *metr-1(ok521)* (B), and *osa51; metr-1(ok521)* (C) strains taken using Zeiss Observer Z1 upright microscope

3.2 Lifespan Analysis

Lifespan analysis showed that *metr-1(ok521)* animals lived longer than wild-type, consistent with previous reports (Amin et al. 2020). However, the *metr-1(ok521);osa51* mutant strain had a significantly shorter lifespan than *metr-1(ok521)* animals. The shortened lifespan of *osa51; metr-1(ok521)* animals was surprising considering that it enhanced the UPR^{mt}. However, the UPR^{mt} is not always associated with lifespan extension as observed by Bennett et al., (2014). In this study, a genome-wide RNAi screen was performed to identify negative regulators of the UPR^{mt}. Nineteen RNAi clones that activated the *hsp-6p::gfp* reporter were found to affect the lifespan of *C. elegans* differently. The transcription factor ATFS-1 is vital to the activation of UPR^{mt}, so it would be expected that loss of *atfs-1* would suppress the extended lifespan of specific mitochondrial stressed animals. Surprisingly, the knockdown of two identified genes, knockdown of the ETC gene *cco-1*, or the mutation of the ETC gene *isp-1* extended lifespan despite the absence of *atfs-1*. Furthermore, after identifying 95 gene knockdowns that activate the UPR^{mt}, it was found that there was no correlation between lifespan extension and the degree to which UPR^{mt} was induced.

It is possible that the combination of *osa51* with the *metr-1(ok521)* background leads to a cellular condition that is not conducive to lifespan extension, despite enhanced UPR^{mt} activity.

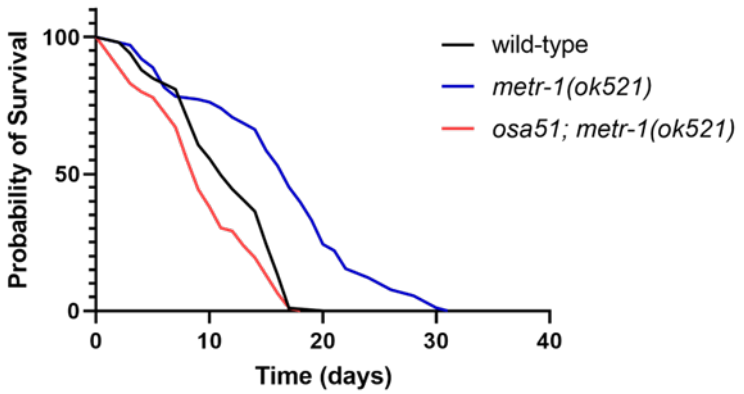

Figure 3.3: Lifespan Analysis data for wild-type, *metr-1(ok521)*, and *osa51; metr-1(ok521)* strains
(*denotes p<0.0001, **denotes p<0.0063)

3.3 Developmental Analysis

Developmental analysis showed that the *osa51; metr-1(ok521)* mutant strain developed slower than the wild-type strain. Because the growth of cells is dependent on different complex mechanisms, mutation of certain genes can result in a slower or faster growth rate (Alberts et al., 2002). The mutation in the *osa51* strain could have altered pathways affecting developmental rate resulting in slower transition through the life stages (Alberts et al., 2002).

Strain	Percent of Worms			
Life Stage	Adults	YA	L4	<L4
wild-type	50	24	20	5.7
metr-1(ok521)	14	8.7	68	9.3
osa51;metr-1(ok521)	0	0	13	87

Table 3.1: Developmental Analysis data for the number of worms at each life stage three days after egg-prepping

Strain	Percent of Worms			
Life Stage	Adults	YA	L4	<L4
wild-type	73.3	23	3.5	0
metr-1(ok521)	22.7	68	9.3	0
osa51;metr-1(ok521)	6.7	61	23	8.7

Table 3.2: Developmental Analysis data for the number of worms at each life stage four days after egg-prepping

A B C

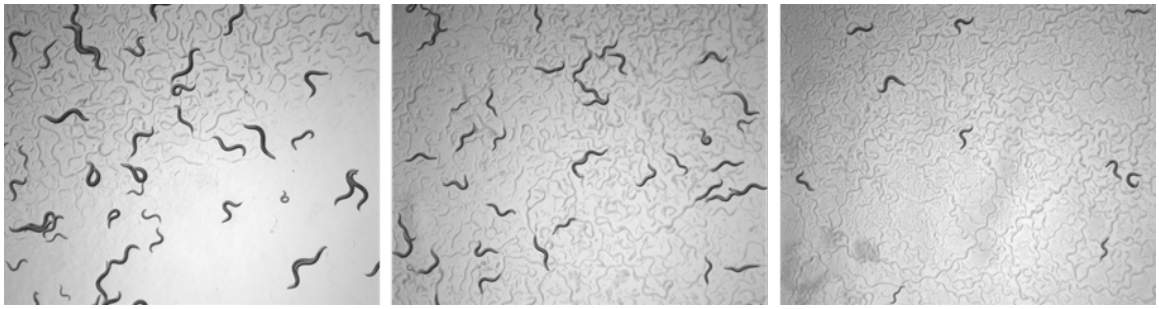

Figure 3.4: 18.2x magnification images of wild-type (A), *metr-1(ok521)* (B), and *osa51*; *metr-1(ok521)* (C) strains three days after synchronization

3.4 Morphological Differences: The Transparent Tail Phenotype

A significantly higher amount of *osa51; metr-1(ok521)* worms developed transparent and paralyzed tails compared to *metr-1(ok521*) single mutants and wild-type animals. Paralysis and transparency of tails observed in the *osa51; metr-1(ok521)* mutants are most likely due to an altered developmental caused by this mutation. The frequency of worms that were observed to have transparent tails increased with age, and animals that developed this phenotype died shortly thereafter. Thus, this transparent tail phenotype is likely linked with the shortened lifespan caused by the *osa51* mutation.

A B C

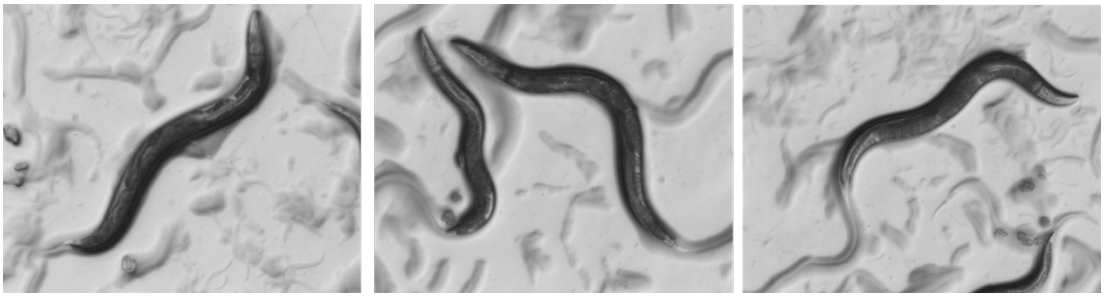

Figure 3.5: 92.6x magnification images of Day 7 wild-type (A), *metr-1(ok521)* (B), and *osa51*; *metr-1(ok521)* (C) adults

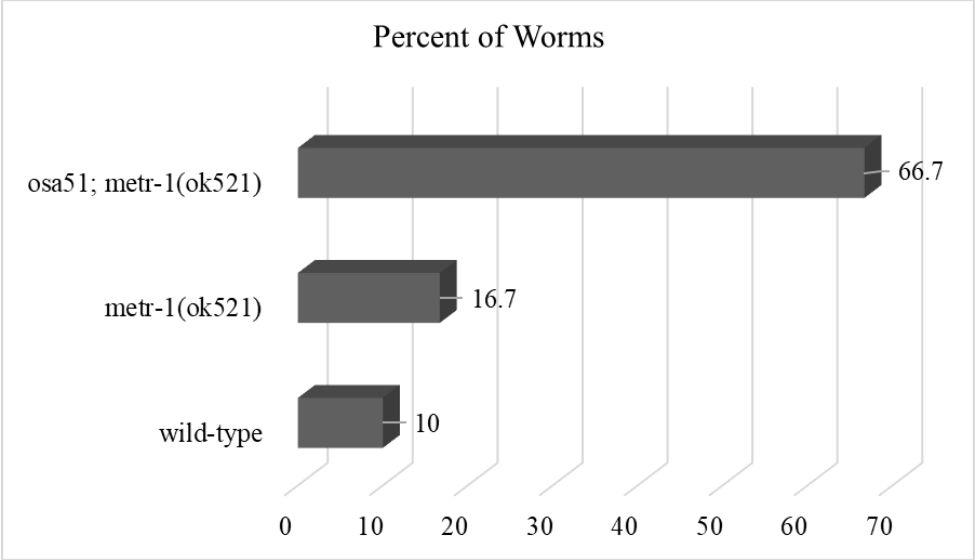

Figure 3.6: Percent of day 7 adult worms showing transparent tails for wild-type, *metr-1*, and *osa51*; *metr-1(ok521)* strains

CHAPTER 4

CONCLUSION

The goal of this experiment was to characterize the *osa51; metr-1(ok521)* mutant strain of *C. elegans* and determine its effects on lifespan and longevity. The next step would be to map the *osa51* causative gene of interest. For this purpose, whole-genome sequencing data will be used to identify all mutations in the *osa51* mutant animal that are not present in the original methionine synthase mutant background, and thus were caused by the EMS mutagenesis. To complement the whole genome sequencing, *osa51* animals will be mapped using classical mapping techniques to a small chromosomal region (Fay, 2005-2018). Once mapped, the gene product will then be characterized based on the predicted function. For example, if the *osa51* gene encodes a transcription factor, RNA sequencing technology will be used to determine the genes it regulates. If the *osa51* gene is a kinase, co-immunoprecipitation will be performed to isolate protein partners that it may be phosphorylating.

The forward genetics strategy was performed in such a way as to isolate dominant mutations. Therefore, we will determine whether the dominant mutation is a gain-of-function mutation or a dominant-negative mutation. A dominant-negative mutation results in the protein folding differently, which may cause it to inhibit itself by binding to a normal copy of the same protein or to other proteins resulting in their inhibition. Gain-of-function mutations would result in an overactive form of the protein. If the mutation is a gain-of-function mutation, we will utilize RNAi to knock down the gene of interest, which should

silence its function and suppress the enhanced UPR^{mt} activity. *E. coli* bacteria harboring a plasmid that synthesizes double-stranded RNA will be fed to the mutant animal to silence gene function (Conte, MacNeil, Walhout, & Mello, 2015). Finally, the *osa51* gene mutation will be confirmed using genome editing by CRISPR/Cas9 (Dickinson & Goldstein, 2016). Here, we will introduce the same *osa51* gene mutation in the *metr-1(ok521)* background to see whether it similarly enhances the UPR^{mt} and reduces its lifespan.

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BIOGRAPHICAL INFORMATION

Heba earned a Bachelor of Science degree in Biological and Biology at the University of Texas at Arlington. Although she didn't intend on researching as an undergraduate student, an opportunity to study cellular and molecular pathways in Dr. Pellegrino's lab allowed her to gain invaluable knowledge and experience that she will undoubtedly use in her future endeavors. Her research involved investigating the mechanistic pathways that affect aging in organisms and how they can be altered in *C. elegans.*

During her time at UTA, she was a part of SNMA/MAPS, an organization on campus that allows pre-medical students to gain the skills needed to be a strong medical school applicant. Through this organization, she was able to gain leadership positions that allowed her to mentor students. She applied and received acceptance to JAMP (Joint Admission Medical Program), which gave her tremendous support to pursue her dream of becoming a physician. She has received acceptance to and plans to attend UT Southwestern Medical school starting in the Fall 2021 semester in hopes of one day becoming an ER physician or surgeon.