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AN EXAMINATION OF JNK SIGNALING DURING CAENORHABDITIS ELEGANS MITOHORMESIS

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by

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ABSTRACT

AN EXAMINATION OF JNK SIGNALING DURING

CAENORHABDITIS ELEGANS

MITOHORMESIS

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Mitohormesis is the biological concept that a mild dose of mitochondrial stress has beneficial effects on an organism, leading to increased longevity. While mitohormesis has been demonstrated to result in a conserved increase in lifespan across multiple species, the molecular players involved in this phenomenon still need to be fully identified. Here, we investigated the role of KGB-1, the *C. elegans* homolog of mammalian c-Jun N-terminal kinase (JNK) in the regulation of mitohormesis. Specifically, what substrate(s) interact downstream of the stress-activated kinase KGB-1 to mediate mitohormesis? Primary methodology was lifespan analysis using *C. elegans* in which each predicted KGB-1 interactor gene's function was reduced by RNA interference (RNAi). For each RNAi strain, lifespan of *C. elegans* was monitored and used to construct survival graphs. Results indicated that the effect of KGB-1 on mitohormesis longevity was dependent on the

specific substrate that was knocked down. Of the KGB-1 substrates that were reduced following RNAi, the GTPase NOG-1 that is required for ribosome assembly showed the greatest effect. Our preliminary results suggest a novel mechanism of mitohormesis by the regulation of ribosome assembly through the interaction of KGB-1 and NOG-1. Biochemical assays will be carried out to further study this in mammalian cells.

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CHAPTER 1

INTRODUCTION

1.1 Quality Control Upon Mitochondrial Dysfunction

Mitochondria are double-membraned organelles best known as the "powerhouses of the cell" since they primarily create energy in the form of ATP through oxidative phosphorylation. In addition, mitochondria have a variety of other core functions, including synthesis of nucleotides and amino acids as well as the regulation of programmed cell death (apoptosis). However, mitochondria are challenged by various stress conditions in the form of toxins accumulating in an organism, harmful free radicals, or mutations within the genome. Accordingly, extensive recovery mechanisms are used to mitigate mitochondrial dysfunction owing to the essential nature of the organelle.

Three main cellular approaches are used to recover damaged mitochondria. First, mitochondrial dynamics, either through mitochondrial fusion or fission/division, can be used to help restore mitochondrial homeostasis. For instance, if a defective mitochondrion fuses with a healthy one, the dysfunction can be dissipated and proper function restored. Second, severely damaged mitochondria can be specifically removed from the cell through the process known as mitophagy, which refers to the selective engulfing, or eating away, of faulty mitochondria. Third, retrograde signaling can be used to communicate the physiological status of the organelle to the nucleus in order to induce changes in gene expression that will help support organelle recovery. Mitochondrial retrograde signaling in metazoans is known as the mitochondrial unfolded protein response (UPRmt). The UPRmt

is activated with various mitochondrial stress conditions and is mediated by the bZIP transcription factor ATFS-1 (Activated Transcription Factor under Stress). ATFS-1 is an important transcription factor that mediates mitochondrial quality control to reestablish homeostasis during times of mitochondrial dysfunction through the transcriptional regulation of a wide range of genes. ATFS-1 has both a nuclear localization sequence (NLS) and a mitochondrial targeting signal sequence (MTS). The MTS allows ATFS-1 to be imported into healthy mitochondria via the mitochondrial import pathway where it is degraded by protease digestion. However, when mitochondria are stressed, ATFS-1 import into mitochondria is impaired since mitochondrial inner membrane potential and ATP levels are reduced. Here, ATFS-1 translocates to the nucleus to transcriptionally regulate approximately 400 genes with predominantly mitochondrial protective properties. Included amongst the nuclear expressed genes are those that promote mitochondrial proteostasis, such as mitochondrial chaperone proteins and proteases, to assist in protein folding and removal, respectively. In addition, ATFS-1 regulates a number of genes involved in glycolysis in an effort to maintain ATP levels when the mitochondrial respiratory capacity is reduced. And, ATFS-1 also regulates genes involved in detoxifying damaging free radicals and those genes involved in mitochondrial dynamics.

1.2 Mitohormesis and JNK MAP Kinase Signaling

Throughout the course of organismal life, mitochondrial function decreases and vital energy-consuming cells are especially affected. While elevated levels of mitochondrial dysfunction are associated with disease onset and reduced longevity, paradoxically, a mild exposure to mitochondrial stress can be beneficial and result in extended lifespan. Known as mitohormesis, this early exposure to mitochondrial stress

allows cells to activate recovery programs to deal with potentially more severe dysfunction to mitochondria later in life.

Insights into the regulation of mitohormesis is developing. Our group has discovered that that kinase signaling by the JNK pathway is involved in this phenomenon that was recently validated independently. Kinases function by phosphorylating their target substrate to activate or deactivate its function. The functions of JNK signaling is quite pleiotropic but it is known to have significant roles in protecting an organism during cellular stress. JNK is a MAP kinase that undergoes a cascade of events. RAS GTPase activates MAP kinase kinase kinase (MAPKKK), which activates MAP kinase kinase (MAPKK), which finally activates the JNK MAP kinase (MAPK). Once JNK finally binds to its target, a response is elicited. The rapid cascade of events influences many responses within the cell such as cell proliferation and differentiation.

1.3 KGB-1 MAP Kinase in *C. elegans*

Mitohormesis is a conserved phenomenon that has been studied in lower eukaryotes like yeast, all the way to higher eukaryotes like mice. The organism *Caenorhabditis elegans* is widely used as a model system to understand longevity pathways because of its many practical advantages. First, it only takes 3-5 days for eggs to become juveniles. This way, a large quantity of worms can be handled at once and experimentation can be repeated multiple times. Second, they contain conserved pathways that are similarly found in humans, including the KGB-1/JNK kinase we study here. Through analysis of these factors in worms, we can make conclusions about mitochondrial stress-related longevity in higher eukaryotes, which can help further our understanding of aging and neurological diseases.

Because KGB-1/JNK signaling is an important mediator of stress recovery, we suspected that it may have a role in regulating mitochondrial dysfunction-induced longevity. Indeed, we have found that hyper-activation of the KGB-1 signaling pathway can effectively attenuate the UPRmt during multiple mitochondrial stress conditions (data not shown). Some propose that the UPRmt pathway is involved with the increased longevity associated with mitohormesis owing to its role in promoting mitochondrial recovery, although this remains controversial. The activation of mitochondrial UPR stress response is correlated with the increased longevity of organisms, but there is currently still some debate as to whether it is required.

We therefore measured the lifespan of a known mitohormesis mutant worm that possesses a missense mutation in the mitochondrial gene *isp-1* in the presence or absence of KGB-1. As expected, *isp-1* mutant animals lived longer compared to wild-type controls, but interestingly, mutation in *kgb-1* (using the *km21* allele of *kgb-1*) completely suppressed this effect (Figure 1.1). This is consistent with KGB-1 in regulating mitochondrial stress induced longevity. Knowing this requirement for KGB-1 and that kinases phosphorylate target proteins, we set out to identify the specific targets that interact with KGB-1 that mediate increased lifespan and combat stress.

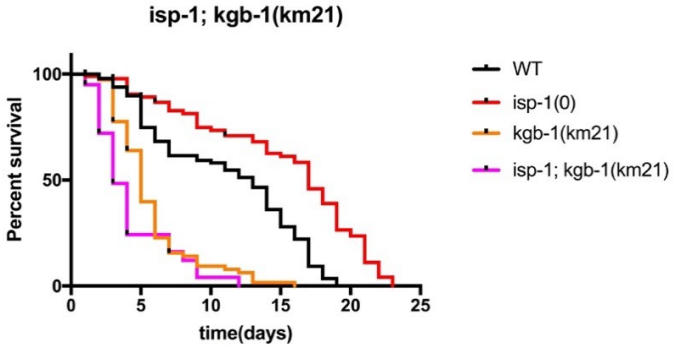

Figure 1.1: Survival graph for wild-type, *isp-1, kgb-1 (km21)*, and *isp-1:kgb-1 (km21)* in *C. elegans*

CHAPTER 2

METHODOLOGY

2.1 Screening for Potential Interactors of KGB-1

We referred to a previously published yeast-two-hybrid screen to identify potential substrates of KGB-1 to test in our longevity assays. Here, a "bait" yeast strain containing an expression plasmid consisting of the KGB-1 coding sequence fused to the LexA DNA binding domain (DBD) was co-transformed with a *C. elegans* "prey" cDNA library, where every C. elegans gene is fused a GAL4 transactivation domain. As a prey, KGB-1 will bind to its target substrates bringing the LexA DBD and GAL4 transactivation domain together to transcribe a DNA promoter that will allow the yeast to grow on minimal media. This method identified nine potential interactors for KGB-1 in the scientific community: FOS-1 transcription factor, EFT-3 translation initiator factor, NOG-1 GTP-binding protein, DNJ-12 heat shock protein, APE-1 transcription regulator, GFAT-1 hexosamine biosynthesis enzyme, RPT-3 proteasome subunit, CCCH-1 zinc finger protein, and endocytic recycling protein SDPN-1.

2.2 RNA Interference

Primary methodology for this project was to perform lifespan analysis using *C. elegans* that had each KGB-1 predicted interactor's gene function reduced by RNA interference (RNAi). *E. coli* bacteria harboring plasmids were used in which one gene was flanked to bacterial inducible T7 promoters. Addition of the T7 promoter inducer (using IPTG) stimulated the transcription of the gene on both strands of the DNA, generating

double-stranded RNA (dsRNA). Since RNA is normally single stranded, the bacterial cell recognizes the dsRNA as foreign and cleaves it into small 22 nt fragments. These small fragments then bind to endogenous RNA transcripts for that corresponding gene, leading to its degradation and silencing. This assay was performed for RNAi strains of six out of the eight predicted KGB-1 interactors (FOS-1, EFT-3, NOG-1, DNJ-12, and APE-1).

The worms were fed these RNAi *E. coli*, which in turn silenced the corresponding genes within the worm's genome as well. *C. elegans* goes through distinct life stages: egg, L1, L2, L3, L4, and adulthood. The RNAi *E. coli* against each gene was grown on agar plates (containing the IPTG T7 promoter inducer) at 25 degrees Celsius for 2-3 days. Worms were then placed onto the plates and feeding was carried out for two days. Worms were grown on the RNAi *E. coli* from the egg stage to the L4 stage (referred to as RNAi-dev) or from the onset of adulthood (RNAi-adult). As a negative control, a RNAi *E. coli* strain was used that carried an empty plasmid which had no gene insert.

Two *C. elegans* genotypes were used: the wild-type strain (N2) and the mitochondrial stress mutant *isp-1(qm150)*. Each worm strain contained the *hsp-6*::GFP reporter plasmid that is a reflection of UPRmt activation. For each RNAi experiment, *C. elegans* development and lifespan was monitored. For lifespan analysis, 100 worms of each genotype were transferred to the respective RNAi bacteria. Experimental worms were transferred to new plates daily to ensure that the same 100 subjects were being tested, as worms produce progeny quickly as they age. A daily log of how many worms were alive/dead/censored was recorded, and the resulting data was used to construct survival graphs. Animals were censored if they died from unnatural causes (e.g. human error) or if the worms crawled off the plate.

2.3 NOG-1 Follow-up Experimentation in *C. elegans*

2.3.1 Localization and Stability of NOG-1 During Times of Stress

We were interested in analyzing the localization and protein stability of NOG-1 in wild-type and *kgb-1* mutant animals in the presence or absence of mitochondrial stress. For these experiments, a translational green fluorescent protein (GFP) reporter for NOG-1 gene was created. Here, the promoter of *nog-1* as well as the full *nog-1* open reading frame (excluding the stop codon) was fused to the coding sequence of GFP. Microinjection transformation was used to incorporate the NOG-1::GFP reporter DNA within the nucleus. The worm was co-injected with *myo-2*::mCherry as a transformation marker. This plasmid contains the *myo-2* promoter fused to the mCherry coding sequence, which results in red fluorescence in the pharyngeal muscle specifically.

NOG-1::GFP translational reporter animals were then fed RNAi E. coli bacteria in which the function of mitochondrial quality control protease SPG-7 had been knocked down. This results in the accumulation of misfolded proteins in mitochondria, causing proteotoxicity and mitochondrial stress. Worms were examined under fluorescent microscope and findings were recorded.

2.3.2 Translation Efficiency During Times of Mitochondrial Stress

We examined translation efficiency with loss or over-activation of KGB-1in the presence or absence of mitochondrial stress using the transcriptional GFP reporter *irg-1*::GFP that increases fluorescence expression when protein translation is reduced. Wild-type and/or *kgb-1(km21)* irg-1::GFP animals were fed with RNAi *E. coli* bacteria that knocked down the function of the mitochondrial quality control protease spg-7 or the phosphatase enzyme *vhp-1*. The phosphatase VHP-1 negative regulates KGB-1 by via

dephosphorylation. Worms were examined under fluorescent microscope and pictures were taken.

CHAPTER 3

RESULTS

3.1 RNA Interference Screen of KGB-1 Interactors

We used an RNAi approach to knockdown the expression of the predicted KGB-1 substrates and tested its effect on the longevity of wild-type and *isp-1* long-lived mitochondrial stressed animals. With regard to knockdown of *ape-1*, *gfat-1*, *eft-3*, and *dnj-12*, we observed no difference in the survival of *isp-1* mutant animals (Figure 3.1). Interestingly, RNAi of *dnj-12* reduced the lifespan of wild-type animals but this increased lethality was suppressed in the *isp-1* mutant background. Amazingly, while knockdown of *fos-1* severely reduced the lifespan of wild-type animals, it further increased the survival of *isp-1* mutant animals (Figure 3.2). Knockdown of *nog-1*was the only condition that reduced the increased lifespan of *isp-1* mutant animals in a similar trend that was observed with loss of KGB-1 (Figure 3.3).

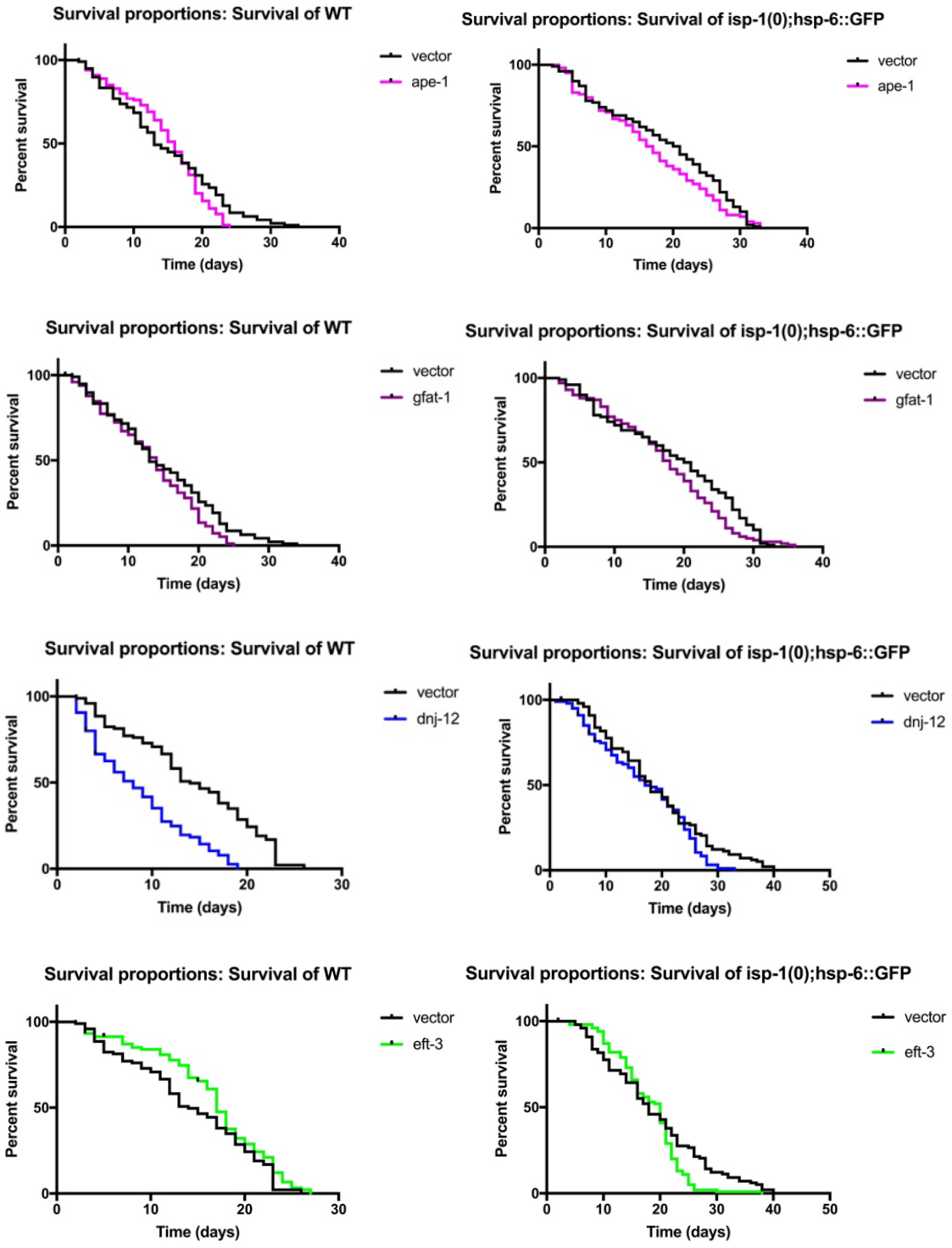

Figure 3.1: Survival graphs for *ape-1, gfat-1, dnj-12*, and *eft-3* RNAi in wild-type and *isp-1* (*qm150*) background

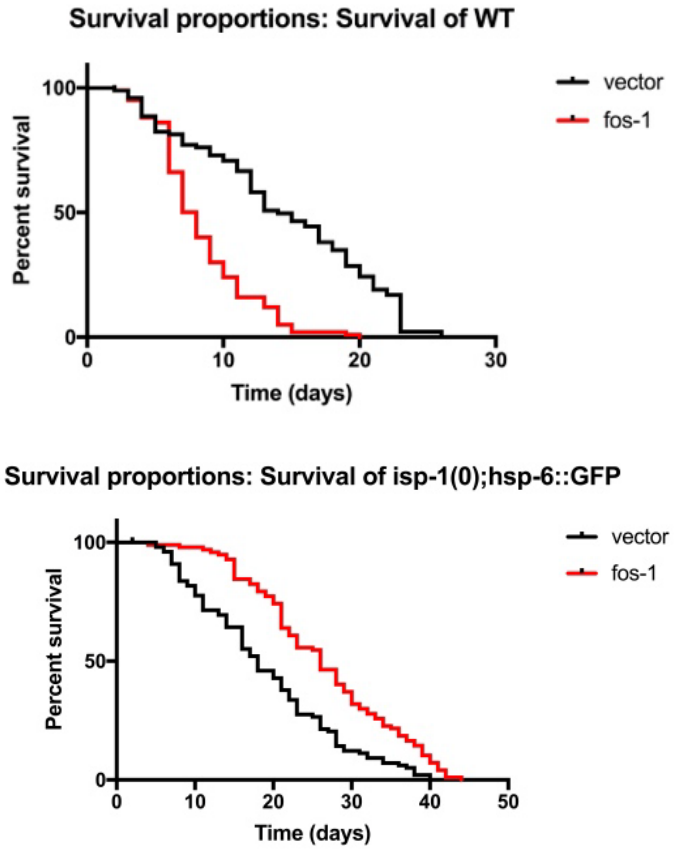

Figure 3.2: Survival graph for *fos-1* RNAi in wild-type and *isp-1* (*qm150*) backgrounds

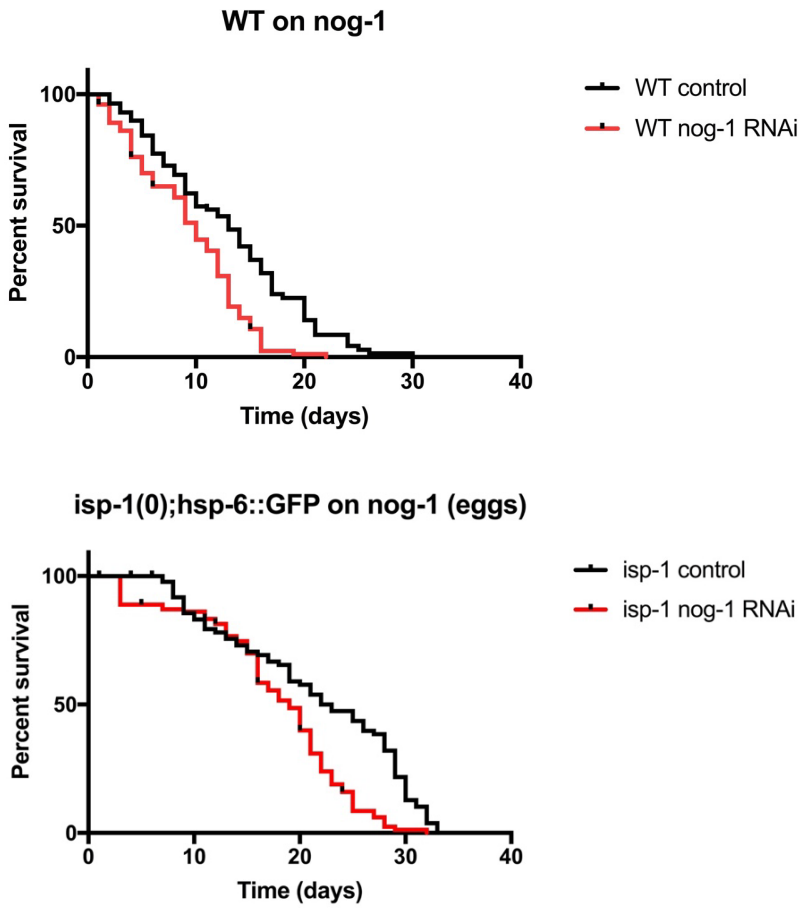

Figure 3.3: Survival graph for *nog-1* RNAi in wild-type and *isp-1* (*qm150*) backgrounds

3.2 NOG-1 Follow-up Experimentation in *C. elegans*

We decided to pursue the investigation of NOG-1 further since it was the only RNAi clone that when knocked down, mimicked the effect of *kgb-1* loss of function mutation on *isp-1(qm150)* longevity (i.e. associated with a reduction in lifespan). Interestingly, *C. elegans* NOG-1 shows considerable homology to other predicted NOG-1 protein including that of humans. Also, the predicted phosphorylation consensus site (S/TP) and docking site is highly conserved (Figure 3.4 and 3.5, respectively).

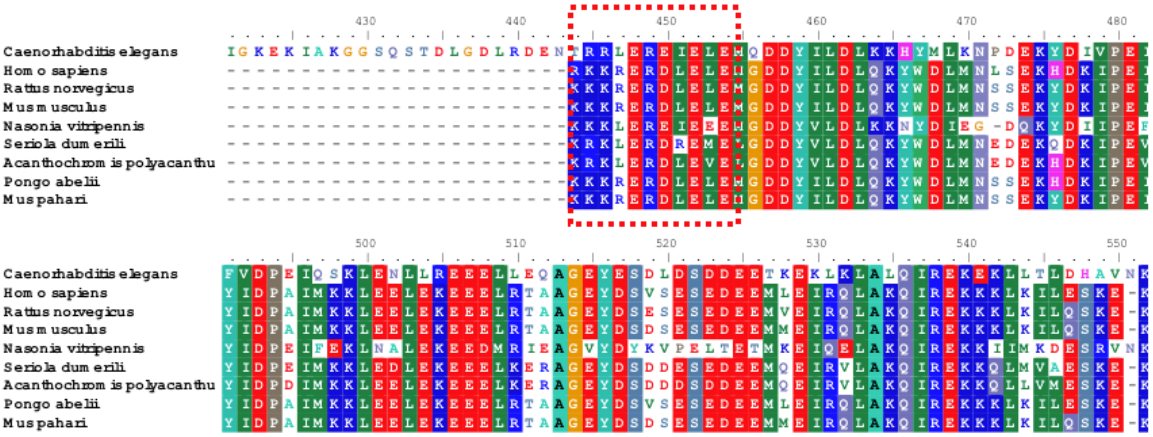

Figure 3.4: Conserved docking site consensus sequence of *nog-1* in *C. elegans* and *Homo Sapiens*

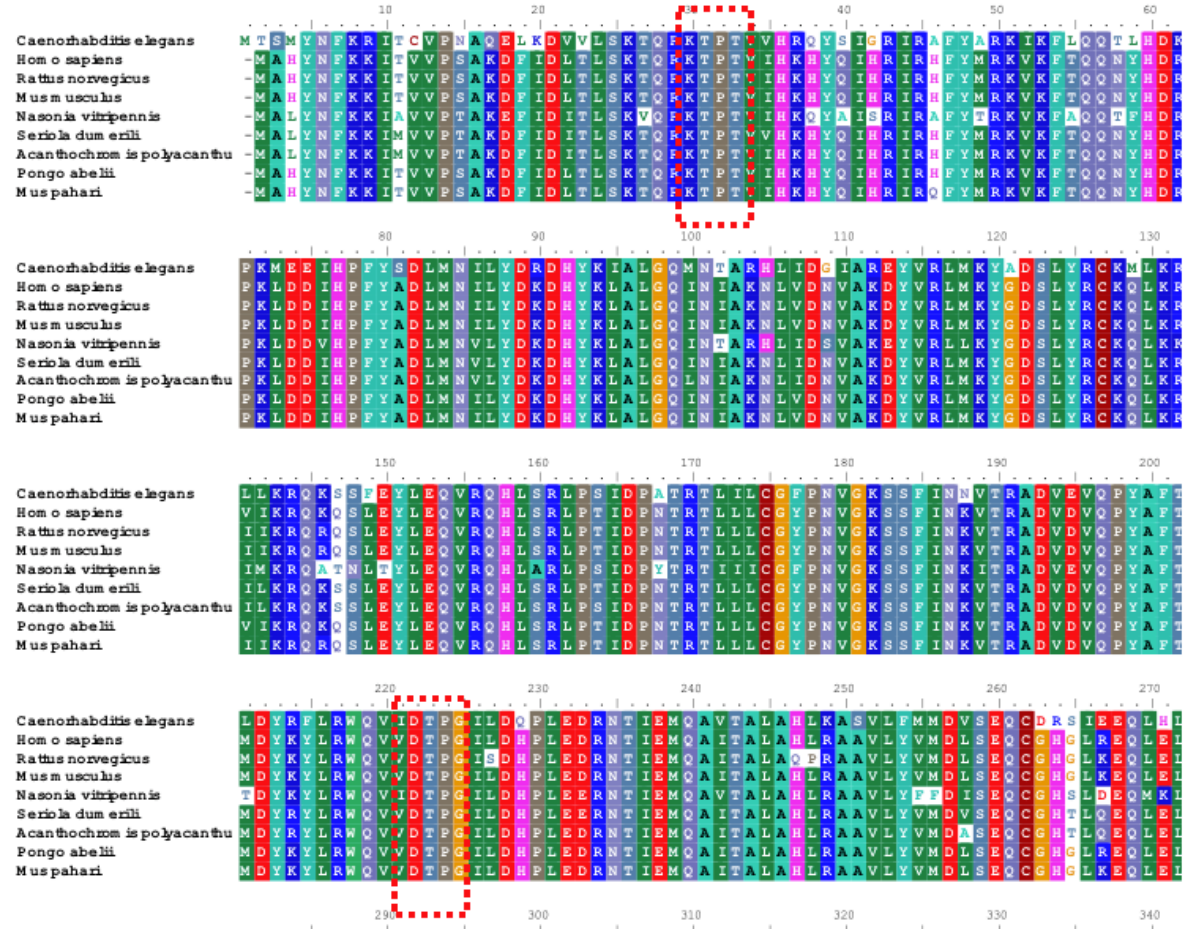

Figure 3.5: Conserved phosphorylation site consensus sequences of *nog-1* in *C. elegans* and *Homo Sapiens*

3.2.1 Localization and Stability of NOG-1 During Times of Stress

We created a transgenic *C. elegans* expressing a NOG-1::GFP translational reporter to examine possible functional relationship between KGB-1 and NOG-1. We hypothesized that potentially KGB-1 activity affected either the localization or stability of NOG-1 during mitochondrial stress. Therefore, we induced mitochondrial stress with our NOG-1::GFP transgenic strain by knocking down the expression of *spg-7*. However, NOG-1::GFP remained localized to its predicted site of localization, the nucleolus in the presence or absence of mitochondrial stress (Figure 3.6). Also, no change in NOG-1::GFP expression was observed, suggesting that mitochondrial stress does not affect the stability of the NOG-1 protein. Similar results were also obtained when we performed RNAi against a negative regulator of KGB-1, the phosphatase VHP-1 (data not shown).

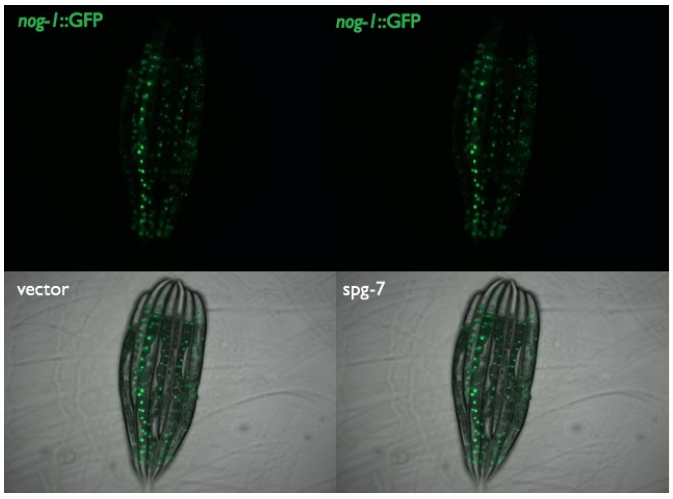

Figure 3.6: NOG-1::GFP under control (vector) and mitochondrial stress (*spg-7*) conditions

3.2.2 Translation Efficiency During Times of Stress

If our hypothesis that KGB-1 and NOG-1 were true, then we should observe a change in translation efficiency owing to NOG-1's predominant role in ribosome assembly. To measure protein translation efficiency, we used the *irg-1*::GFP transcriptional reporter

transgenic animal that increases its expression dramatically during conditions that attenuate protein translation. Interestingly, both mitochondrial stress (*spg-7* RNAi) and KGB-1 over-activation (*vhp-1* RNAi) were able to induce irg-1::GFP expression in a KGB-1-depenedent manner (Figure 3.7). Thus, KGB-1 appears to negatively regulate protein synthesis during mitochondrial stress.

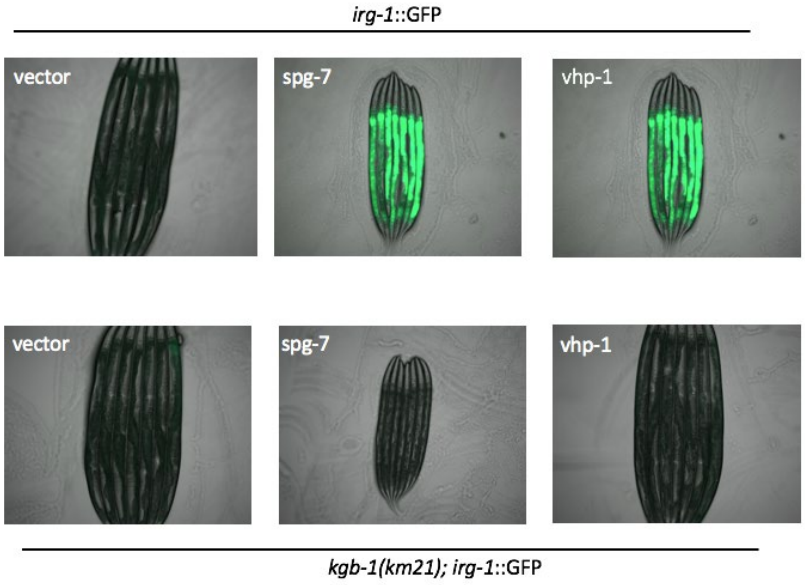

Figure 3.7: Translational efficiency in wild-type and *kgb-1 (km21)* mutants in the presence and absence of mitochondrial stress (*spg-7*) or KGB-1 over-activation (*vhp-1*) using the *irg-1*::GFP transgenic strain

CHAPTER 4

DISCUSSION

Mitohormesis is an exciting avenue for investigation because there is an undeniable correlation between the status of mitochondria and organismal aging. Consistent with our hypothesis that JNK signaling was involved in the regulation of mitohormesis, we observed a strict requirement for the *C. elegans* JNK homolog, KGB-1, during mitochondrial stress-induced longevity. Interestingly, KGB-1 possesses age-dependent effects on longevity and stress resistance. Here, loss of KGB-1 early in the life of *C. elegans* reduces their lifespan while loss of KGB-1 function, beginning at the adult stage, increases lifespan. This suggests that KGB-1 function can be altered through the life of the animal and with remarkably different downstream effects. While a connection between KGB-1 and longevity is appreciated, the substrates of KGB-1that mediate these effects are not know. Therefore, this research project aimed to identify and characterize the KGB-1 effectors that may have a role during mitohormesis.

4.1 RNA Interference Analysis

Using the list of predicted KGB-1 interactors as a reference, we wished to test which one, when knocked down, could mimic knockdown of *kgb-1* in the context of mitochondrial stress induced longevity. Unexpectedly, each independent substrate exhibited specific, and sometimes contradictory, effects on the lifespan of our long-lived mitochondrial stressed mutant *isp-1(qm150)*. This should be expected though considering

that KGB-1 phosphorylates multiple substrates, and each may have its own particular influence on wild-type and *isp-1(qm150)* longevity.

RNAi against *ape-1*, *dnj-12*, *gfat-1*, and *eft-3* had no significant effect on *isp-1(qm150)* longevity. APE-1 is a negative regulator of p53, a regulator of mitohormesis. Knockdown of *ape-1* on both WT and *isp-1(qm150)* backgrounds had no overall effect. It is possible that RNAi against ape-1 was not effective enough to efficiently knockdown its expression. Therefore, ape-1 transcript levels should be quantified following ape-1 RNAi using quantitative PCR methods. Also, an *ape-1* with a loss of function mutation bearing a deletion in the coding sequence is available. Therefore, we could follow up on APE-1 further using this mutant to validate the results obtained by RNAi. Interestingly, while *dnj-12* RNAi had no effect on *isp-1(qm150)* longevity, it reduced the lifespan of wild-type animals. Possibly, the benefits of *isp-1(qm150)* on longevity may dissipate the ill effects of dnj-12 RNAi in the wild-type background.

Fos-1 RNAi had the interesting effect on longevity whereby, in the wild-type background, *fos-1* RNAi decreased lifespan, whereas in the *isp-1(qm150)* background it further enhanced their already extended lifespan. The only other described condition that can further enhance mitohormesis related longevity is through protein translation repression. Therefore, it is possible that FOS-1 regulates protein synthesis indirectly through transcriptional regulation of its target genes. FOS-1 is known to associate with the histone deacetylase HDAC to control promoter activity of its gene targets during cellular stress. Perhaps the FOS-1/HDAC complex is involved with controlling transcription related to protein translation that may impact longevity during mitochondrial stress.

Of all the predicted KGB-1 interactors tested, knockdown of *nog-1*was the only condition shown to reduce the lifespan of *isp-1(qm150)*. It should be noted that *nog-1* RNAi similarly reduced the lifespan of wild-type *C. elegans* so it may be possible that the effect on *isp-1(qm150)* may be due to a reduction in viability. Nonetheless, we decided to pursue follow-up studies on NOG-1 considering its effect on longevity.

There are various potential human errors associated with RNAi that must be considered as they could lead to inaccurate results. When transferring the live worms for the longevity assays, worms could accidently be killed if poked too hard. Also, cuts could be created in the agar during the transfer process allowing the worms to crawl into the solid agar media making it very difficult to recover them. These fall into the "censored" category of the daily lifespan logs. Repeats for every interactor gene will be conducted in the future to ensure reliability of data.

4.2 Significance of NOG-1

We decided to focus on NOG-1 further because of its decreased longevity on RNAi and because of its conserved consensus sequences with other species. Every kinase has two types of protein sequence motifs that it recognizes on its substrate: the phosphorylation site and the docking site. The MAPK phosphorylation site amino acid consensus sequence consists of a serine or threonine, followed by a proline (S/TP). The docking site for worms is a longer consensus sequence (K/R-X-X/K/R-K/R-X(1-4)-L/I-X-L/I) that helps physically dock the kinase to its protein. Amazingly, both the phosphorylation sites and the longer docking site consensus sequence are also conserved with worms and humans. This serves as an additional source of evidence that KGB-1 and NOG-1 physically and functionally interact in the cell.

4.3 NOG-1 Follow-up Experimentation in *C. elegans* Analysis

How might KGB-1 functionally interact with NOG-1? Considering that NOG-1 has a known role in ribosome assembly, we hypothesized that KGB-1 might regulate protein NOG-1 to affect protein translation levels. Presumably, KGB-1 would negatively regulate NOG-1 in order to reduce protein synthesis which can further harm an already stressed cell through the trafficking of proteins through dysfunctional organelles and the enormous energy that is consumed to support the act of protein translation.

Three possible scenarios exist with regard to our main hypothesis. First, KGB-1 might affect the nucleolar localization of NOG-1. Second, KGB-1 might affect the stability or turnover rate of NOG-1. Third, NOG-1 affects the activity of NOG-1 independent of its localization or protein activity. To test the first two hypotheses, we created a transgenic *C. elegans* strain that expressed a NOG-1::GFP translation reporter. By fusing the coding of GFP to the open reading frame of nog-1, we can visualize not only the amount of NOG-1 present in the cell but also the localization of the NOG-1 protein.

4.3.1 Localization and Stability of NOG-1 During Times of Stress

A ribosome uses about 200 assembly factors, including NOG-1, to aid in its biosynthesis. NOG-1 travels with the assembling ribosome from the nucleolus, to the nucleus, and then finally to the cytoplasm where translation occurs. KGB-1, however, remains cytoplasmic. One possibility therefore is that KGB-1 and NOG-1 physically interact in the cytoplasm during mitochondrial stress and the interaction exists in order to refrain NOG-1 re-entry into the nucleus/nucleolus, thus preventing ribosome assembly and reducing protein synthesis. If true, then NOG-1::GFP localization should remain partially or fully retained in the cytoplasm in the presence of mitochondrial stress conditions such

as RNAi against the mitochondrial quality control protease *spg-7*. However, while our NOG-1::GFP fusion protein showed expected localization to the nucleus/nucleolus, we observed no difference in localization during exposure to mitochondrial stress. Furthermore, no obvious change in the intensity of the NOG-1::GFP reporter was observed, suggesting that the stability of NOG-1 is not affected as well.

4.3.2 Translation Efficiency During Times of Mitochondrial Stress

Finally, if KGB-1 were to affect NOG-1 function during mitochondrial stress, then protein translation rates would expected to be reduced. As an alternative to measuring ribosome activity and global translation rates that can be challenging and time-consuming, we decided to examine protein efficiency using a GFP reporter system. Here, a transgenic *C. elegans* carrying an *irg-1* transcriptional reporter consisting of the *irg-1* promoter fused to the coding sequence of GFP was used. *Irg-1* was initially identified as a a gene that was strongly induced at the transcriptional level during infection. Later, the mechanism of *irg-1* induction was revealed to be related to translation efficiency whereby conditions that reduce protein synthesis induce the expression of *irg-1*. Therefore, *irg-1*::GFP shows minimal expression when grown under homeostatic conditions but shows dramatic increase in fluorescence when protein translation is reduced.

Interestingly, mitochondrial stress via *spg-7* RNAi strongly induced the *irg-1*::GFP reporter, consistent with reduced protein synthesis. Amazingly, the induction of *irg-1*::GFP during mitochondrial stress was completely dependent on KGB-1, suggesting that KGB-1 mediates a mechanism to attenuate protein translation during stress. Consistently, over-activation of KGB-1 via the RNAi knockdown of its negative regulator *vhp-1* also induced the expression of *irg-1*::GFP. Thus, KGB-1 appears to help mitochondrial function

recovery through the attenuation of protein translation. Reducing protein translation rates during mitochondrial stress has previously been shown to help recovery mitochondrial activity by lowering the transit of proteins being trafficked through the organelle. Here, the kinase GCN-2 phosphorylates the eukaryotic initiator factor eIF2α to reduce protein translation rates as part of the integrative stress response (ISR). In this study, we uncover a novel mechanism of protein translation attenuation via the JNK homolog KGB1. Whether KGB-1 mediates protein translation control through the regulation of NOG-1 and ribosome assembly still remains to be determined. However, our proposed follow-up experiments should help shed light on this possible mechanism.

4.4 NOG-1 Follow-up Experimentation in Mammalian Cells

Our genetic data suggest that possibly KGB-1 and NOG-1 physically interact with a functional significance of reducing protein translation by attenuating ribosome assembly. We now propose the following experiments in order to validate this hypothesis further:

First, one would validate the physical interaction of KGB-1 and NOG-1 that was observed in the yeast-two-hybrid assay. Here, epitope tagged versions of KGB-1 and NOG-1 will be created and used for transient transfection in mammalian cells. Mammalian cells cells will then be lysed and centrifuged for protein isolation.

Next, co-immunoprecipitation (co-IP) will be used to assess the interaction between KGB-1 and NOG-1. Here, antibodies against one epitope tag will be added to the cell lysate, followed by the addition of agarose coupled-Protein A that will bind the epitope primary antibody. Centrifugation will be then we performed to pull down the putative KGB-1::NOG-1 complex. SDS-PAGE and Western Blot analysis of co-IP samples will then be used to confirm the successful pulldown of each respective partner.

Following a validation of the interaction between KGB-1 and NOG-1, we examine whether NOG-1 is truly phosphorylated by KGB-1 using a biochemical approach. In this experiment, we will simply express our epitope-tagged KGB-1 and NOG-1 in mammalian cells to detect any changes in migration of NOG-1 that could be due to phosphorylation by KGB-1 (i.e. addition of phosphates to NOG-1 would expect to alter its separation during electrophoresis). If NOG-1 is truly phosphorylated by KGB-1, we would expect to see a laddering effect consisting of multiple molecular weight species of NOG-1 (phosphorylated and non-phosphorylated forms alike). Alkaline phosphatase would be added in order to confirm phosphorylation of NOG-1 whereby addition of the phosphatase would expect to remove the phosphates from NOG-1 and thus reduce the laddering effect during electrophoresis.

Lastly, if KGB-1 and NOG-1 interaction is confirmed, as well as its phosphorylation, we would then pursue a more detailed examination of the effects KGB-1 on NOG-1 activity. Here, we would assay ribosome assembly and global translation rates using established biochemical assays.

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BIOGRAPHICAL INFORMATION

Romeeka Arfeen Siddiqui graduated in the Spring of 2018 with an Honors Bachelor of Science in Biology and minors in Biochemistry and Sociology, *Magna Cum Laude*. She has volunteered at free health clinics in the DFW area for the past ten years with American Muslim Women Physician Association (AMWPA) and has both volunteered and scribed at the Emergency Department at Methodist Charlton while being a full-time college student.

Miss Siddiqui was a part of countless organizations while attending The University of Texas at Arlington, including the Muslim Student Association (MSA), the Medical and Dental Preparatory Association (MDPA), and the National Society of Leadership and Success (NSLS). She traveled to the Dominican Republic with Global Medical Training (GMT) and conducted researched for an ecology lab with Dr. Matthew Walsh prior to joining Dr. Mark Pellegrino's lab. She also tutored through the University Tutorial and Supplemental Instruction (UTSI) for biology and chemistry, and was a recitation leader for BIOL1441 labs. Furthermore, she was selected to receive a committee letter on behalf of the Health Professional Advisory Committee (HPAC) while applying to medical school.

Romeeka will follow her mother and sister's footsteps in the medical field by starting her medical school journey in Fall 2018 at Texas Tech University Health Sciences Center. She dreams to open her own practice one day in an underserved area in Texas. She will also continue to volunteer at health clinics and travel to third-world countries to provide the indigent free medications and healthcare, with a focus on prevention education.