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Investigating Transcriptional Regulation of C. elegans Insulin-like Genes

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Investigating Transcriptional Regulation of *C. elegans* Insulin-like Genes

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Abstract

The highly conserved insulin/insulin-like growth factor signaling (IIS) pathway plays a critical role in development, lifespan, and metabolism. In the nematode, *Caenorhabditis elegans*, there are 40 insulin-like genes. Here, I investigate the transcriptional regulation of one insulin-like gene, *ins-27*, by knocking down transcription factor expression via RNA interference. I identified sixty five transcription factors as potential regulators of *ins-27*. By investigating regulators of *C. elegans* insulin-like genes, we may learn more about the biological processes that modulate insulin expression. This may provide greater insight into the regulation of human insulin-like genes.

Acknowledgements

I would like to thank Dr. Marian Walhout for sponsoring my MQP. I've learned so much from working in your lab and appreciate this opportunity. Thanks to Ashlyn Ritter, who guided and mentored me throughout the year, along with providing the transgenic strains that I've worked with. I would like to thank Lesley MacNeil for also providing some worm strains and demonstrating the RNAi 96-well plate set-up. I also would like to give a big thanks to Professor Ryder for your advice and dedication to my project.

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Introduction

The insulin/insulin-like growth factor signaling (IIS) pathway is highly conserved in *Caenorhabditis elegans* and humans and plays a critical role in development, lifespan, stress resistance, and metabolism for both species (Ritter et al., 2013; Kenyon 2011). There have been several studies that have linked individuals with reduced somatic signaling with a longer lifespan on average compared to those without genetic variation. Specifically, variants of the Forkead box O (FOXO) gene, known to be under IIS control, are known for their longevity amongst unrelated human populations (Anisimov and Bartke, 2013). While the human genome contains ten genes that encode for insulin-like peptides, including insulin, insulin-like growth factors (IGFs) and relaxins, *C. elegans* encodes 40 insulin-like genes (Ritter et al., 2013; Li et al., 2003). Other downstream components of the IIS pathway in *C. elegans* include the DAF-2 receptor, and a downstream signaling cascade that acts on the FOXO/DAF-16 transcription factor (Narasimhan et al., 2009; Kenyon, 2011).

Despite the high conservation of the IIS pathway, much is still left unknown. Learning more about the participants involved in this pathway, can give insight into the biological processes that modulate insulin expression and the regulation of human insulin-like genes. This question can be approached on a systems level, which helps to understand complex interactions within biological systems. Since *C. elegans* is a transparent animal, green fluorescent protein (GFP) reporters can be used to determine when and where there insulin-like genes are expressed. The worm also contains roughly 940 known transcription factors, which have been used to create an RNA interference (RNAi) library (Arda and Walhout, 2009). In this project, this library was used to identify transcription factors involved in the activation of *ins-27*, one of the insulin-like

genes. Thus, by using model organisms such as *C. elegans*, we can learn more about the IIS pathway in both *C. elegans* and humans.

Insulin/Insulin-like Signaling Pathway

There are forty insulin-like genes in *C. elegans*, compared to ten in humans. This raises questions as to why the worm needs so many different insulin-like genes and how their different expression patterns are regulated. These questions were examined in a recent study of the insulin-like genes (Ritter et al., 2013). It was originally proposed that there is a certain level of redundancy amongst the genes. Thirty one of the insulin-like genes are expressed in at least two tissue types, with three genes expressed in as many as 15. The major tissues that the insulins are expressed in are the neurons, muscle, and intestine (Ritter et al., 2013). Many of these genes have similar expression patterns, but each gene may have varying degrees of expression intensities in specific tissues during development or when exposed to environmental changes. For instance, insulin expression in the pharynx decreases as the worm age, and expression increases in the distal tip cell in older worms. Factors such as aging, starvation, dauer formation, and heat stress can also change the insulin-expression patterns (Ritter et al., 2013). Of the forty genes, *ins-4* was one of the genes that stood out as showing high expression overlap with seven different insulin genes including *ins-1*, *ins-5*, *ins-7*, *ins-8*, *ins-10*, *ins-17*, and *ins-18*. This was calculated by a Tissue Overlap Coefficient (TsOC) score, which calculates the degree of expression overlap for each pair-wise combination of inulins at each developmental stage (Ritter et al., 2013). With no complete overlap in expression patterns between any two insulin-like genes, there is no absolute redundancy amongst the insulin-like genes.

The insulin-like gene family shares 25-40% of amino acid sequence identity, and thirty four of the studied thirty seven genes are shown to have conserved intron and exon patterning, as

seen in vertebrates (Nelson and Padgett, 2003). As a result of their structural similarity, ten proteins, including *ins-4*, have been identified as ligands for DAF-2, a receptor in the signaling pathway (Duret et al., 1998).

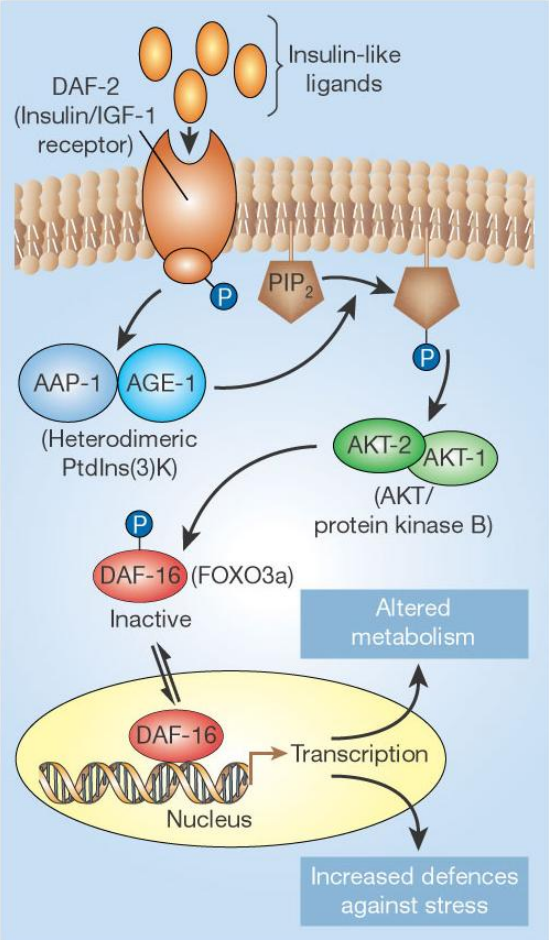

Figure 1: IIS pathway (Nemoto and Finkel, 2004). Insulin-like ligands mediate cellular responses such as metabolism and defenses against stress via a signaling pathway.

DAF-2 is a type of receptor tyrosine kinases and is the only homolog in *C. elegans* of the mammalian insulin/IGF receptor family. In its activated state in *C. elegans*, DAF-2 promotes several processes including reproductive growth. If this receptor is not activated, it leads to developmental arrest in the dauer stage. Some of the downstream molecules of DAF-2 include AGE-1, PIP_2, AKT-1/AKT-2, and DAF-16 (Figure 1). This pathway seen in *C. elegans* is also

conserved in mammals, with homologs for each of the mentioned molecules (Pierce et al., 2000). The human homologs of DAF-16 are FKHR and AFX, which may be misregulated in some forms of diabetes (Nelson et al., 2003). DAF-16 is responsible for the up-regulation of transcription for genes that encode for heat shock proteins and antioxidants. There was a study that showed that *daf-2* mutants live twice as long as wild-type *C. elegans*, showing that the IIS pathway is also connected to longevity (Kenyon et al., 1993).

Caenorhabditis elegans

C. elegans is a small nematode that serves as a model organism for biological research including genomics, cell biology, neuroscience, and aging. These animals have short life cycles, compact genomes, consistent development, large number of progeny, and genes that are easily manipulated (Altun and Hall, 2009). With only 959 somatic cells, *C. elegans* was the first multicellular eukaryote to have its genome completely sequenced, yielding 97 Mb of sequence encoding approximately 19,000 genes (Kamath and Ahringer, 2003). A notable feature of this organism is its transparency (Figure 2). Their tissues and organs can be seen under a microscope, which is another reason why they are a good organism to study. Gene expression can be examined using RNA interference (RNAi) or genetic mutations.

An easy method for looking at gene expression in *C. elegans* is by creating a transgenic strain with a GFP insert. Endogenous gene expression patterns can be mimicked by creating transgenic animals with a promoter of interest driving GFP expression. Therefore, when a transcription factor binds to a promoter, the GFP will fluoresce under ultraviolet light. One method of creating these transgenic strands is through the use of microparticle bombardment (Pratis et al., 2001).

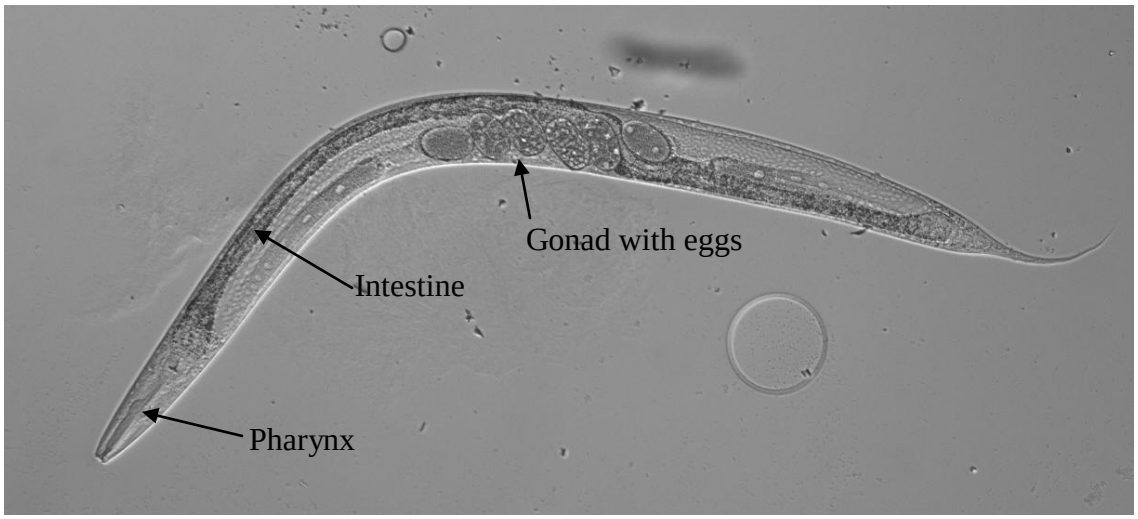

Figure 2: Adult *C. elegans* with eggs. The organs of the worm can be seen, as well as any eggs when the worm is in the adult stage. The anterior of the worm is on the left and the posterior is to the right. Some noticeable features are its pharynx, intestine, and gonad.

RNAi

Studying the genetics of an organism can be achieved through two different approaches. The first type of approach is to randomly mutagenize an organism, looking for a desired phenotype. Once the organism displays this phenotype, one can go back and try to identify the gene. This process is known as forward genetics, or classical genetics. Alternatively, reverse genetics starts with a gene of interest, and once mutated, its phenotype can be studied (Griffiths et al., 2008). Forward genetics may work with organisms with small genomes, but larger eukaryotic genomes make screening and mapping every mutable gene unrealistic. With the complete genome of *C. elegans* sequenced, the use of reverse genetics is possible with the use of RNAi. This technique results in protein knock-down typically by interfering with translation (Kamath and Ahringer, 2003).

Methods for RNAi screening

There are three different methods of introducing RNAi into *C. elegans*. As Fire et al. describe, the first studies that demonstrated the effectiveness of RNAi used injection of dsRNA

into the head or tail of the animal. This produced significant interference throughout the body and germ line, showing the ability of RNAi to cross cellular boundaries (Fire et al., 1998). Another study showed that soaking the worms in dsRNA from the maternal *pos-1* gene for 24 hours resulted in the distinct embryonic lethal phenotype in 86% of the F1 progeny (Tabara et al., 1998). The final method is through feeding. The worms are fed *E. coli* transformed with a vector containing cDNA of the desired gene to knock down. Also in the vector are T7 promoter sites flanking each side of the multiple cloning site (Kamath and Ahringer, 2003, Timmons et al., 2001). The T7 promoters transcribe the sense and antisense strands to produce dsRNA. The feeding method is the most effective for large-scale RNAi screening since the bacterial libraries of these RNAi strains exist.

RNAi through feeding not only requires less laborious efforts, but it also allows many genes to be tested on a large population of worms. Another advantage to feeding is that it is cheaper than soaking or microinjections. This method requires that each bacterial strain to be created contains the correct DNA insert. Each gene to be tested must have the DNA cloned into a special plasmid vector, which then needs to be inserted into a particular bacterial strain (Kamath and Ahringer, 2003). This project will utilize the transcription factor RNAi library created by the Walhout laboratory at UMass.

RNAi mechanism

RNAi is a process of post-transcriptional gene silencing (PTGS) that has been conserved throughout evolution. Fire et al. determined that dsRNA induced gene silencing when injected into *C. elegans* was substantially more effective than either the single sense or antisense strands (1998). They also found that only the exons of the gene affect the phenotypic change. Figure 3 is a guide from Lima et al. to explain the process of RNAi. The RNAi library should produce

exact small interfering RNA (siRNA) for each transcription factor, inducing the cleavage pathway as shown, rather than translational repression.

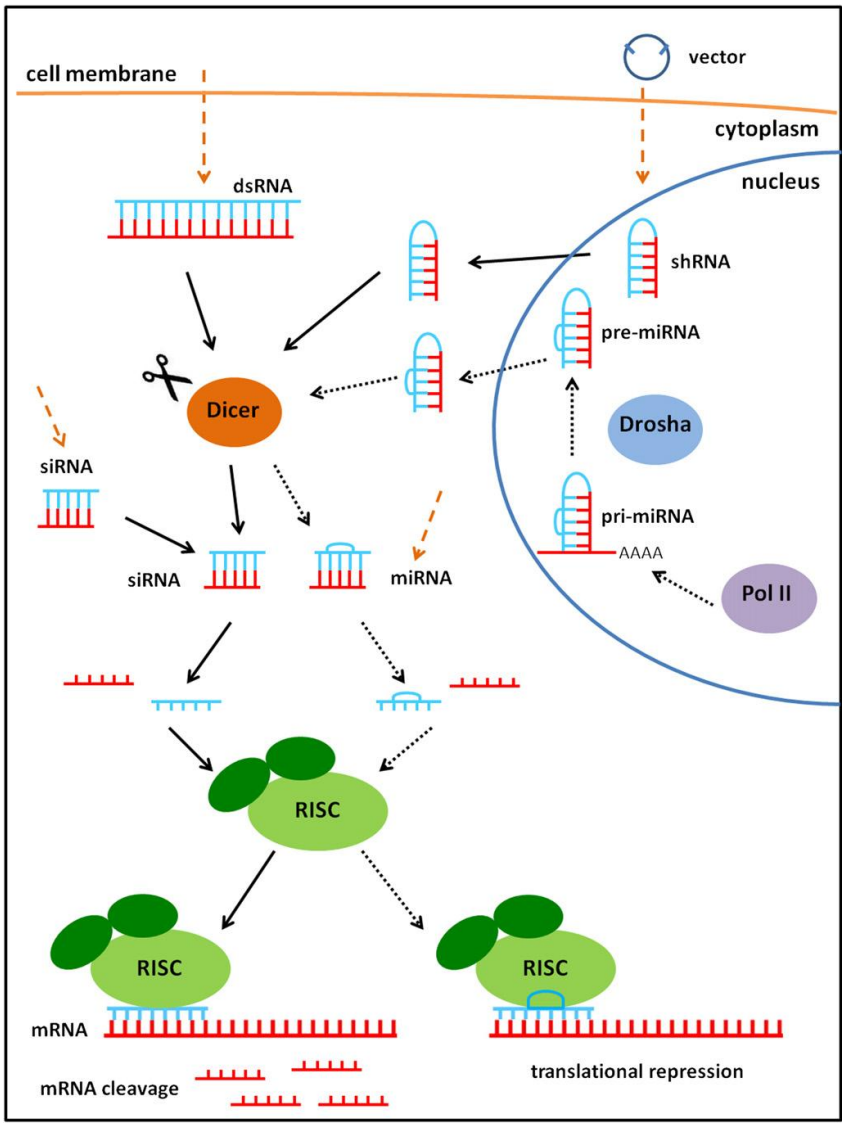

Figure 3: Mechanism of RNAi (Lima et al., 2013). One means of RNAi is when dsRNA enters the cell through the membrane via endogenous, transgenic, or viral trancripts. It is then processed into siRNA approximately 21-22 bp in length by a Ribonuclease III enzyme. This enzyme, referred to as Dicer, recognizes and cleaves dsRNA at specific positions or sequences (Lima et al., 2013). The siRNA molecules are now able to be incorporated into a multi-subunit RNA-induced silencing complex (RISC), which targets the specific RNA transcript for degradation or repression (Shuey et al., 2002). RISC contains a helicase domain comprised of Argonaute, which allows it to separate the siRNA into its sense and antisense strands. The antisense strand serves as a template for degradation, where RNA hydrolysis occurs within the region of homology with the mRNA (Shuey et al., 2002; Lima et al., 2013).

Goals

The overall goal of the project was to understand the regulation of *ins-4* and *ins-27*. The first step towards the goal was to set up the high throughput method of testing the entire transcription factor RNAi library. The next step was to use the system to identify transcription factors regulating *ins-4* and *ins-27* using transgenes containing a GFP reporter. These goals were attempted using the transcription factor RNAi library as mentioned in Watson et al. (2013).

Materials and Methods

The protocol for setting up the RNAi library for screening was the same as that used by Watson et al. (2013).

Strain maintenance

The strains were maintained using methods as described by Brenner (1974). Worms were fed *Escherichia coli* (OP50) and grown on NGM-agar plates. They were kept at a temperature of 20°C or 15°C, until eggs could be harvested. When the plates became starved, worms were transferred to new plates by cutting a chunk from the starved plate and laying it face-down on the new plate.

Egg isolation protocol

To isolate the eggs, worms and any laid eggs were washed from six 6 cm nearly starved plates with M9 buffer and collected in a 15ml conical tube. The tubes were centrifuged at 2000rpm for 2 minutes and the supernatant was removed. To digest the worms, 4ml of M9, 500µl of 5M NaOH, and 500µl of bleach were added to the tubes. They were rocked gently, and were centrifuged at 2000rpm for 2 minutes when the carcasses were mostly dissolved, usually

after 5-10 minutes of bleaching. This was followed by a series of four washes: 6ml of M9 was added to the tubes, and they were centrifuged at 2000rpm for 2 minutes, removing the supernatant after each wash.

Preparing the 96-well plates

The 96-well plates were poured using NGM-agar enriched with 0.5µM IPTG. A repeat multi-channel pipette was used to dispense 200µl of media into each well. Plates were cooled slowly to prevent cracking, and were stored at 4°C once the agar solidified (Watson et al., 2013).

Bacterial cultures

Bacterial RNAi cultures were grown from frozen stocks stored at -80°C. The whole transcription factor library is contained in 12 96-well blocks. An overnight culture was prepared in a 96-well block with 1ml of LB with 100µg/ml ampicillin (AMP) in each well. To transfer bacteria, pipette tips were used to scrape bacteria from the frozen stock and mix it into the new 96-well block with a multi-channel pipette. The overnight culture was stored at 37°C.

Then, 50µl of the overnight culture was inoculated in a new 96-well block containing 1ml LB with 100µg/ml AMP for 6 hours or until it reached an optical density of around 1. The cells were then centrifuged at 3000rpm for 15 minutes. The supernatant was removed, and the bacteria were suspended in 100µl of 1x M9 (Watson et al., 2013).

Setting up the RNAi plates

Using a multi-channel pipette, 10µl of the concentrated bacterial cultures was added to the appropriate well of the 96-well agar plates. Precautions were used to not touch the surface of the agar, since that could cause the worms to burrow. The plates were dried in the hood for around 15 minutes or until dry.

The eggs, prepared using the Egg isolation protocolEgg isolation protocol, were added a day after bacteria was seeded onto the plates. The eggs were suspended in M9 at about 20 eggs/10µl of buffer. 10µl of suspended eggs was added to each well using a multi-channel pipette and dried in the hood (Watson et al., 2013).

Screening the plates

Once the worms reached the young adult stage, expression of the GFP transgene could be examined. A chart was used to visually score the GFP expression patterns. Each well was given a numerical value ranging from 0 to 3. A score of 3 indicated wild-type expression, a score of 2 indicated a slight loss of expression, a score of 1 indicated a major loss of expression, and a score of 0 indicated complete loss of GFP expression. It was also possible to score a ND (not discernible) when the well could not be given an accurate score. The final column of the plates alternated between a negative control and a positive control. The negative control was an empty vector and the positive control contained RNAi specific to GFP.

Results

In order to determine potential regulators of *ins-4* and *ins-27* expression, RNAi was used to knockdown individual transcription factors in *C. elegans*. The 96-well set up allowed for quick screening under a dissecting scope. After scoring each well in triplicate with a numerical value from 0 to 3, the expression patterns were averaged to determine the highest knockdowns for each gene. Once a list was compiled of the total RNAi scores, the top candidate genes were further examined.

ins-4

Before beginning the RNAi screening for *ins-4*, the wild-type expression pattern needed to be recorded. When grown at 15°C on OP50 bacteria, expression could be seen in the head neurons and in the posterior end of the intestine (Figure 4). At different temperatures, throughout development, and under starvation, no changes in the tissue of expression were seen or major changes in GFP intensities.

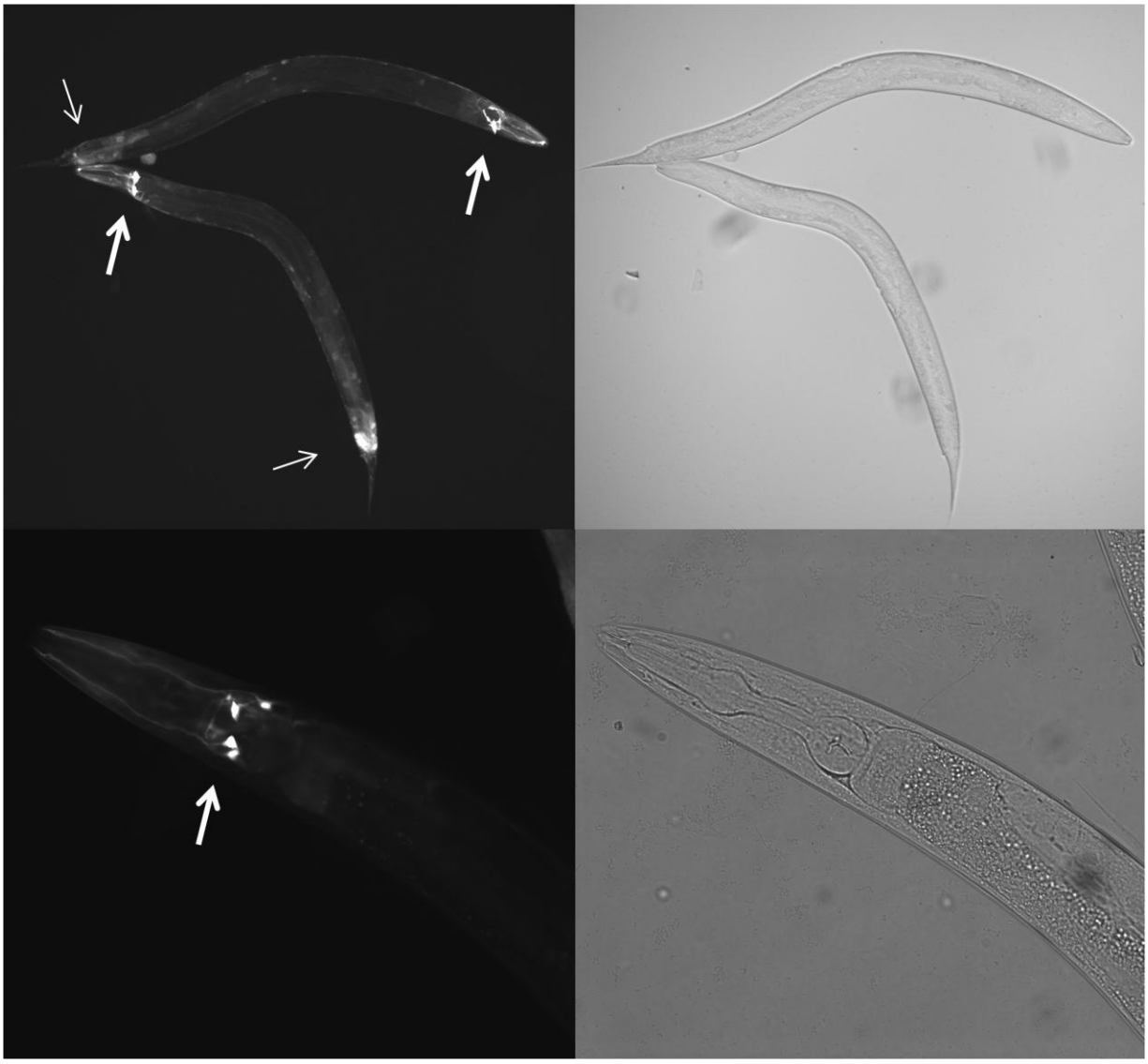

Figure 4: Wild-type expression pattern for *ins-4*. The bold arrows depict the head neurons and the thin arrows depict the expression in the intestine.

After a screen, it was apparent that RNAi did not affect the genes in the neurons. This meant that the scoring for *ins-4* expression would be based on the expression in the intestine. It was difficult to clearly see noticeable differences for RNAi knockdown in the intestine since the wild-type expression was already weak. This made accurately scoring each RNAi nearly impossible given the number of transcription factors that needed to be examined with the given time constraints. Thus, no further work was performed on *ins-4* expression.

ins-27

The expression pattern of *ins-27* when grown on OP50 bacteria at 15°C was visible in the body wall muscle (Figure 5). There were no major changes in expression as a result of starvation effects or when grown at different temperatures.

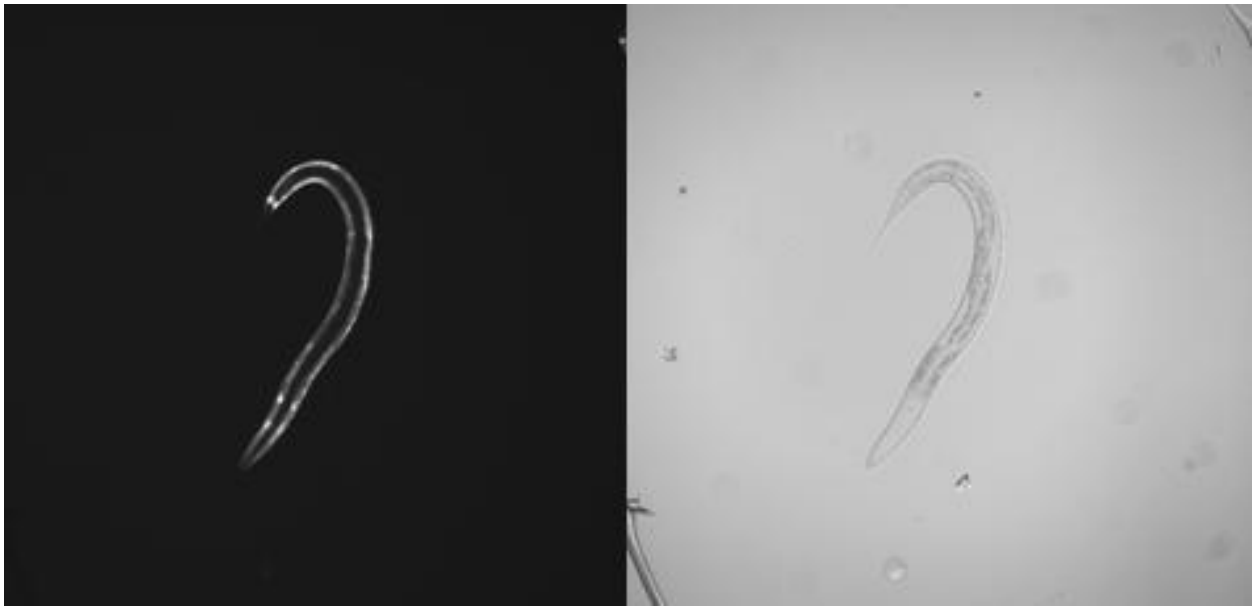

Figure 5: Wild-type expression for *ins-27*. This gene is expressed in the body wall muscle, and increases in intensity during development.

RNAi knockdown is effective in the muscle, and because this gene showed bright expression, the changes were able to be quantified by the scoring range described in the

Materials and Methods. Each well was scored in triplicate and three of the twelve plates from the RNAi library were tested. An example of RNAi is shown below (Figure 6). Since panel C shows wild-type expression, it would be assigned a score of 3. Panel D showed a major decrease in expression in two worms and very little expression in the leftmost worm. This resulted in a score of 1. The positioning of the worms was taken into account. Worms lying on their back, such as in panel B would show expression patterns that were brighter than if they were on their sides, such as those in panel D.

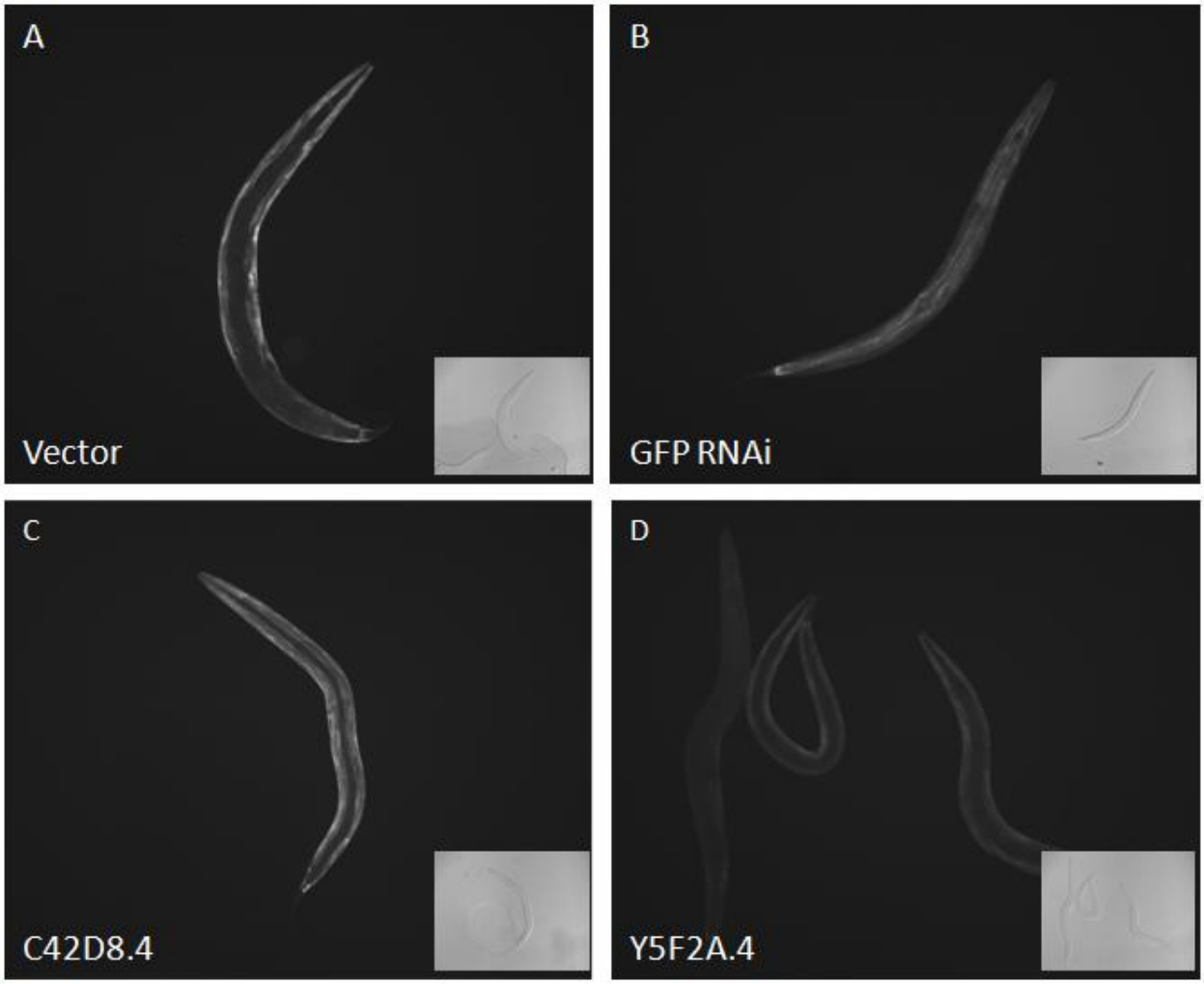

Figure 6: GFP knockdown examples from the RNAi plates. Wild-type GFP expressed in the muscle is seen in A and C, and a loss of GFP is seen in B and D. A and B were the respective negative and positive controls, and C and D are genes labeled by their open reading frame.

Once the averages for each gene were calculated from the three scores, the data were plotted to determine the ranges of expression patterns (Figure 7). It is shown that most of the genes displayed nearly wild-type expression patterns. Based on the spread of this data, candidate genes were considered if they scored an average, calculated from at least two wells, below 2.00.

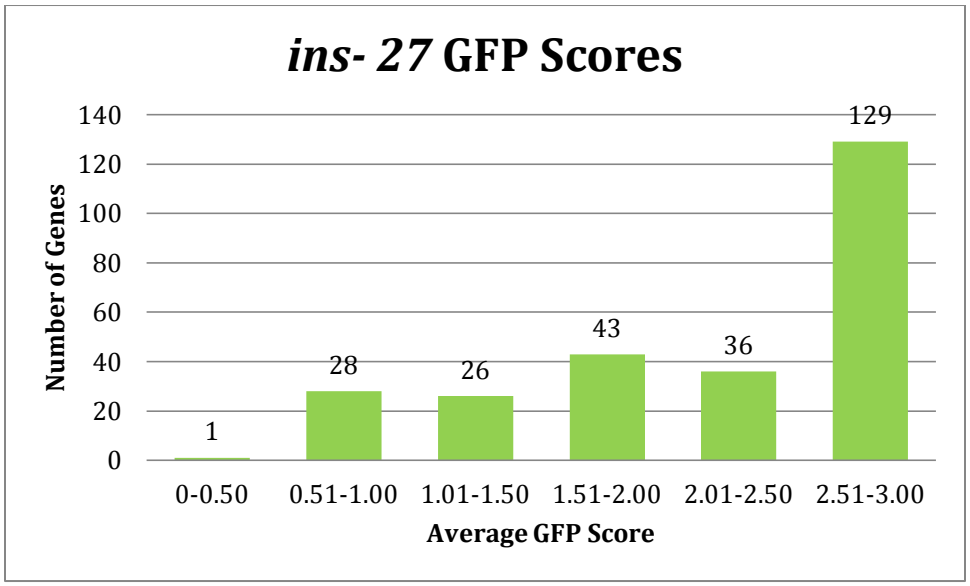

Figure 7: Average GFP scores for the 262 genes tested. Scored 262 genes, 65 (25%) transcription factors influenced *ins-27* expression.

Characterization of transcription factors

Once the data from the RNAi screens for *ins-27* were collected, the transcription factors were further examined. The first step in learning more about the transcription factors scoring below a 2.00 was to compare them to known transcription factor regulators of another gene. The *acdh-1* gene is expressed in the intestine and already had its transcription factors studied using the same RNAi approach (MacNeil et al., 2013). From this comparison, thirteen transcription factors were found to activate both genes (Figure 8). This result suggests that these transcription factors are most likely general transcription factors, since they are active in both muscle and intestine.

Figure 8: TF comparison between ins-27 and acdh-1. The *ins-27* TFs with expression patterns below 2.0 were compared to tested transcription factors of *acdh-1*, expressed in the intestine.

The general overlap between the two genes suggests that these potential candidate transcription factors are general transcription factors, but looking into each gene's assigned Gene Ontology (GO) provided greater insight into the function of the transcription factors. GO terms are assigned descriptions for each gene. Generalizing GO terms allowed the clear distinction that most of these genes have been labeled as general transcription factors (Figure 9). The overlapping transcription factors have all been assigned GO terms indicating that they act as general transcription factors. This supports the hypothesis that they are potentially general transcription factors in the worm.

A

Gene Name	GO Terms	Ave Score
sptf-3	D/A, Re, GT, CP, R	0.50
C27A12.2	D/A, GT, R	0.67
F37D6.2	GT, R	0.67
nhr-74	GT, R	0.67
tbx-34	GT, R	0.67
zip-8	Unknown	0.67
ZK337.2	GT, R	0.67
atg-4.1	GT, R	1.00
ceh-22	D/A, GT, CP, R	1.00
dnj-11	D/A, GT, CP	1.00
dpl-1	D/A, Re, GT, CP, R	1.00
egl-13	D/A, Re, GT, CP, R, O	1.00
ets-5	GT	1.00
hlh-8	Re, GT, R	1.00
nhr-181	GT, CP, R	1.00
nhr-80	D/A, Re, GT, R	1.00
tab-1	GT, R	1.00

B

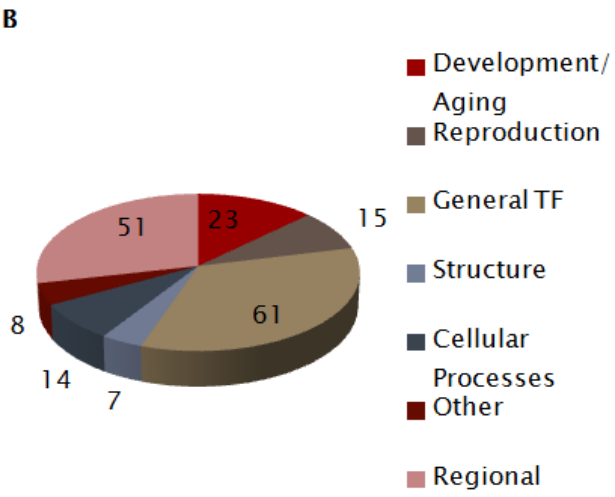

Figure 9: Generalized GO terms from the 65 candidate TFs. (A) The top 17 RNAi TFs with their GO terms and average GFP scores. D/A- Development/Aging, Re- Reproduction, GT- General Transcription Factor, S- Structure, CP- Cellular Processes, O- Other, R- Regional. (B) All but 1 gene had multiple GO terms, and most fell into the general TF category. Some TF are involved with development and aging, processes in the IIS pathway.

Although 94% of the transcription factors were labeled as general transcription factors, twenty-three are seen to be involved in processes such as development and aging. Since these processes are also known to be influenced by the IIS pathway, it can indicate there may be some more specific functions of the transcription factors. There wasn't an apparent pattern seen between the GO terms and the RNAi knockdown scores. Just as the GO terms seen amongst the top seventeen transcription factors vary, this is the same throughout the candidate transcription factors. The development and aging GO terms, for example, are assigned to genes in the top seventeen as well as genes that scored 1.67.

The top 65 transcription factors expression profiles were also examined. Each gene was searched on WormBase to determine the tissue that they are expressed in. Several have been indentified to be produced in the muscle. Nearly half of the transcription factors didn't have

their expression fully annotated, so a complete analysis of the tissues that these genes are expressed in cannot be completed.

Discussion

In order to learn more about the IIS pathway in *C. elegans*, the transcription factors of one insulin-like gene were examined. The worms used have promoter driven GFP for the *ins-27* gene, so when a transcription factor binds to the promoter to stimulate transcription, they will fluoresce under UV light. A library exists that contains RNAi specific to each transcription factor in the worm. By testing each transcription factor and scoring GFP knockdown, potential regulators of *ins-27* were identified.

After screening 262 transcription factors, 65 have been scored below a 2.00, indicating that they have a significant loss of GFP expression. Of these, nearly all have been identified as general transcription factors by GO terms. Even when the activators of *ins-27* and *acdh-1* were compared, there were thirteen in common, further suggesting that these genes are most likely general transcription factors.

Utilizing RNAi knockdown of genes doesn't necessarily show that these transcription factors are directly activating *ins-27*. It is possible that some of these transcription factors could be involved in a pathway upstream of *ins-27* direct activation. Epistasis tests can determine the order of genes in a pathway. In epistasis with a double mutation, the gene upstream in the pathway will mask the phenotype of the downstream gene. This test will not yield results if the two genes are in separate pathways.

Future recommendations

After attempting to screen the *ins-4* worms for potential transcription factors that regulate expression, it was clear that it would be extremely difficult to score. RNAi knockdown isn't effective in neurons, and the weak expression in the intestine cannot yield accurate scores. One possible method to achieve knockdown in the neurons is to cross the marker into a sensitized strain, such as *rrf-3* or other strains expressing SID-1 in particular neurons (Calixton et al., 2010; Asikainen et al., 2005).

Since all the plates weren't able to be tested from the RNAi library, the first step is to complete the scoring for the remaining plates of the RNAi library for *ins-27*. Once this is completed, random retesting of the transcription factors using RNAi as followed in this experiment should be implemented to eliminate any false positives. The next step is to sequence the bacteria containing the cDNA of the candidate transcription factors. This is to verify that they contain the correct open reading frames for the correct gene knockdowns. The final step is to use RT-PCR to quantify the level of *ins-27* expression to validate GFP observations.

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Appendix

Appendix A: Scoring for *ins-27*

Plate Number	TF ORF	Gene Name	Score 1	Score 2	Score 3	Ave
4-A01	Y40B1A.4	sptf-3	ND	0	1	0.50
1-A11	ZK337.2		0	1	1	0.67
1-B01	F23F12.9	zip-8	1	0	1	0.67
1-H04	C27C7.3	nhr-74	0	1	1	0.67
2-A07	C27A12.2		0	1	1	0.67
2-A08	F37D6.2		0	1	1	0.67
2-A09	Y47D3A.10	tbx-34	1	0	1	0.67
4-A07	Y52B11A.9		1	ND	1	1.00
4-A11	Y87G2A.3	atg-4.1	0	1	2	1.00
4-D01	M02H5.1	nhr-99	1	1	ND	1.00
4-F01	F38A5.13	dnj-11	1	1	ND	1.00
4-F10	Y56A3A.18		1	0	2	1.00
4-F11	Y53G8AR.9		2	0	1	1.00
4-G01	F29F11.5	ceh-22	1	1	ND	1.00
4-G02	F56D1.1		1	ND	1	1.00
4-H10	Y17G7B.20		1	1	1	1.00
4-H11	T01B10.4	nhr-14	1	ND	1	1.00
1-A10	C02B8.4	hlh-8	1	1	1	1.00
1-B05	T22B7.1	egl-13	0	1	2	1.00
1-F01	T23G7.1	dpl-1	1	1	1	1.00
1-H08	H10E21.3	nhr-80	1	1	1	1.00
1-H10	M03D4.4		1	1	1	1.00
2-A01	C34C6.6	prx-5	1	1	ND	1.00
2-B01	F31E8.3	tab-1	1	1	1	1.00
2-H01	C03G6.8	nhr-147	1	ND	ND	1.00
2-H05	F38H12.3	nhr-181	1	1	1	1.00
2-H06	T27B7.1	nhr-59	ND	1	ND	1.00
2-H10	F57A8.5	nhr-192	1	ND	1	1.00
2-H11	C42D8.4	ets-5	1	ND	1	1.00
4-G04	F16B4.12	nhr-117	1	0	3	1.17
4-A10	Y74C9A.4	rcor-1	1	2	1	1.33
4-C01	C25G4.4	tag-347	1	2	1	1.33
4-G07	F44E7.8	nhr-142	2	1	1	1.33
4-H08	K06B4.5	nhr-196	1	1	2	1.33

1-D01	C16A3.4		1	1	2	1.33
1-E01	W03C9.4	lin-29	1	1	2	1.33
1-G02	F32A11.6	moe-3	1	1	2	1.33
1-H02	W10D9.4	nfyb-1	2	1	1	1.33
1-H03	C54C8.1	nhr-169	2	1	1	1.33
1-H05	C17D12.1	dhhc-7	2	1	1	1.33
1-H06	ZK418.1	nhr-9	2	1	1	1.33
1-H07	F43C1.4	nhr-20	2	1	1	1.33
1-H11	T08H4.3	ast-1	2	1	1	1.33
2-A03	T26A5.8		2	1	1	1.33
2-C01	Y80D3A.3	dlx-1	1	1	2	1.33
2-E01	F15C11.1	sem-4	1	1	2	1.33
2-F01	C24A1.2		1	2	1	1.33
2-G01	K06B4.11	nhr-53	1	2	1	1.33
2-H07	R11G11.1	nhr-132	1	2	1	1.33
4-A08	Y54E10BR.8	ztf-3	2	ND	1	1.50
4-B11	ZK616.10	ehn-3	2	ND	1	1.50
1-G01	T23F11.4		1	ND	2	1.50
1-H01	F52F12.4	lsl-1	ND	2	1	1.50
2-H02	F47C10.3	nhr-186	ND	1	2	1.50
2-H03	ZK6.4	nhr-253	ND	1	2	1.50
4-D10	C56E10.4	nhr-137	2	1	2	1.67
4-G03	Y116A8C.19		1	2	2	1.67
4-G05	F42G4.3	zyx-1	2	1	2	1.67
4-G06	F36F12.8	ztf-28	2	1	2	1.67
4-G08	Y37E11B.1		2	2	1	1.67
1-F09	C27A12.5	ceh-2	0	2	3	1.67
1-G10	Y48C3A.17	efl-2	2	2	1	1.67
2-A02	C28H8.9		3	1	1	1.67
2-A04	R05D3.3		2	1	2	1.67
2-A06	Y5F2A.4		2	1	2	1.67
2-A10	Y75B8A.1	php-3	2	2	1	1.67
2-A11	F26C11.2	unc-4	2	2	1	1.67
2-D01	F38A6.1	pha-4	2	2	1	1.67
2-H09	T27B7.3	nhr-226	1	2	2	1.67
4-A02	F59C6.2		2	ND	2	2.00
4-A05	T07D10.3		2	2	2	2.00
4-D11	C47C12.3	ref-2	3	1	2	2.00
4-E01	F09B12.2	dhhc-1	2	2	ND	2.00
4-E11	T10B11.3	ztf-4	2	2	2	2.00

4-F09	T23G5.6		3	1	2	2.00
4-G10	T27C4.4	lin-40	2	2	2	2.00
4-G11	C01F6.9		2	2	2	2.00
4-H01	C33G8.9	nhr-140		2	ND	2.00
4-H02	K12H6.12		2	2	ND	2.00
4-H03	C29F7.4	fkh-3	ND	ND	2	2.00
4-H04	ZK1128.6	ttll-4	ND	2	2	2.00
4-H05	C28G1.4		2	ND	2	2.00
4-H06	T10B5.10		2	ND	ND	2.00
4-H07	ZK652.6		2	ND	ND	2.00
4-H09	R07B7.15	nhr-208	2	ND	2	2.00
1-A01	C25A1.11	aha-1	2	ND	2	2.00
1-A04	Y116A8C.20		2	ND	ND	2.00
1-A07	C04G2.7	egl-38	ND	3	1	2.00
1-C10	F38C2.5	ccch-2	1	2	3	2.00
1-D11	K08B4.1	lag-1	2	3	1	2.00
1-E09	F47D12.4	hmg-1.2	2	2	2	2.00
1-F08	F13G3.1	ztf-2	2	2	ND	2.00
1-G03	F10C1.5	dmd-5	2	2	2	2.00
1-G06	B0280.4	odd-1	2	1	3	2.00
1-G11	E02H1.7	nhr-19	2	2	2	2.00
1-H09	F40H6.4	tbx-11	2	ND	ND	2.00
2-G11	C38C3.9	nhr-260	1	3	ND	2.00
2-H08	C49A1.4	eya-1	ND	2	2	2.00
4-A04	Y47H9C.2		3	2	2	2.33
4-A09	F27D4.4		2	2	3	2.33
4-B10	F55B11.4		2	2	3	2.33
4-D02	M02H5.7	nhr-123	3	2	2	2.33
4-D06	ZC376.7		2	3	2	2.33
4-E02	C43H6.7		3	2	2	2.33
4-E06	F23C8.4	ubxn-1	3	2	2	2.33
4-F06	T11G6.8		3	2	2	2.33
4-G09	F42G4.3	zyx-1	3	2	2	2.33
1-A02	C33D3.1	elt-2	2	3	2	2.33
1-A03	F35H8.3	zfp-2	3	3	1	2.33
1-A06	T08D10.1	nfya-1	2	3	2	2.33
1-A08	F44A6.2	sex-1	2	3	2	2.33
1-C01	F56E3.4	fax-1	3	2	2	2.33
1-D03	C36F7.1	irx-1	3	2	2	2.33
1-D07	F55B12.1	ceh-24	1	3	3	2.33

1-E11	F21D12.1	nhr-21	3	3	1	2.33
1-F02	T15H9.3	hlh-6	3	1	3	2.33
1-F03	C35D6.4		2	2	3	2.33
1-F10	C47F8.8	nhr-81	2	2	3	2.33
1-G04	F22D3.1	ceh-38	2	3	2	2.33
1-G05	F45H11.1	sptf-1	2	2	3	2.33
1-G08	T23H4.2	nhr-69	2	2	3	2.33
1-G09	B0336.3		2	2	3	2.33
2-A05	T05G5.2	hlh-4	3	2	2	2.33
2-B11	C09G9.7		3	2	2	2.33
2-G07	F47C10.4	nhr-187	2	3	2	2.33
4-A03	T22E7.2		3	ND	2	2.50
4-A06	C47F8.2	nhr-165	3	2	ND	2.50
4-B01	T05G5.6	ech-6	3	ND	2	2.50
4-B06	F12E12.5	sdz-12	3	ND	2	2.50
4-F03	F40G9.11	mxl-2	2	3	ND	2.50
4-F04	K01G5.1	rnf-113	2	3	ND	2.50
1-A05	T22C8.5	sptf-2	3	2	ND	2.50
1-D02	F28C6.1		2	ND	3	2.50
1-E02	Y75B8A.2	nob-1	2	ND	3	2.50
4-B02	B0261.1		3	3	2	2.67
4-B03	F45C12.2		3	3	2	2.67
4-B07	F37H8.1	lir-3	3	3	2	2.67
4-B08	W01D2.2	nhr-61	3	3	2	2.67
4-C02	Y42H9AR.3	rabs-5	3	3	2	2.67
4-C06	Y116A8C.22		3	3	2	2.67
4-C07	Y55F3AM.6		3	3	2	2.67
4-C08	R02D3.7		3	3	2	2.67
4-C09	C29E6.5	nhr-43	3	3	2	2.67
4-C10	W06H12.1	ztf-6	3	3	2	2.67
4-C11	M04B2.1	mep-1	3	3	2	2.67
4-D03	Y105E8A.17	ekl-4	3	3	2	2.67
4-D04	W05B10.2		3	3	2	2.67
4-D05	F17A9.6	ceh-49	3	3	2	2.67
4-D07	Y113G7B.23	psa-1	3	3	2	2.67
4-D09	F31A3.4	hlh-29	3	3	2	2.67
4-E03	K09A11.1		3	3	2	2.67
4-E04	T05A7.4	hmg-11	3	3	2	2.67
4-E05	Y17G7A.1	hmg-12	3	3	2	2.67
4-E07	W02A2.7	mex-5	3	3	2	2.67

4-E08	F09G2.9	attf-2	3	3	2	2.67
4-E09	D1046.2		3	3	2	2.67
4-E10	T07G12.11	zim-3	3	3	2	2.67
4-F02	T10D4.6		3	2	3	2.67
4-F05	W05H7.4	zfp-3	3	3	2	2.67
4-F07	F54F2.9		3	3	2	2.67
4-F08	Y75B8A.29		3	3	2	2.67
1-B03	K02B9.4	elt-3	3	2	3	2.67
1-B04	C10A4.8	mnm-2	2	3	3	2.67
1-B09	F52E1.1	pos-1	2	3	3	2.67
1-C06	H01A20.1	nhr-3	3	3	2	2.67
1-C07	H14A12.4	mls-1	2	3	3	2.67
1-C09	F43G9.11	ces-1	2	3	3	2.67
1-D04	T13C5.4		3	2	3	2.67
1-D06	F32B6.1	nhr-4	2	3	3	2.67
1-E03	R03E9.1	mdl-1	3	2	3	2.67
1-E04	F18A1.4	lir-2	3	2	3	2.67
1-E07	R74.3	xbp-1	2	3	3	2.67
1-E08	Y41C4A.4	crh-1	3	2	3	2.67
1-F07	F40H3.4	fkh-8	3	2	3	2.67
1-F11	F58A4.7	hlh-11	3	3	2	2.67
1-G07	B0280.8	nhr-10	2	3	3	2.67
2-B02	F21D12.5		3	3	2	2.67
2-B03	C06G3.1	nhr-105	2	3	3	2.67
2-B04	C08F8.8	nhr-67	2	3	3	2.67
2-B05	Y37D8A.11		3	3	2	2.67
2-B07	F45B8.4	pag-3	3	2	3	2.67
2-D09	C07H6.7	lin-39	2	3	3	2.67
2-D10	Y94H6A.1	nhr-277	3	2	3	2.67
2-D11	Y55F3AM.14		3	3	2	2.67
2-E06	Y57G11C.25		2	3	3	2.67
2-E08	ZK370.2	sma-2	3	3	2	2.67
2-E10	W02D7.6		3	2	3	2.67
2-F10	F35E8.12	nhr-108	3	2	3	2.67
2-F11	C33G8.8	nhr-139	3	3	2	2.67
2-G04	C34H3.2	odd-2	3	3	2	2.67
2-G09	F47C10.7	nhr-188	3	3	2	2.67
4-B04	Y38E10A.18	nhr-234	3	3	ND	3.00
4-B05	Y48C3A.4	ztf-22	3	3	3	3.00
4-B09	Y51H1A.6	mcd-1	3	3	3	3.00

4-C03	F25H8.6	zbed-6	3	3	3	3.00
4-C04	Y51H4A.17	sta-1	3	3	3	3.00
4-C05	R13.4	miz-1	3	3	3	3.00
4-D08	T27A8.2		3	3	3	3.00
1-A09	R07B1.1	vab-15	ND	3	ND	3.00
1-B02	F34D10.5	lin-48	3	3	3	3.00
1-B06	F18A1.3	lir-1	3	3	3	3.00
1-B07	F54A5.1		3	3	3	3.00
1-B08	C24G6.4	nhr-47	3	3	3	3.00
1-B10	C01H6.5	nhr-23	3	3	3	3.00
1-B11	W09C2.1	elt-1	3	3	3	3.00
1-C02	T19B10.11	mxl-1	3	3	3	3.00
1-C03	W02D9.3		3	3	3	3.00
1-C04	T27A1.6	mab-9	3	3	3	3.00
1-C05	T22C8.3		3	3	3	3.00
1-C08	ZC204.2		3	3	3	3.00
1-C11	F54D1.4	nhr-7	3	3	3	3.00
1-D05	Y48B6A.14	hmg-1.1	3	3	3	3.00
1-D08	B0035.1		3	3	3	3.00
1-D09	K10G6.1	lin-31	3	3	3	3.00
1-D10	F33D11.4	ceh-12	3	3	3	3.00
1-E05	B0414.2	rnt-1	3	3	3	3.00
1-E06	Y75B8A.35	zip-1	3	3	3	3.00
1-E10	C41G7.5	ahr-1	3	3	3	3.00
1-F04	F41D3.1	nhr-82	3	3	3	3.00
1-F05	Y47D3A.6	tra-1	3	3	3	3.00
1-F06	F54F2.5	ztf-1	3	3	3	3.00
2-B06	C32F10.6	nhr-2	3	3	3	3.00
2-B08	F57G8.6	nhr-193	3	3	3	3.00
2-B09	B0286.5	fkh-6	3	3	ND	3.00
2-B10	ZK909.4	ces-2	3	3	3	3.00
2-C02	F54H5.4	mua-1	3	3	3	3.00
2-C03	C49D10.2	nhr-166	3	3	3	3.00
2-C04	R11E3.6	eor-1	3	3	3	3.00
2-C05	Y53C12B.5	mab-3	3	ND	3	3.00
2-C06	C17E7.1	nhr-156	3	3	3	3.00
2-C07	F58E10.5	end-3	3	3	3	3.00
2-C08	B0304.1	hlh-1	3	3	3	3.00
2-C09	Y73F8A.16	tbx-39	3	3	3	3.00
2-C10	R04B5.3	nhr-205	3	3	3	3.00

2-C11	F10B5.3		3	3	3	3.00
2-D02	Y51H4A.4		3	3	3	3.00
2-D03	F20D12.6	ceh-19	3	3	3	3.00
2-D04	F38C2.7		3	3	3	3.00
2-D05	F58E10.2	end-1	3	3	3	3.00
2-D06	C06B8.1	nhr-150	3	3	3	3.00
2-D07	T06C12.7	nhr-84	3	3	3	3.00
2-D08	R12B2.1	sma-4	3	3	3	3.00
2-E02	C54F6.8	nhr-171	3	3	3	3.00
2-E03	F58G1.2		3	3	3	3.00
2-E04	ZK682.4	hlh-10	3	3	3	3.00
2-E05	Y52E8A.2		3	3	3	3.00
2-E07	C45E5.6	nhr-46	3	3	3	3.00
2-E09	F19F10.1		3	3	3	3.00
2-E11	Y116A8C.17	dct-13	3	3	3	3.00
2-F02	F44E2.7		3	3	3	3.00
2-F03	C03G6.10	nhr-148	3	3	3	3.00
2-F04	T13F3.3	nhr-127	3	3	3	3.00
2-F05	F45E4.9	hmg-5	3	3	3	3.00
2-F06	ZC64.3	ceh-18	3	3	3	3.00
2-F07	T03E6.3	nhr-271	3	3	3	3.00
2-F08	C33G8.12	nhr-163	3	3	3	3.00
2-F09	F44G3.9	nhr-111	3	3	3	3.00
2-G02	K06B4.2	nhr-52	3	3	3	3.00
2-G03	T20F10.2		3	3	3	3.00
2-G05	F44C8.11	nhr-96	3	3	3	3.00
2-G06	F44C8.4	nhr-103	3	3	3	3.00
2-G08	C33G8.10	nhr-162	3	3	3	3.00
2-G10	F36D3.2	nhr-54	3	3	3	3.00

Appendix B: GO terms for each gene scoring below a 2.00

Gene WB ID	Gene Public Name	GO Term ID	Term
WBGene00020368	ast-1	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00020368	ast-1	GO:0040007	growth
WBGene00020368	ast-1	GO:0002119	nematode larval development
WBGene00020368	ast-1	GO:0018991	oviposition
WBGene00020368	ast-1	GO:0045944	positive regulation of transcription from RNA

			polymerase II promoter
WBGene00020368	ast-1	GO:0006355	regulation of transcription, DNA-dependent
WBGene00020368	ast-1	GO:0043565	sequence-specific DNA binding
WBGene00020368	ast-1	GO:0003700	transcription factor activity
WBGene00020368	ast-1	GO:0007411	axon guidance
WBGene00020368	ast-1	GO:0007413	axonal fasciculation
WBGene00020368	ast-1	GO:0071542	dopaminergic
WBGene00013595	atg-4.1	GO:0008270	zinc ion binding
WBGene00013595	atg-4.1	GO:0005622	intracellular
WBGene00015809	C16A3.4	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00015809	C16A3.4	GO:0003676	nucleic acid binding
WBGene00015809	C16A3.4	GO:0000003	reproduction
WBGene00015809	C16A3.4	GO:0040010	positive regulation of growth rate
WBGene00015809	C16A3.4	GO:0008270	zinc ion binding
WBGene00015809	C16A3.4	GO:0016246	RNA interference
WBGene00015809	C16A3.4	GO:0005622	intracellular
WBGene00016029	C24A1.2	GO:0006355	regulation of transcription, DNA-dependent
WBGene00016029	C24A1.2	GO:0043565	sequence-specific DNA binding
WBGene00016029	C24A1.2	GO:0003700	transcription factor activity
WBGene00016154	C27A12.2	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00016154	C27A12.2	GO:0003676	nucleic acid binding
WBGene00016154	C27A12.2	GO:0040010	positive regulation of growth rate
WBGene00016154	C27A12.2	GO:0008270	zinc ion binding
WBGene00016154	C27A12.2	GO:0005622	intracellular
WBGene00016200	C28H8.9	GO:0005515	protein binding
WBGene00016200	C28H8.9	GO:0008270	zinc ion binding
WBGene00016200	C28H8.9	GO:0005622	intracellular
WBGene00000445	ceh-22	GO:0043282	pharyngeal muscle development
WBGene00000445	ceh-22	GO:0003677	DNA binding
WBGene00000445	ceh-22	GO:0040010	positive regulation of growth rate
WBGene00000445	ceh-22	GO:0045944	positive regulation of transcription from RNA polymerase II promoter
WBGene00000445	ceh-22	GO:0045449	regulation of transcription
WBGene00000445	ceh-22	GO:0006355	regulation of transcription, DNA-dependent
WBGene00000445	ceh-22	GO:0043565	sequence-specific DNA binding
WBGene00000445	ceh-22	GO:0032569	specific transcription from RNA polymerase II promoter
WBGene00000445	ceh-22	GO:0003700	transcription factor activity
WBGene00000445	ceh-22	GO:0030528	transcription regulator activity
WBGene00000445	ceh-22	GO:0006898	receptor-mediated endocytosis
WBGene00000445	ceh-22	GO:0005634	nucleus
WBGene00001029	dnj-11	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00001029	dnj-11	GO:0003677	DNA binding
WBGene00001029	dnj-11	GO:0031072	heat shock protein binding
WBGene00001061	dpl-1	GO:0009792	embryonic development ending in birth or egg

			hatching
WBGene00001061	dpl-1	GO:0040035	hermaphrodite genitalia development
WBGene00001061	dpl-1	GO:0000003	reproduction
WBGene00001061	dpl-1	GO:0040027	negative regulation of vulval development
WBGene00001061	dpl-1	GO:0006355	regulation of transcription, DNA-dependent
WBGene00001061	dpl-1	GO:0003700	transcription factor activity
WBGene00001061	dpl-1	GO:0005667	transcription factor complex
WBGene00001061	dpl-1	GO:0016564	transcription repressor activity
WBGene00001061	dpl-1	GO:0040011	locomotion
WBGene00001061	dpl-1	GO:0046580	negative regulation of Ras protein signal transduction
WBGene00001061	dpl-1	GO:0045749	negative regulation of S phase of mitotic cell cycle
WBGene00001061	dpl-1	GO:0016481	negative regulation of transcription
WBGene00001061	dpl-1	GO:0012501	programmed cell death
WBGene00001061	dpl-1	GO:0005634	nucleus
WBGene00001162	efl-2	GO:0040027	negative regulation of vulval development
WBGene00001162	efl-2	GO:0006355	regulation of transcription, DNA-dependent
WBGene00001162	efl-2	GO:0003700	transcription factor activity
WBGene00001162	efl-2	GO:0005667	transcription factor complex
WBGene00001182	egl-13	GO:0001708	cell fate specification
WBGene00001182	egl-13	GO:0008406	gonad development
WBGene00001182	egl-13	GO:0040035	hermaphrodite genitalia development
WBGene00001182	egl-13	GO:0040025	vulval development
WBGene00001182	egl-13	GO:0018991	oviposition
WBGene00001182	egl-13	GO:0000003	reproduction
WBGene00001182	egl-13	GO:0003677	DNA binding
WBGene00001182	egl-13	GO:0003700	transcription factor activity
WBGene00001182	egl-13	GO:0009653	anatomical structure morphogenesis
WBGene00001182	egl-13	GO:0051301	cell division
WBGene00001182	egl-13	GO:0045026	plasma membrane fusion
WBGene00001182	egl-13	GO:0005634	nucleus
WBGene00001223	ehn-3	GO:0008406	gonad development
WBGene00001223	ehn-3	GO:0010628	positive regulation of gene expression
WBGene00001223	ehn-3	GO:0008270	zinc ion binding
WBGene00001223	ehn-3	GO:0005622	intracellular
WBGene00001223	ehn-3	GO:0005634	nucleus
WBGene00016600	ets-5	GO:0006355	regulation of transcription, DNA-dependent
WBGene00016600	ets-5	GO:0043565	sequence-specific DNA binding
WBGene00016600	ets-5	GO:0003700	transcription factor activity
WBGene00009508	F37D6.2	GO:0008270	zinc ion binding
WBGene00009508	F37D6.2	GO:0005622	intracellular
WBGene00018959	F56D1.1	GO:0000003	reproduction
WBGene00018959	F56D1.1	GO:0003676	nucleic acid binding
WBGene00018959	F56D1.1	GO:0008270	zinc ion binding
WBGene00018959	F56D1.1	GO:0005622	intracellular
WBGene00001953	hlh-8	GO:0018991	oviposition

WBGene00001953	hlh-8	GO:0040010	positive regulation of growth rate
WBGene00001953	hlh-8	GO:0045449	regulation of transcription
WBGene00001953	hlh-8	GO:0030528	transcription regulator activity
WBGene00001953	hlh-8	GO:0005634	nucleus
WBGene00009937	lsl-1	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00009937	lsl-1	GO:0003676	nucleic acid binding
WBGene00009937	lsl-1	GO:0008270	zinc ion binding
WBGene00009937	lsl-1	GO:0005622	intracellular
WBGene00019751	M03D4.4	GO:0003676	nucleic acid binding
WBGene00019751	M03D4.4	GO:0008270	zinc ion binding
WBGene00019751	M03D4.4	GO:0005622	intracellular
WBGene00003388	moe-3	GO:0048599	oocyte development
WBGene00003388	moe-3	GO:0003676	nucleic acid binding
WBGene00003388	moe-3	GO:0008270	zinc ion binding
WBGene00021132	nfyb-1	GO:0003677	DNA binding
WBGene00021132	nfyb-1	GO:0043565	sequence-specific DNA binding
WBGene00021132	nfyb-1	GO:0005622	intracellular
WBGene00003707	nhr-117	GO:0006355	regulation of transcription, DNA-dependent
WBGene00003707	nhr-117	GO:0043565	sequence-specific DNA binding
WBGene00003707	nhr-117	GO:0003707	steroid hormone receptor activity
WBGene00003707	nhr-117	GO:0003700	transcription factor activity
WBGene00003707	nhr-117	GO:0008270	zinc ion binding
WBGene00003707	nhr-117	GO:0005634	nucleus
WBGene00003722	nhr-132	GO:0006355	regulation of transcription, DNA-dependent
WBGene00003722	nhr-132	GO:0043565	sequence-specific DNA binding
WBGene00003722	nhr-132	GO:0003707	steroid hormone receptor activity
WBGene00003722	nhr-132	GO:0003700	transcription factor activity
WBGene00003722	nhr-132	GO:0008270	zinc ion binding
WBGene00003722	nhr-132	GO:0005634	nucleus
WBGene00003727	nhr-137	GO:0006355	regulation of transcription, DNA-dependent
WBGene00003727	nhr-137	GO:0043565	sequence-specific DNA binding
WBGene00003727	nhr-137	GO:0003707	steroid hormone receptor activity
WBGene00003727	nhr-137	GO:0003700	transcription factor activity
WBGene00003727	nhr-137	GO:0008270	zinc ion binding
WBGene00003727	nhr-137	GO:0016021	integral to membrane
WBGene00003727	nhr-137	GO:0019915	lipid storage
WBGene00003727	nhr-137	GO:0005634	nucleus
WBGene00003613	nhr-14	GO:0008340	determination of adult life span
WBGene00003613	nhr-14	GO:0003677	DNA binding
WBGene00003613	nhr-14	GO:0006355	regulation of transcription, DNA-dependent
WBGene00003613	nhr-14	GO:0043565	sequence-specific DNA binding
WBGene00003613	nhr-14	GO:0003707	steroid hormone receptor activity
WBGene00003613	nhr-14	GO:0003700	transcription factor activity
WBGene00003613	nhr-14	GO:0008270	zinc ion binding
WBGene00003613	nhr-14	GO:0004879	ligand-dependent nuclear receptor activity
WBGene00003613	nhr-14	GO:0005634	nucleus

WBGene00018430	nhr-142	GO:0006355	regulation of transcription, DNA-dependent
WBGene00018430	nhr-142	GO:0043565	sequence-specific DNA binding
WBGene00018430	nhr-142	GO:0003707	steroid hormone receptor activity
WBGene00018430	nhr-142	GO:0003700	transcription factor activity
WBGene00018430	nhr-142	GO:0008270	zinc ion binding
WBGene00018430	nhr-142	GO:0005634	nucleus
WBGene00008289	nhr-169	GO:0006355	regulation of transcription, DNA-dependent
WBGene00008289	nhr-169	GO:0043565	sequence-specific DNA binding
WBGene00008289	nhr-169	GO:0003707	steroid hormone receptor activity
WBGene00008289	nhr-169	GO:0003700	transcription factor activity
WBGene00008289	nhr-169	GO:0008270	zinc ion binding
WBGene00008289	nhr-169	GO:0005634	nucleus
WBGene00018189	nhr-181	GO:0006355	regulation of transcription, DNA-dependent
WBGene00018189	nhr-181	GO:0043565	sequence-specific DNA binding
WBGene00018189	nhr-181	GO:0003707	steroid hormone receptor activity
WBGene00018189	nhr-181	GO:0003700	transcription factor activity
WBGene00018189	nhr-181	GO:0008270	zinc ion binding
WBGene00018189	nhr-181	GO:0006915	apoptosis
WBGene00018189	nhr-181	GO:0005634	nucleus
WBGene00018541	nhr-186	GO:0006355	regulation of transcription, DNA-dependent
WBGene00018541	nhr-186	GO:0043565	sequence-specific DNA binding
WBGene00018541	nhr-186	GO:0003707	steroid hormone receptor activity
WBGene00018541	nhr-186	GO:0003700	transcription factor activity
WBGene00018541	nhr-186	GO:0008270	zinc ion binding
WBGene00018541	nhr-186	GO:0005634	nucleus
WBGene00010180	nhr-192	GO:0006355	regulation of transcription, DNA-dependent
WBGene00010180	nhr-192	GO:0043565	sequence-specific DNA binding
WBGene00010180	nhr-192	GO:0003707	steroid hormone receptor activity
WBGene00010180	nhr-192	GO:0003700	transcription factor activity
WBGene00010180	nhr-192	GO:0008270	zinc ion binding
WBGene00010180	nhr-192	GO:0005634	nucleus
WBGene00010600	nhr-196	GO:0006355	regulation of transcription, DNA-dependent
WBGene00010600	nhr-196	GO:0043565	sequence-specific DNA binding
WBGene00010600	nhr-196	GO:0003707	steroid hormone receptor activity
WBGene00010600	nhr-196	GO:0003700	transcription factor activity
WBGene00010600	nhr-196	GO:0008270	zinc ion binding
WBGene00010600	nhr-196	GO:0005634	nucleus
WBGene00003619	nhr-20	GO:0006355	regulation of transcription, DNA-dependent
WBGene00003619	nhr-20	GO:0043565	sequence-specific DNA binding
WBGene00003619	nhr-20	GO:0003707	steroid hormone receptor activity
WBGene00003619	nhr-20	GO:0003700	transcription factor activity
WBGene00003619	nhr-20	GO:0008270	zinc ion binding
WBGene00003619	nhr-20	GO:0005634	nucleus
WBGene00020850	nhr-226	GO:0006355	regulation of transcription, DNA-dependent
WBGene00020850	nhr-226	GO:0043565	sequence-specific DNA binding
WBGene00020850	nhr-226	GO:0003707	steroid hormone receptor activity
WBGene00020850	nhr-226	GO:0003700	transcription factor activity

WBGene00020850	nhr-226	GO:0008270	zinc ion binding
WBGene00020850	nhr-226	GO:0005576	extracellular region
WBGene00020850	nhr-226	GO:0005634	nucleus
WBGene00022639	nhr-253	GO:0006355	regulation of transcription, DNA-dependent
WBGene00022639	nhr-253	GO:0043565	sequence-specific DNA binding
WBGene00022639	nhr-253	GO:0003707	steroid hormone receptor activity
WBGene00022639	nhr-253	GO:0003700	transcription factor activity
WBGene00022639	nhr-253	GO:0008270	zinc ion binding
WBGene00022639	nhr-253	GO:0040011	locomotion
WBGene00022639	nhr-253	GO:0005634	nucleus
WBGene00003643	nhr-53	GO:0006355	regulation of transcription, DNA-dependent
WBGene00003643	nhr-53	GO:0043565	sequence-specific DNA binding
WBGene00003643	nhr-53	GO:0003707	steroid hormone receptor activity
WBGene00003643	nhr-53	GO:0003700	transcription factor activity
WBGene00003643	nhr-53	GO:0008270	zinc ion binding
WBGene00003643	nhr-53	GO:0005634	nucleus
WBGene00003664	nhr-74	GO:0006355	regulation of transcription, DNA-dependent
WBGene00003664	nhr-74	GO:0043565	sequence-specific DNA binding
WBGene00003664	nhr-74	GO:0003707	steroid hormone receptor activity
WBGene00003664	nhr-74	GO:0003700	transcription factor activity
WBGene00003664	nhr-74	GO:0008270	zinc ion binding
WBGene00003664	nhr-74	GO:0005634	nucleus
WBGene00003670	nhr-80	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00003670	nhr-80	GO:0007275	multicellular organismal development
WBGene00003670	nhr-80	GO:0000003	reproduction
WBGene00003670	nhr-80	GO:0045923	positive regulation of fatty acid metabolic process
WBGene00003670	nhr-80	GO:0040010	positive regulation of growth rate
WBGene00003670	nhr-80	GO:0045944	positive regulation of transcription from RNA polymerase II promoter
WBGene00003670	nhr-80	GO:0006355	regulation of transcription, DNA-dependent
WBGene00003670	nhr-80	GO:0043565	sequence-specific DNA binding
WBGene00003670	nhr-80	GO:0003707	steroid hormone receptor activity
WBGene00003670	nhr-80	GO:0003700	transcription factor activity
WBGene00003670	nhr-80	GO:0008270	zinc ion binding
WBGene00003670	nhr-80	GO:0005634	nucleus
WBGene00003608	nhr-9	GO:0040010	positive regulation of growth rate
WBGene00003608	nhr-9	GO:0006355	regulation of transcription, DNA-dependent
WBGene00003608	nhr-9	GO:0043565	sequence-specific DNA binding
WBGene00003608	nhr-9	GO:0003707	steroid hormone receptor activity
WBGene00003608	nhr-9	GO:0003700	transcription factor activity
WBGene00003608	nhr-9	GO:0008270	zinc ion binding
WBGene00003608	nhr-9	GO:0005634	nucleus
WBGene00003689	nhr-99	GO:0006355	regulation of transcription, DNA-dependent
WBGene00003689	nhr-99	GO:0043565	sequence-specific DNA binding
WBGene00003689	nhr-99	GO:0003707	steroid hormone receptor activity

WBGene00003689	nhr-99	GO:0003700	transcription factor activity
WBGene00003689	nhr-99	GO:0008270	zinc ion binding
WBGene00003689	nhr-99	GO:0005634	nucleus
WBGene00004013	pha-4	GO:0030154	cell differentiation
WBGene00004013	pha-4	GO:0060573	cell fate specification involved in pattern specification
WBGene00004013	pha-4	GO:0008340	determination of adult life span
WBGene00004013	pha-4	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00004013	pha-4	GO:0048598	embryonic morphogenesis
WBGene00004013	pha-4	GO:0040007	growth
WBGene00004013	pha-4	GO:0040035	hermaphrodite genitalia development
WBGene00004013	pha-4	GO:0032038	myosin II heavy chain binding
WBGene00004013	pha-4	GO:0002119	nematode larval development
WBGene00004013	pha-4	GO:0060465	pharynx development
WBGene00004013	pha-4	GO:0000003	reproduction
WBGene00004013	pha-4	GO:0040010	positive regulation of growth rate
WBGene00004013	pha-4	GO:0040018	positive regulation of multicellular organism growth
WBGene00004013	pha-4	GO:0045727	positive regulation of translation
WBGene00004013	pha-4	GO:0006355	regulation of transcription, DNA-dependent
WBGene00004013	pha-4	GO:0043565	sequence-specific DNA binding
WBGene00004013	pha-4	GO:0016563	transcription activator activity
WBGene00004013	pha-4	GO:0003700	transcription factor activity
WBGene00004013	pha-4	GO:0040011	locomotion
WBGene00004013	pha-4	GO:0005634	nucleus
WBGene00004024	php-3	GO:0003677	DNA binding
WBGene00004024	php-3	GO:0045449	regulation of transcription
WBGene00004024	php-3	GO:0006355	regulation of transcription, DNA-dependent
WBGene00004024	php-3	GO:0043565	sequence-specific DNA binding
WBGene00004024	php-3	GO:0003700	transcription factor activity
WBGene00004024	php-3	GO:0030528	transcription regulator activity
WBGene00004024	php-3	GO:0005634	nucleus
WBGene00004194	prx-5	GO:0008340	determination of adult life span
WBGene00004194	prx-5	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00004194	prx-5	GO:0040007	growth
WBGene00004194	prx-5	GO:0002119	nematode larval development
WBGene00004194	prx-5	GO:0005488	binding
WBGene00004194	prx-5	GO:0040010	positive regulation of growth rate
WBGene00004194	prx-5	GO:0019915	lipid storage
WBGene00004194	prx-5	GO:0016558	protein import into peroxisome matrix
WBGene00004194	prx-5	GO:0000268	peroxisome targeting sequence binding
WBGene00004773	sem-4	GO:0048333	mesodermal cell differentiation
WBGene00004773	sem-4	GO:0042692	muscle cell differentiation
WBGene00004773	sem-4	GO:0014016	neuroblast differentiation
WBGene00004773	sem-4	GO:0018991	oviposition
WBGene00004773	sem-4	GO:0003676	nucleic acid binding

WBGene00004773	sem-4	GO:0040010	positive regulation of growth rate
WBGene00004773	sem-4	GO:0008270	zinc ion binding
WBGene00004773	sem-4	GO:0048675	axon extension
WBGene00004773	sem-4	GO:0005622	intracellular
WBGene00012735	sptf-3	GO:0010171	body morphogenesis
WBGene00012735	sptf-3	GO:0040002	collagen and cuticulin-based cuticle development
WBGene00012735	sptf-3	GO:0008340	determination of adult life span
WBGene00012735	sptf-3	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00012735	sptf-3	GO:0040007	growth
WBGene00012735	sptf-3	GO:0040035	hermaphrodite genitalia development
WBGene00012735	sptf-3	GO:0002009	morphogenesis of an epithelium
WBGene00012735	sptf-3	GO:0002119	nematode larval development
WBGene00012735	sptf-3	GO:0000003	reproduction
WBGene00012735	sptf-3	GO:0003676	nucleic acid binding
WBGene00012735	sptf-3	GO:0008270	zinc ion binding
WBGene00012735	sptf-3	GO:0040011	locomotion
WBGene00012735	sptf-3	GO:0006898	receptor-mediated endocytosis
WBGene00012735	sptf-3	GO:0005622	intracellular
WBGene00011956	T23F11.4	GO:0008270	zinc ion binding
WBGene00011956	T23F11.4	GO:0019915	lipid storage
WBGene00011956	T23F11.4	GO:0005622	intracellular
WBGene00020823	T26A5.8	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00020823	T26A5.8	GO:0000003	reproduction
WBGene00020823	T26A5.8	GO:0003677	DNA binding
WBGene00020823	T26A5.8	GO:0043565	sequence-specific DNA binding
WBGene00020823	T26A5.8	GO:0035046	pronuclear migration
WBGene00020823	T26A5.8	GO:0005622	intracellular
WBGene00006380	tab-1	GO:0003677	DNA binding
WBGene00006380	tab-1	GO:0045449	regulation of transcription
WBGene00006380	tab-1	GO:0006355	regulation of transcription, DNA-dependent
WBGene00006380	tab-1	GO:0043565	sequence-specific DNA binding
WBGene00006380	tab-1	GO:0003700	transcription factor activity
WBGene00006380	tab-1	GO:0030528	transcription regulator activity
WBGene00006380	tab-1	GO:0005634	nucleus
WBGene00007732	tag-347	GO:0005488	binding
WBGene00007732	tag-347	GO:0003677	DNA binding
WBGene00007732	tag-347	GO:0005634	nucleus
WBGene00006553	tbx-34	GO:0006355	regulation of transcription, DNA-dependent
WBGene00006553	tbx-34	GO:0003700	transcription factor activity
WBGene00006553	tbx-34	GO:0005634	nucleus
WBGene00006744	unc-4	GO:0007400	neuroblast fate determination
WBGene00006744	unc-4	GO:0003677	DNA binding
WBGene00006744	unc-4	GO:0050770	regulation of axonogenesis
WBGene00006744	unc-4	GO:0050803	regulation of synapse structure and activity

WBGene00006744	unc-4	GO:0045449	regulation of transcription
WBGene00006744	unc-4	GO:0006355	regulation of transcription, DNA-dependent
WBGene00006744	unc-4	GO:0043565	sequence-specific DNA binding
WBGene00006744	unc-4	GO:0003700	transcription factor activity
WBGene00006744	unc-4	GO:0030528	transcription regulator activity
WBGene00006744	unc-4	GO:0030424	axon
WBGene00006744	unc-4	GO:0040011	locomotion
WBGene00006744	unc-4	GO:0007416	synaptogenesis
WBGene00006744	unc-4	GO:0005634	nucleus
WBGene00013796	Y116A8C.19	GO:0003676	nucleic acid binding
WBGene00013796	Y116A8C.19	GO:0008270	zinc ion binding
WBGene00012471	Y17G7B.20	GO:0010171	body morphogenesis
WBGene00012471	Y17G7B.20	GO:0000003	reproduction
WBGene00012471	Y17G7B.20	GO:0040010	positive regulation of growth rate
WBGene00012471	Y17G7B.20	GO:0040018	positive regulation of multicellular organism growth
WBGene00021374	Y37E11B.1	GO:0003676	nucleic acid binding
WBGene00021374	Y37E11B.1	GO:0008270	zinc ion binding
WBGene00021374	Y37E11B.1	GO:0005622	intracellular
WBGene00013128	Y52B11A.9	GO:0010171	body morphogenesis
WBGene00013128	Y52B11A.9	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00013128	Y52B11A.9	GO:0040007	growth
WBGene00013128	Y52B11A.9	GO:0002009	morphogenesis of an epithelium
WBGene00013128	Y52B11A.9	GO:0002119	nematode larval development
WBGene00013128	Y52B11A.9	GO:0000003	reproduction
WBGene00013128	Y52B11A.9	GO:0040010	positive regulation of growth rate
WBGene00013128	Y52B11A.9	GO:0008270	zinc ion binding
WBGene00013128	Y52B11A.9	GO:0005840	ribosome
WBGene00013128	Y52B11A.9	GO:0003735	structural constituent of ribosome
WBGene00013128	Y52B11A.9	GO:0040011	locomotion
WBGene00013128	Y52B11A.9	GO:0006412	translation
WBGene00013128	Y52B11A.9	GO:0005622	intracellular
WBGene00021816	Y53G8AR.9	GO:0002009	morphogenesis of an epithelium
WBGene00021816	Y53G8AR.9	GO:0003676	nucleic acid binding
WBGene00021816	Y53G8AR.9	GO:0008270	zinc ion binding
WBGene00013236	Y56A3A.18	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00013236	Y56A3A.18	GO:0040007	growth
WBGene00013236	Y56A3A.18	GO:0002119	nematode larval development
WBGene00013236	Y56A3A.18	GO:0000003	reproduction
WBGene00013236	Y56A3A.18	GO:0040010	positive regulation of growth rate
WBGene00013236	Y56A3A.18	GO:0008270	zinc ion binding
WBGene00013236	Y56A3A.18	GO:0006898	receptor-mediated endocytosis
WBGene00013236	Y56A3A.18	GO:0005622	intracellular
WBGene00012385	Y5F2A.4	GO:0003676	nucleic acid binding
WBGene00012385	Y5F2A.4	GO:0008270	zinc ion binding

WBGene00012385	Y5F2A.4	GO:0005622	intracellular
WBGene00013970	ZK337.2	GO:0003676	nucleic acid binding
WBGene00013970	ZK337.2	GO:0008270	zinc ion binding
WBGene00013970	ZK337.2	GO:0005622	intracellular
WBGene00018099	ztf-28	GO:0008270	zinc ion binding
WBGene00018099	ztf-28	GO:0005622	intracellular
WBGene00016905	ztf-3	GO:0040002	collagen and cuticulin-based cuticle development
WBGene00016905	ztf-3	GO:0009792	embryonic development ending in birth or egg hatching
WBGene00016905	ztf-3	GO:0003676	nucleic acid binding
WBGene00016905	ztf-3	GO:0040018	positive regulation of multicellular organism growth
WBGene00016905	ztf-3	GO:0008270	zinc ion binding
WBGene00016905	ztf-3	GO:0005622	intracellular
WBGene00020399	ztf-4	GO:0003676	nucleic acid binding
WBGene00020399	ztf-4	GO:0000166	nucleotide binding
WBGene00020399	ztf-4	GO:0008270	zinc ion binding
WBGene00020399	ztf-4	GO:0005622	intracellular
WBGene00006999	zyx-1	GO:0000003	reproduction
WBGene00006999	zyx-1	GO:0005515	protein binding
WBGene00006999	zyx-1	GO:0008270	zinc ion binding
WBGene00006999	zyx-1	GO:0015629	actin cytoskeleton
WBGene00006999	zyx-1	GO:0005925	focal adhesion
WBGene00006999	zyx-1	GO:0055120	striated muscle dense body
WBGene00006999	zyx-1	GO:0017151	DEAD/H-box RNA helicase binding
WBGene00006999	zyx-1	GO:0031430	M band
WBGene00006999	zyx-1	GO:0005634	nucleus