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GENE EXPRESSION REGULATORS *lin-11* AND *let-711*, IN MODULATING THE RATE OF AGING AND LIFESPAN, IN *C. elegans*.

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ABSTRACT OF DISSERTATION

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The Graduate School
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A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Arts and Sciences at the
University of Kentucky.

By

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Lexington, Kentucky

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Lexington, Kentucky

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ABSTRACT OF DISSERTATION

GENE EXPRESSION REGULATORS *lin-11* AND *let-711*, IN MODULATING THE RATE OF AGING AND LIFESPAN, IN *C. elegans.*

lin-11 and *let-711* are early-developmental gene expression regulators with no previously known roles in aging regulation. Yet, they show strong aging-correlated expression profiles (Lund, Tedesco et al. 2002). *lin-11* is strongly upregulated in very old worm populations, and *let-711* is progressively downregulated in aging worm populations. Microarray studies were performed to identify their genome-wide targets, which were then subjected to further lifespan and genetic analysis to investigate their role in *C. elegans* aging.

The results indicate that the target pools of both *lin-11* and *let-711* are enriched for aging genes, since a significant number of tested genes increased lifespan. This enrichment of aging genes in their target pools provides strong evidence that *lin-11* and *let-711* are indeed regulating the expression of aging genes in adult *C. elegans.* The data suggests that increased *lin-11* expression as well as reduced *let-711* expression may be promoting longevity by downregulating the insulin/IGF-1 pathway. Decreasing *let-711* may also be contributing to longevity by downregulating the germline signaling pathway.

K11E4.2, R53.5, C49A9.2 and Y82E9BR.5 are four genes from the *lin-11* target pool, whose knockdown produced increases inlifespan. These are unannotated genes, and the details of their roles in aging regulation are not known at this point. *ins-3* expression was downregulated two-fold upon knockdown of *lin-11,* suggesting the possible involvement of *lin-11* in regulation of the insulin/IGF-1 pathway. An RNAi construct for *ins-3* was not available and it is not known whether loss of *ins-3* leads to lifespan extension.

let-711 knockdown resulted in an almost four-fold reduction in *pdk-1* expression. *pdk-1* is an integral part of the insulin/IGF-1 pathway and its knockdown by RNAi extended lifespan. Four other genes from the *let-711* target pool that increased lifespan, *cdc-25.1, gna-2, meg-1* and *ooc-3,* all have germline specific functions. The extensions in lifespan generated by these genes were completely dependent on DAF-16. Furthermore,

for *gna-2*, *meg-1* or *ooc-3*, they were independent of DAF-2. These results agree with previously established mechanisms for germline regulation of aging, suggesting the involvement of *let-711* in regulating the germline-signaling pathway.

KEYWORDS: *C. elegans*, aging, *lin-11, let-711*,germline signaling.

Tseten Yeshi Jamling

25 Febuary 2011

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25 Febuary 2011

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FOR MY SON, TO BE BORN JUNE 29th 2011,
MY WIFE, PHUNTSOK DOLMA,
AND MY PARENTS,
BUMO TSERING AND TSEWANG YESHI JAMLING.

THIS ACHEIVEMENT IS AS MUCH YOURS AS IT IS MINE.

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CHAPTER 1: Background and Significance

Introduction

Aging and death are something we are very familiar with, and accept as universal biological phenomena that cannot be avoided. Nonetheless, human life expectancy has increased by decades due to modern medicine and technology over the last century. Infant mortality rates have declined, cures for deadly diseases have been discovered, and our cars and buildings have become safer, allowing us to live longer. As such, the elderly population is growing at a rapid rate and diseases associated with aging, including Alzheimer's disease and cancer, are gaining notoriety. The US Department of Health and Human Services estimates that 20% of Americans, more than 70 million individuals, will be over the age of 65, by the year 2030. With ever-greater numbers living to grow old, there is strong interest in understanding the aging process at a fundamental level.

But what do we really know about the biology of aging? The increased lifespan expectancy of the modern man is due to a reduction in risks or external factors and not due to an intimate understanding of the biology of aging. All organisms age, and the similar vulnerability to deleterious effects of aging across phyla is remarkable, hinting at a conserved mechanism that regulates the aging process. Aging exponentially increases the mortality rate, producing a characteristic population survival curve observed in aging populations of a wide range of animals with lifespans ranging from days to years. Physiological changes such as organ function decline, loss of muscle mass, and weakened stress resistance are common aging phenotypes. The similarities are even more remarkable at the cellular level. Accumulation of DNA damage, lipofuscin deposits, and accumulation of misfolded and damaged proteins are common cellular changes observed with aging in all living organisms.

Over the past twenty years, genetic mutations have been discovered that can dramatically extend lifespan in healthy model lab organisms (reviewed in (Guarente and Kenyon 2000). These genetic effects are conserved over large evolutionary distances in that mutations in orthologous genes can extend lifespan in *S. cerevisiae*, *C. elegans, D. melanogaster* and *M. musculus*, despite their very different physiology and lifestyles.These observations suggest that aging mechanisms are conserved across phyla, and therefore, understanding the biology of aging in fast living model organisms can provide valuable insights for understanding human aging, as well as diseases whose primary risk factor is increased age.

Caenorhabditis elegans, a hermaphroditic soil nematode, was pioneered in the 1960s as a model organism for molecular biology (Brenner 1974). *C. elegans* has since emerged as the primary model organism for studying the genetics of aging and lifespan regulation. Many genes found to affect lifespan in *C. elegans* have been shown to have similar effects in a wide range of organisms, including the mouse (reviewed in (Guarente and Kenyon 2000). Also, alleles of these genes are associated with longevity in humans.

*C. elegans*has a relatively short average lifespan of approximately two weeks under standard laboratory conditions at 25°C,and it is easy to generate age-synchronized populations. These properties allow experiments measuring changes in lifespan of worm populations to be completed reasonably quickly. *C. elegans* reproduces by hermaphrodite self-fertilization; therefore isogenic populations of viable mutant strains are easily maintained. *C. elegans* also exhibit XO/XX sexual reproduction, which allows for easy crossing of strains as needed. The efficiency and ease of RNA interference to disrupt gene function is an invaluable tool for *C. elegans* genetics (Fire, Xu et al. 1998; Timmons, Court et al. 2001) and two libraries of dsRNA producing bacteria have been produced, which target 11,942 and16,757 of 19,477 predicted open reading frames in *C. elegans* genome(Kamath and Ahringer 2003; Kamath, Fraser et al. 2003; Rual, Ceron et al. 2004). This genetic tractability and short lifespan make *C. elegans* a very attractive model organism for studying the biology of aging.

Studies in model organisms have clearly and repeatedly shown that single-gene mutations can cause dramatic changes in lifespan and aging. Many of these genes encode signaling and transcriptional regulators with conserved roles in aging regulation from simple metazoa to mammals. These findings support the existence of organismal and cellular processes that regulate the rate of aging. How these genes and signaling pathways regulate aging is an area of active research.

Cellular pathways that regulate aging

The DAF-2 Insulin/IGF-1 Pathway

The insulin/IGF-1 pathway is a well-characterized aging regulatory pathway that influences lifespan in worms, flies and mammals (Tatar, Bartke et al. 2003). This pathway was first linked to aging in *C. elegans*, where loss-of-function mutations in the insulin/IGF-1 receptor ortholog,*daf-2*, were found to double the animal's lifespan (Kenyon, Chang et al. 1993). *daf-2* worms are not only long-lived, but all phases of lifespan, rather than just old age,are extended, allowing them to remain active and youthful when wild-type animals are dying. *daf-2* encodes the only *C. elegans* insulin/IGF-1 receptor ortholog (Kimura, Tissenbaum et al. 1997), pointing to the importance of insulin or IGF-1-like hormones in aging regulation. Lifespan extension due to various perturbations of this pathway, including *daf-2* mutations, is entirely dependent on the nuclear localization of *daf-16*(Kenyon, Chang et al. 1993), a FOXO family transcription factor (Lin, Dorman et al. 1997; Ogg, Paradis et al. 1997).

daf-2 and *daf-16* were first identified as regulating dauer formation during *C. elegans* development (Riddle 1997). *C elegans* develops through a series of four larval stages, L1 through L4, before reaching reproductive adulthood. During development, under adverse conditions such as high temperature, over-crowding and food shortage, L2 animals exit normal development to form dauer larvae, which are stress-resistant, growth-

arrested and can survive for several months to outlast the adverse conditions(Cassada and Russell 1975). Once favorable conditions are restored, animals exit dauer state and continue development to adulthood. Dauer entry is regulated in part by downregulating insulin/IGF signaling. *daf-2* mutants are dauer constitutive (Daf-c), meaning they become dauers even under favorable environmental conditions, while *daf-16* mutants are dauer defective (Daf-d) or incapable of forming dauers even under inhospitable conditions (Riddle, Swanson et al. 1981). Loss of *daf-2* function after the dauer checkpoint, i.e. in L3 or older worms, leads to normal development but extended adult lifespan. Like lifespan, theDaf-c phenotype of *daf-2* mutants is dependent on *daf-16,* and many stress-response genes whose expression change in long-lived adults, also change in dauers (Jones, Riddle et al. 2001; McElwee, Schuster et al. 2004).Two other pathways, the *daf-7*/TGF-β and the *daf-11*/cGMP pathways, are also involved in dauer formation, but these pathways do not play a role in longevity (Kenyon, Chang et al. 1993).

DAF-2 activates a conserved PI3-kinase/Akt signaling pathway (Figure 1.1) that activates kinases AKT-1, AKT-2 and SGK-1 (Alessi, James et al. 1997; Brunet, Park et al. 2001; Scheid, Marignani et al. 2002; Hertweck, Gobel et al. 2004), which,in turn, inhibit DAF-16 nuclear localization (Lin, Dorman et al. 1997; Henderson and Johnson 2001; Lee, Hench et al. 2001). These kinases phosphorylate DAF-16 (Tatar, Bartke et al. 2003; Hertweck, Gobel et al. 2004), leading to cytosolic sequestration of DAF-16 by virtue of its association with 14-3-3 proteins (Berdichevsky, Viswanathan et al. 2006; Li, Tewari et al. 2007). *daf-16* is expressed in all cells and under normal conditions, DAF-16 displays a diffuse cytoplasmic expression (Lin, Dorman et al. 1997; Ogg, Paradis et al. 1997; Henderson and Johnson 2001). When the insulin-like signaling pathway is inhibited, in *daf-2* or *age-1* (PI3 Kinase) mutants, DAF-16 localizes to the nuclei of neuronal, intestinal, muscle and hypodermal cells (Lin, Hsin et al. 2001). DAF-16 can also become nuclear localized under conditions of starvation or oxidative stress or heat stress (Henderson and Johnson 2001; Lin, Hsin et al. 2001). Once nuclear localized, DAF-16/FOXO transcription factor activates, both directly and indirectly, transcription of a wide range of stress-response, metabolic, antimicrobial, hormone signaling or processing, and novel genes, leading to an extended lifespan of the animal (McElwee,

Bubb et al. 2003; Murphy, McCarroll et al. 2003; Halaschek-Wiener, Khattra et al. 2005). Therefore, in wild-type worms, DAF-16 nuclear localization leads to lifespan extension and DAF-2 (and the insulin-like signaling pathway) functions to restrict lifespan by inhibiting DAF-16 activity.

Insulin/IGF-1 signaling regulates aging in higher organisms: The finding that inhibiting insulin-like signaling extends lifespan in *C. elegans* has since been recapitulated in flies and mice, demonstrating that the role of insulin/IGF-1 signaling in aging regulation is evolutionarily conserved (Tatar, Bartke et al. 2003). In *Drosophila*, mutants expressing heteroallelic combinations of hypomorphicinsulin-like receptor (InR)gene live up to 85% longer than wild-type animals (Tatar, Kopelman et al. 2001), and mutations in the insulin receptor substrate-like gene, chico, also significantly increases lifespan (Clancy, Gems et al. 2001). Furthermore, overexpression of dFOXO, the fly homolog of *daf-16*, also extends fly lifespan (Giannakou, Goss et al. 2004; Hwangbo, Gershman et al. 2004). Similar results have also been reported in mice. Mice cannot survive without the insulin receptor; however, mosaic mice that lack the insulin receptor specifically in adipose tissue live 18% longer than normal (Bluher, Kahn et al. 2003). These mosaic mice displayed a 50-70% reduction in fat mass with no diabetic symptoms. Another study showed that female mice heterozygous for IGF-1 receptor knockout live up to 30% longer than normal, and displayed no changes in metabolism, fertility, or sexual maturation (Holzenberger, Dupont et al. 2003). Thus, the regulation of aging and lifespan by the insulin/IGF-1 pathway is conserved across evolution from nematodes to mammals.

Role of the reproductive system in aging regulation

The reproductive system and aging: The “disposable soma theory” proposes that aging is the outcome of an optimal fitness balance between investment in reproduction and maintenance of the soma (Kirkwood 1977; Partridge and Barton 1993;

Kirkwood 2001). This theory postulates that reproduction and somatic maintenance are antagonistic to each other, and favoring one comes at cost to the other. Accordingly, relationships between germline and lifespan have been observed in various organisms, establishing a conserved regulation of lifespan by the reproductive system. Germline ablated worms live up to 60% longer than wild type worms (Hsin and Kenyon 1999) and female *Drosophila* carrying the dominant ovo^{D1} mutation, which halts oogenesis, have an extended lifespan (Sgro and Partridge 1999). Similarly, eliminating germline stem cells through over-expression of *bam* in *Drosophila* also extends lifespan (Flatt, Min et al. 2008). Involvement of the reproductive system in aging regulation has also been demonstrated in mice. Transplanting ovaries from sexually mature two-month old mice into middle-aged 11-month old ovariectomized mice, increased the life span of the older recipients by up to 60% (Cargill, Carey et al. 2003). Since the germ cells in these transplanted ovaries die in the process, the recipient ends up with somatic gonad tissue lacking germ cells, which, as dexcribed in the next section, also causes extension of lifespan in *C. elegans.*

Germline removal extends lifespan in *C. elegans:* The influence of the germline on organismal lifespan is an intriguing story that is still very much under active investigation. In *C. elegans,* the reproductive system develops from four precursor cells that are present at the time of hatching: Z1, Z2, Z3 and Z4 (Figure 1.2). Z2 and Z3 are the primordial germ cells that give rise to the germline, whereas Z1 and Z4 give rise to the somatic reproductive tissues (somatic gonad) (Kimble and Hirsh 1979). Worms that lack a germline, either as a result of mutations resulting in loss of germline proliferation (notch receptor *glp-1)* or as a result of laser ablation of Z2 and Z3, are longer lived by as much as 60% (Hsin and Kenyon 1999; Arantes-Oliveira, Apfeld et al. 2002). This effect on lifespan is dependent on the presence of an intact somatic gonad, since laser ablation of gonadal precursor cells (Z1 and Z4)in germline ablated worms, reverts lifespan to wild type levels (Figure 1.4). Further analysis has shown that it is the proliferating germline stem cells, rather than differentiated sperm or oocytes, which must be absent for this lifespan extension (Arantes-Oliveira, Apfeld et al. 2002). The implications of these

findings are twofold. First, counterbalancing signals from the reproductive system regulate longevity; proliferating germ cells signal to shorten lifespan whereas somatic gonad signals to lengthen lifespan. Second, sterility, in and of itself, does not extend lifespan since removal of the whole gonad has no effect on lifespan.

Lifespan extension by germline ablation is DAF-16 dependent: The reproductive system influences longevity by means of insulin/IGF-1 signaling, steroid hormone signaling, and transcriptional activity (Figure 1.5). Germline ablation in *daf-16* null mutants fails to generate long-lived *C. elegans*,indicating that the well-established aging regulator, *daf-16*/FOXO transcription factor is essential for this extension in lifespan (Hsin and Kenyon 1999). Removal of germline precursor cells results in nuclear localization of DAF-16,which occurs primarily in the intestinal nuclei (Lin, Hsin et al. 2001), where it activates transcription of genes that promote longevity (Ghazi, Henis-Korenblit et al. 2009). Furthermore, expression of *daf-16* specifically in the intestine also fully restores the longevity phenotype in germline ablated *daf-16* animals (Libina, Berman et al. 2003). Therefore, DAF-16 activity in the intestinal cells of *C. elegans* is necessary and sufficient for lifespan extension through germline ablation.

Two genes have been identified that are involved in the regulation of DAF-16 activity in response to germline removal. *kri-1,* which encodes a conserved intestinal ankyrin-repeat protein, is required for the localization of DAF-16 to intestinal nuclei in germline ablated animals (Berman and Kenyon 2006). Once DAF-16 is nuclear localized, its transcriptional activity is modulated by TCER-1. *tcer-1* encodes a transcription elongation factor that is required specifically for the upregulation of a subset of known *daf-16* regulated genes under these conditions (Ghazi, Henis-Korenblit et al. 2009).

Lifespan extension by germline ablation is independent of *daf-2* activity: DAF-16 activity is also downregulated by the insulin/IGF-1 receptor DAF-2, through the insulin-like signaling pathway. Germline ablation in *daf-2* mutants shows a synergistic

effect and further extends the lifespan of these long-lived mutants (Hsin and Kenyon 1999). Furthermore, while both *kri-1* and *tcer-1* are important for germline ablation to extend lifespan, neither gene is required for the activation of DAF-16 when the insulin-like signaling pathway is impaired (Berman and Kenyon 2006; Ghazi, Henis-Korenblit et al. 2009). These results suggest that ablation of germline affects DAF-16 independently of the DAF-2 receptor (Figure 1.5).

The somatic gonad regulates longevity in a manner that is distinct from that of the germline. While ablation of germline extends lifespan independently of *daf-2*, *daf-2* is required for the somatic gonad to regulate aging (Hsin and Kenyon 1999). In wild-type worms, the lifespan extension produced by germline ablation can be rescued by removing the whole gonad. This is not true in *daf-2* mutants. Germline ablation increases lifespan of *daf-2* mutants, and this extension cannot be restored back to intact-gonad levels by removal of the whole gonad. The effect of somatic gonad therefore appears sensitive to DAF-2 activity in the animal (Figure 1.5).

To summarize, in wild-type worms, germline cells produce a signal that inhibits the activity of DAF-16, leading to a shorter lifespan. Removal of the germline therefore, results in the removal of this DAF-16 inhibitory signal, which leads to DAF-16 nuclear localization in the intestinal cells to upregulate expression of longevity genes. This germline-signaling pathway is independent of the *daf-2*/insulin-like signaling.

Other pathways that regulate aging

Aside from the DAF-2 pathway, a number of other pathways and biological processes have been implicated in aging regulation, highlighting the complexity of the regulation of aging. Although not the focus of my thesis research, these are important aging pathways and the following is a brief description of these pathways.

JNK pathway: The c-Jun N-terminal Kinase (JNK) pathway is associated with regulation of various biological processes such as apoptosis, cell survival and development (Weston and Davis 2007). In *C. elegans*, environmental stresses such as heat and oxidative stress activate the JNK pathway (Oh, Mukhopadhyay et al. 2005; Wolf, Nunes et al. 2008) leading to lifespan extension and increased stress resistance in a DAF-16 dependent manner (Oh, Mukhopadhyay et al. 2005; Wang, Bohmann et al. 2005). JNK physically interacts with and phosphorylates DAF-16 at sites different from the AKT phosphorylation sites, leading to DAF-16 nuclear localization(Essers, Weijzen et al. 2004; Oh, Mukhopadhyay et al. 2005).

Sensory Signals:*C. elegans* has numerous chemosensory receptors to detect environmental cues, and mutations in these receptors can extend lifespan by up to 50% (Apfeld and Kenyon 1999; Alcedo and Kenyon 2004). In addition, removal of specific neurons can either positively or negatively regulate lifespan, implying the existence of neuronal circuits that influence longevity (Alcedo and Kenyon 2004). The longevity of these sensory mutants is largely, but not entirely,*daf-16* dependent (Apfeld and Kenyon 1999) and DAF-16 is nuclear localized in these sensory mutants (Lin, Hsin et al. 2001). Also, many sensory neurons produce insulin-like peptides that influence longevity (Pierce, Costa et al. 2001; Li, Kennedy et al. 2003; Murphy, McCarroll et al. 2003) suggesting that sensory neurons regulate lifespan, largely by modulating the insulin-like pathway. It remains to be seen if sensory perception affects aging in mammals.

Mitochondria and Reactive Oxygen Species: Mitochondrial function and generation of reactive oxygen species (ROS) have also been implicated in *C. elegans* aging regulation. An RNAi screen for longevity genes identified multiple components of the mitochondrial electron transport chain (Dillin, Hsu et al. 2002; Lee, Lee et al. 2003), indicating the role of mitochondrial function in lifespan regulation. The longevity phenotypes of these genes were independent of both *daf-2* and *daf-16*, implying that the mitochondria do not signal through the insulin-like signaling pathway to regulate aging

(Dillin, Hsu et al. 2002).

The cellular use of oxygen under normal conditions generates reactive oxygen species (ROS) that are deleterious to the cell. ROS cause extensive damage to bio-molecules through peroxidation of membrane lipids, modification of DNA, carbonylation and other protein modifications (Sohal and Weindruch 1996). The amount of cellular oxidative damage that accumulates as the organism ages is thought to be a major causal factor in the natural decline of cellular functions and death. Consistent with the hypothesis, *C. elegans* lifespan is extended with growth in low oxygen and shortened when exposed to a high oxygen environment (Honda, Ishii et al. 1993).Also, in *C. elegans,* electron transport chain mutants, *mev-1* and *gas-1* overproduce ROS, and hence, are hypersensitive to oxidative stress and have shortened lifespans (Adachi, Fujiwara et al. 1998; Kayser, Morgan et al. 1999).

Overexpression of Cu-Zn-superoxide dismutase (SOD) and catalase, which act in tandem to sequester ROS, extends lifespan in flies (Orr and Sohal 1994; Sohal, Agarwal et al. 1995).These transgenic animals also exhibited less age related accumulation of oxidative damage to DNA and proteins, and were more resistant to oxidative stress in response to X-ray exposure (Orr and Sohal 1994; Sohal, Agarwal et al. 1995). SOD and catalase are partly regulated by DAF-16, as activation of DAF-16 leads to increased levels of SOD and catalase among other stress response genes (Halaschek-Wiener, Khattra et al. 2005). Oxidative stress also induces nuclear localization of DAF-16 (Henderson and Johnson 2001; Lin, Hsin et al. 2001), indicating crosstalk between these pathways and adding to the complexity of lifespan regulation.

skn-1 also responds to oxidative stress, in this case by localizing to intestinal nuclei in *C. elegans* (An and Blackwell 2003). Many of the genes for detoxifying enzymes that function to sequester ROS, such as SOD and catalase, contain a SKN-1 binding site within 1kb of their transcriptional start sites(An and Blackwell 2003). An and Blackwell also demonstrated that the expression of oxidative stress response gene *gcs-1* is under *skn-1* regulation. Hence these two transcription factors, *skn-1* and *daf-16,* may be

working in tandem under oxidative stress to regulate the oxidative stress response mechanism.

Dietary Restriction: Dietary restriction (DR) extends lifespan in many animals, including yeast, worms, flies and rodents (Reviewed in (Guarente and Picard 2005). While lifespan extension by DR does not seem to be *daf*-16 dependent in *C. elegans*(Lakowski and Hekimi 1998; Houthoofd, Braeckman et al. 2003), it is regulated through the insulin/IGF-1 pathway in flies (Clancy, Gems et al. 2002). In mice, as in worms, it seems that the DR response may be independent of *daf-16* and the insulin/IGF-1 pathway (Bartke, Wright et al. 2001).

SIR2(SIRT1 in mammals), an NAD dependent histone deacytelase involved in silencing mating-type loci in yeast, has emerged as a central regulator of DR mediated lifespan extension. Yeast and flies exposed to DR are dependent on *SIR2* and dSir2 respectively, for longevity (Lin, Defossez et al. 2000; Rogina and Helfand 2004), and the SIR2-activator resveratrol extends the lifespan of yeast, worms and flies (Howitz, Bitterman et al. 2003; Wood, Rogina et al. 2004). Overexpression of SIR2 orthologs also increases lifespan in both worms and flies (Tissenbaum and Guarente 2001; Rogina and Helfand 2004). However, in worms, lifespan extension by overexpression of *sir-2.1* is *daf-16* dependent suggesting that *sir-2.1* is unlikely to mediate the longevity response to DR in *C. elegans*. *sir-2.1* is also involved in potentiating dauer formation, strengthening *sir-2.1* as a part of the insulin-like signaling pathway in *C. elegans* (Tissenbaum and Guarente 2001).

The nutrient sensing TOR (target of rapamycin) pathway may also be involved in DR response. TOR is a protein kinase that phosphorylates ribosomal S6 kinase and translation initiation factor 4E binding protein (inhibitor of eukaryotic initiation factor 4E) in response to nutrients to promote growth (Inoki, Ouyang et al. 2005). Decreasing TOR activity increases lifespan in both flies and worms (Jia, Chen et al. 2004; Kapahi, Zid et al. 2004). Moreover, DR cannot further extend the lifespan of these mutant

flies,suggesting that longevity response to DR involves down-regulation of TOR.

Gene Expression Regulators *lin-11* and *let-711*

Expression profiles of *lin-11* and *let-711* are aging co-regulated

As discussed above, aging is a complex biological process involving contributions from numerous signaling pathways. Organismal aging and lifespan is subject to regulation by gene expression regulators that control the expression of a multitude of aging genes. Transcription factors such as *daf-16, skn-1* and *hsf-1* have been shown to dramatically extend lifespan in a range of organisms. Indentifying other regulators of aging genes would provide valuable insights into the complex science of aging and lifespan regulation.

I am interested in studying the role of two gene expression regulators,*lin-11* and *let-711,* in *C. elegans* aging. They were selected from a pool of 164 genes that showed strong aging correlated expression profiles (Lund, Tedesco et al. 2002). Of the genes that were known to have gene expression regulatory functions within that pool,*lin-11* and *let-711* showed the strongest aging correlated expression profiles, and hence, were selected for further study. *lin-11* is strongly upregulated in very old worm populations, whereas *let-711* shows a progressively downregulated expression profile as the worm population ages. Neither gene had a previously known role in aging regulation. Since aging degrades cellular defenses and increases cellular deterioration, the complement of expressedgenes ideal for a young worm is probably not optimal for an old worm. Gene expression regulators such as *lin-11* and *let-711*,which show aging correlated expression profiles,may be involved in reshaping the cell to better fit this changing internal environment to allow cells to combat the effects of aging.

lin-11, an early-developmental transcription factor

lin-11 encodes a LIM homeodomain transcription factor that affects vulval development, uterine morphogenesis, neuronal development and fate specification (Ferguson and Horvitz 1985; Freyd, Kim et al. 1990; Garriga, Desai et al. 1993; Newman, Acton et al. 1999; Sarafi-Reinach, Melkman et al. 2001). Accordingly, it is expressed in some neurons, vulval cells and the spermatheca, and*lin-11* mutants display many phenotypes, including vulvaless and egg-laying defective. It was first identified in a screen for mutants that are defective for vulval induction and development, which led to a vulvaless phenotype (Ferguson and Horvitz 1985; Freyd, Kim et al. 1990). The *C. elegans* vulva develops post-embryonically from Vulval Precursor Cells (VPCs). During the L3 stage, a signal from the gonad (anchor cell) and signaling among the VPCs specifies three VPCs to generate 22 vulval cells (Sulston and Horvitz 1977). These mature vulval cells are grouped into seven categories that differ in their patterns of gene expression and cell biology: vulA, vulB1, vulB2, vulC, vulD, vulE and vulF(Burdine, Branda et al. 1998; Inoue, Sherwood et al. 2002; Kirouac and Sternberg 2003; Inoue, Wang et al. 2005). *lin-11* is one of the transcription factors responsible for the identities of specific vulval cell types. It is more highly expressed in vulC and vulD cells than in vulA and vulB cells, and regulates gene expression in these vulval cell types for proper development of the vulva (Ferguson and Horvitz 1985; Gupta, Wang et al. 2003). *lin-11* is also required for differentiation of pi cell progeny during uterine development (Newman, Acton et al. 1999).Sumoylation of LIN-11 is necessary for its function in pi cell specification (Broday, Kolotuev et al. 2004).

Apart from vulval and uterus morphogenesis, *lin-11* has also been demonstrated to be involved in neuronal cell fate specification and axonal pathfinding. Genetic studies show that *lin-11* regulates differentiation of AWA olfactory neurons, while misexpression studies reveal *lin-11* is sufficient to promote ASG chemosensory neuron fate in a subset of neuron types (Sarafi-Reinach, Melkman et al. 2001).

***let-711,* an early-developmental gene expression regulator**

let-711 is progressively downregulated in worm populations older than six days (Lund, Tedesco et al. 2002). *let-711* was first identified in screens for defects in positioning of the mitotic spindle during cleavage (Rose and Kemphues 1998). Cellular studies reveal that *let-711* is essential in *C. elegans* for proper spindle positioning, microtubule length and centrosome morphology in early embryos(DeBella, Hayashi et al. 2006). *let-711* is homozygous lethal at the early or mid larval stage (Stewart, O'Neil et al. 1998), whereas partial loss of function leads to defects in centration and rotation movements that position the first mitotic spindle in the embryo (DeBella, Hayashi et al. 2006). *let-711* embryos also have longer microtubules, which contrasts the short microtubule phenotype caused by loss-of-function mutations in the C elegans XMAP215 homolog,*zyg-9*(Kemphues, Wolf et al. 1986; Matthews, Carter et al. 1998). RNAi knockdown experiments further demonstrated that simultaneous reduction of ZYG-9 and LET-711 could rescue the centration and rotation defects of both single mutants leading to speculation that *let-711* may be downregulating *zyg-9* expression (DeBella, Hayashi et al. 2006). RNAi knockdown of *let-711* has also been reported to produce multiple defects, including larval arrest, sterility, embryonic lethality and osmotically sensitive eggs (Gonczy, Bellanger et al. 2001; Maeda, Kohara et al. 2001; Kamath, Fraser et al. 2003; Simmer, Moorman et al. 2003; Sonnichsen, Koski et al. 2005).

Molecular identification of LET-711 shows it to be an ortholog of NOT1, the core component of the CCR4/NOT complex, which has been implicated in both positive and negative control of transcription, repression of gene expression through mRNA deadenylation and targeting proteins to the proteasome for degradation via ubiquitination (Daugeron, Mauxion et al. 2001; Tucker, Valencia-Sanchez et al. 2001; Collart and Timmers 2004). NOT1 is the largest subunit and the only protein of the complex that is essential for yeast viability (Maillet, Tu et al. 2000). It functions, most likely, as a scaffold for the CCR4/NOT complex as all subunits interact with NOT1 in yeast two-hybrid experiments (reviewed in Collart and Timmers 2004). In *C. elegans, let-711* is expressed broadly in the gonadal tissue, as determined through insituhybridization

procedures (Kohara 2005).

What is the adult function of these early-developmental gene expression regulators,and why do they show an aging correlated expression profile? Are these genes involved in aging regulation in the adult worm? These questions are the driving force of my study and thesis research. Through my research, I show that aging genes are indeed mis-regulated when either gene is knocked down, and propose mechanisms by which they may be regulating aging in adult *C. elegans.*

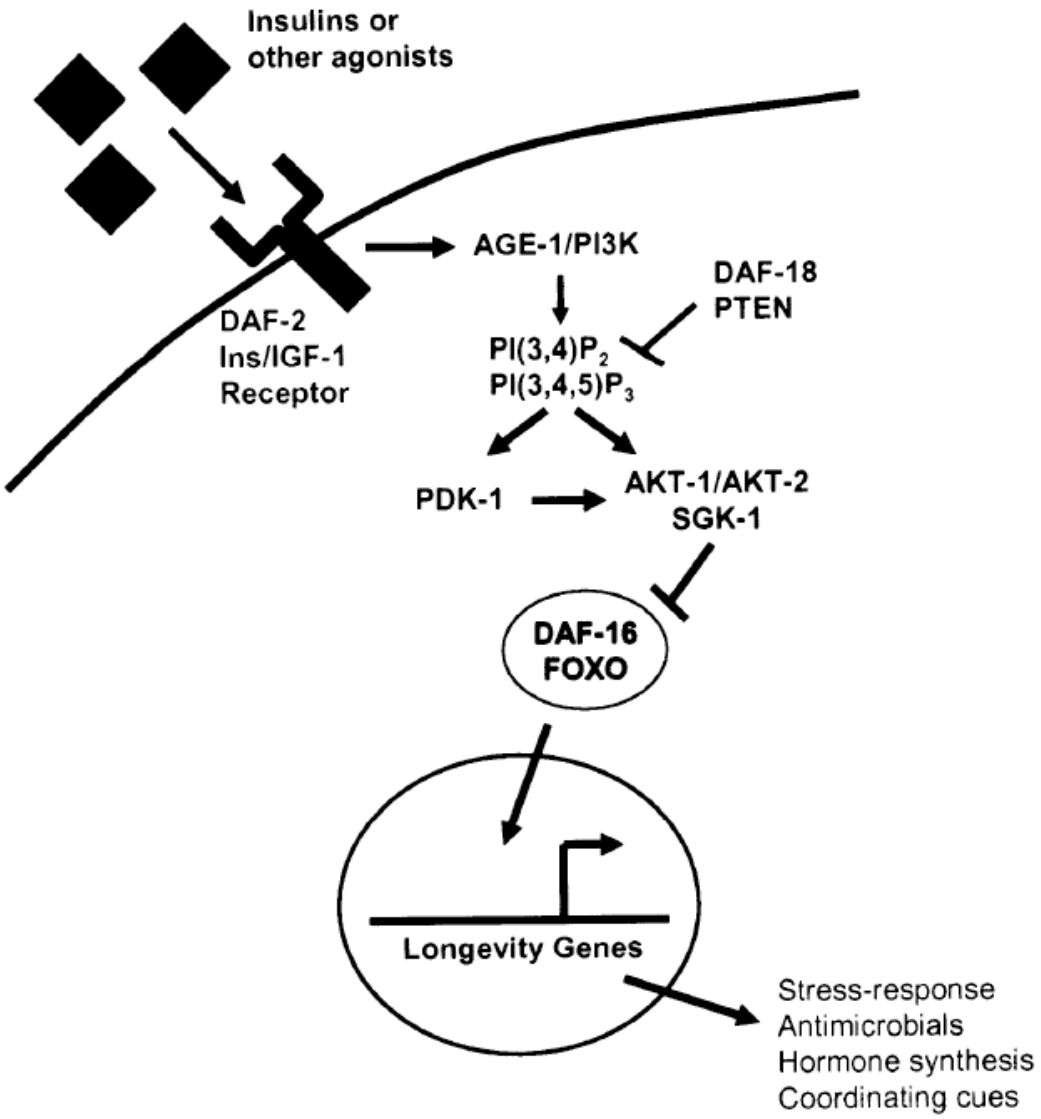


Figure 1.1 The Insulin-like signaling pathway: A simplified schematic of the conserved DAF-2 insulin/IGF-1 pathway. Activation of DAF-2 by insulin-like agonists triggers a PI 3-kinase cascade that blocks the nuclear localization and activity of the DAF-16/FOXO transcription factor. Upon reduction of pathway activity, DAF-16 enters the nucleus where it activates transcription of genes that promote longevity and genes that coordinate the aging response across cells. Some *daf-2* mutants live over twice as long as wild type. Adapted from (Tatar, Bartke et al. 2003).

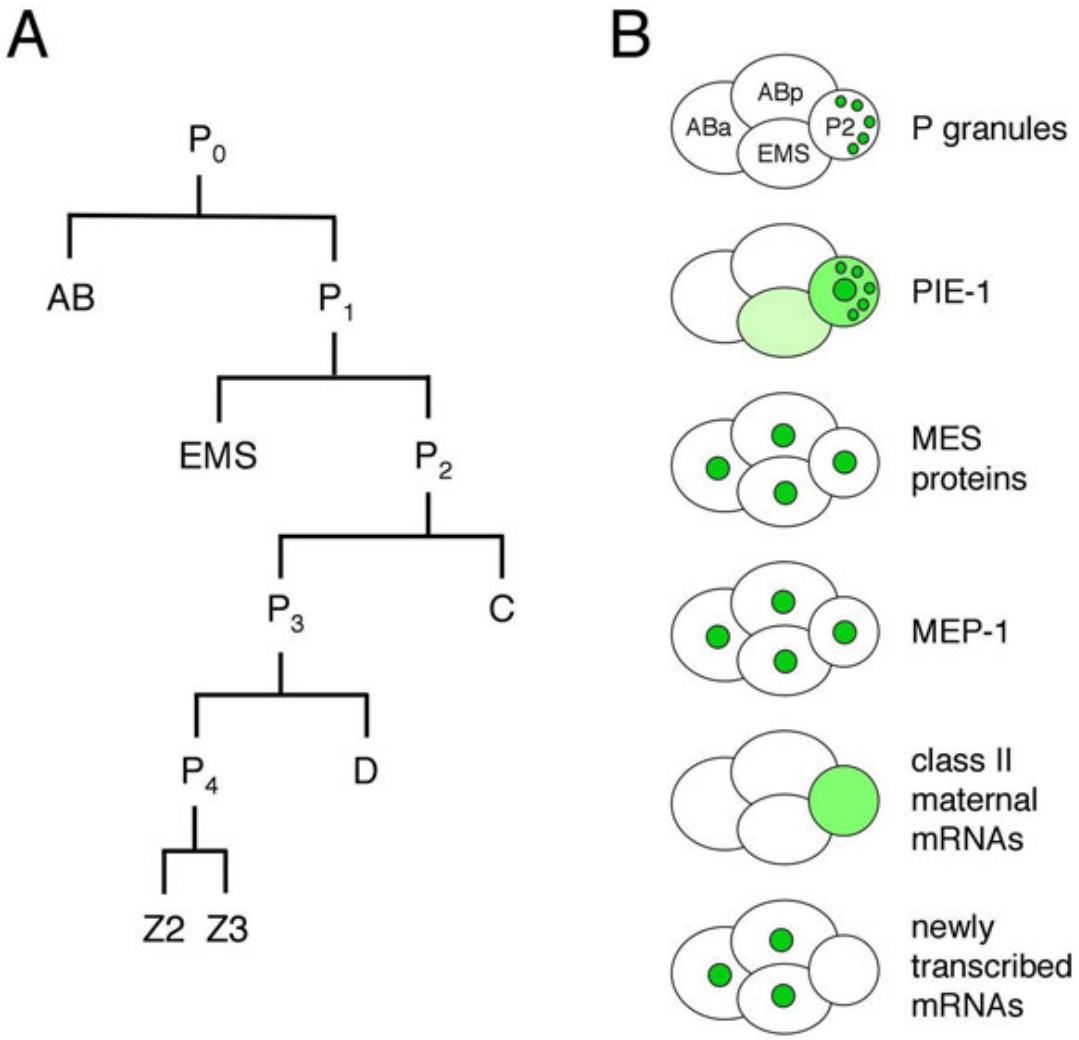


Figure 1.2 The early embryonic lineage and summary of the summary of asymmetric distribution of various cell-fate determinants in 4-cell embryos: (A) Unequal divisions of the germline blastomeres, P0, P1, P2 and P3 generate the somatic blastomeres AB, EMS, C and D, and the primordial germ cell P4. P4 divides equally into Z2 and Z3 at the ~100-cell stage. (B) The cartoons of 4-cell embryos illustrate that P granules are partitioned to the germline blastomeres, PIE-1 resides in the germline cytoplasm and nucleus and also associates with P granules, the MES proteins and MEP-1 are in the nuclei of all cells in early embryos, class II maternal mRNAs persist in the germline blastomeres and are degraded in the somatic blastomeres and new transcription turns on in the somatic blastomeres and not in the germline blastomeres (Adapted from WormBook chapter: Specification of the germ line (Strome 2005))

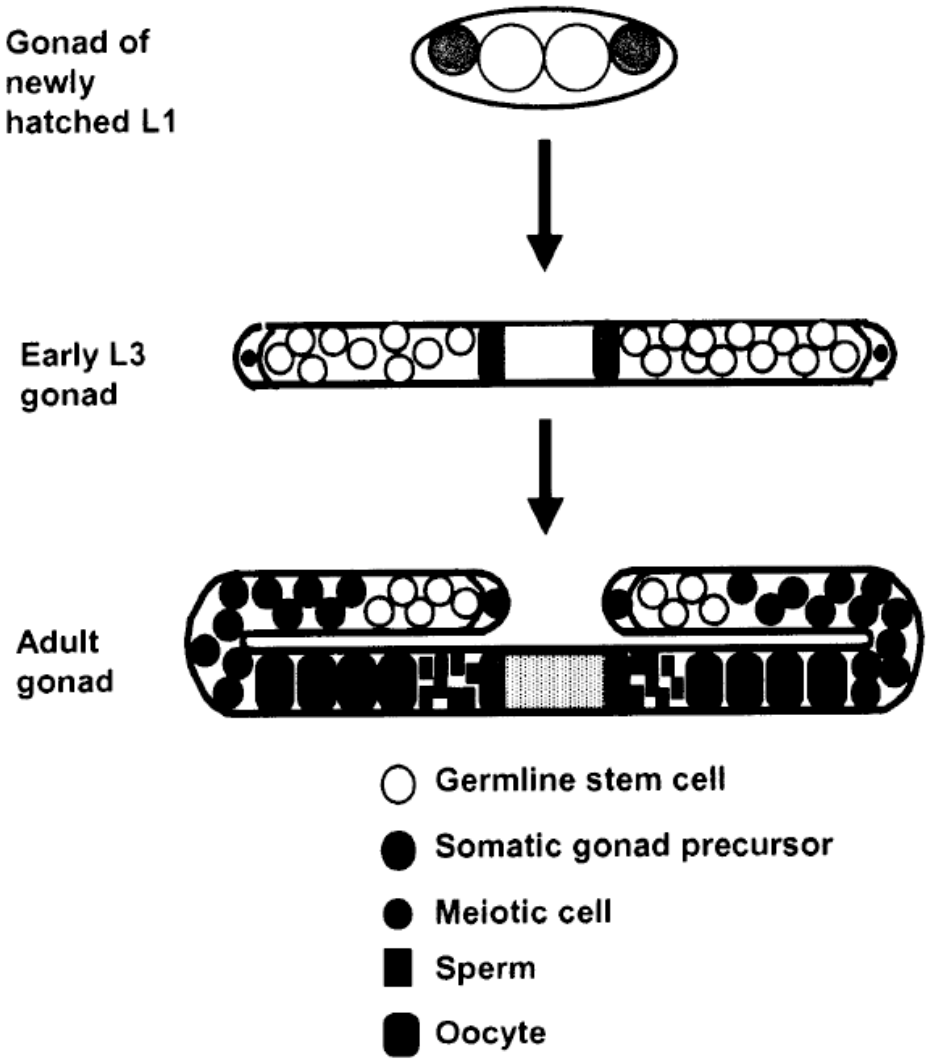


Figure 1.3 Development of the reproductive system in *C. elegans:* At hatching, the L1 gonad contains two somatic gonad precursors Z1 and Z4 (shaded circles) and two germline precursors Z2 and Z3 (open circles). Over time, the somatic gonad precursors give rise to a U-shaped reflexed tube that contains the germline. During L3/L4 stage the most proximal germline stem cells (GSCs) give rise to sperm, which get stored in the spermatheca. GSCs then give rise to oocytes, and proliferate into adulthood. Hermaphrodite reproductive system development is shown. Adapted from (Schedl 1997).

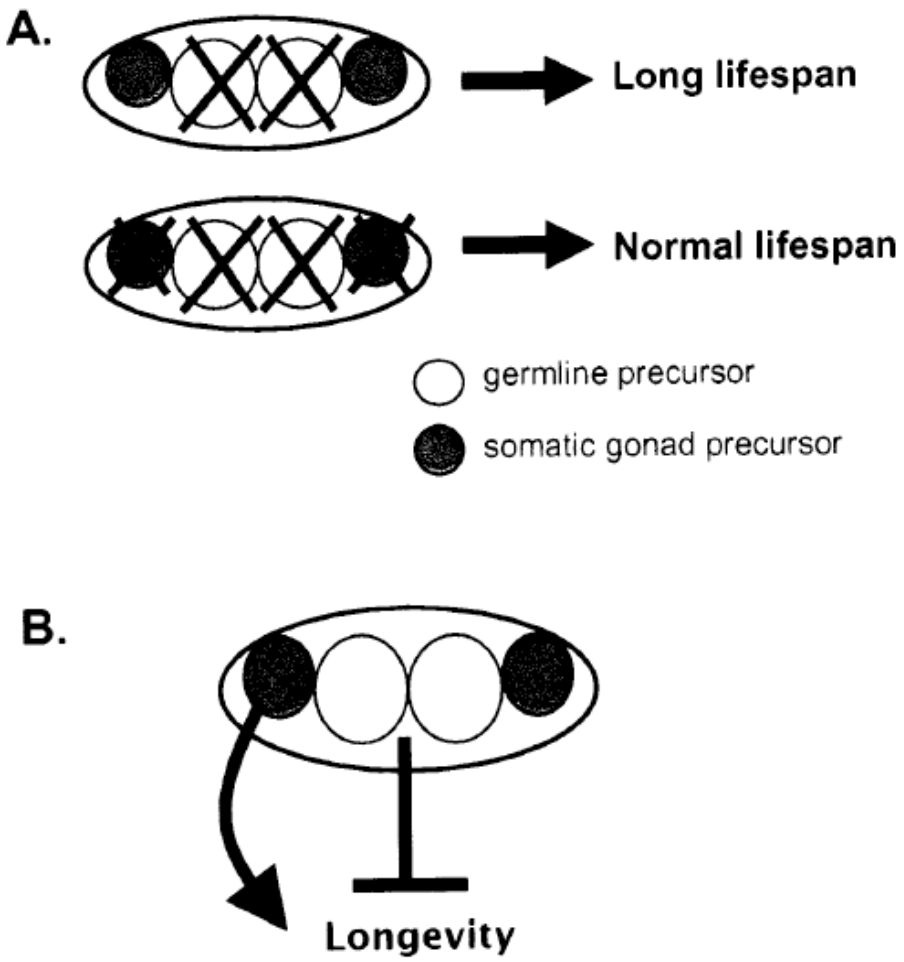


Figure 1.4 Effects of germline and whole gonad ablation on *C. elegans* lifespan: A) When the germline precursors (open circles) are removed using a laser or mutations, adult worms live ~50-60% longer than wild type controls. This effect is dependent on the somatic gonad, since removal of the gonad precursors restores lifespan to wild type levels. B) A simplified model of how the reproductive system regulates aging in *C. elegans*. Proliferating germ cells produce a signal that shortens lifespan, while a signal from the somatic gonad acts to promote longevity. Although the L1 gonad is shown, the pathway refers to effect from adult tissues. Adapted from (Hsin and Kenyon 1999).

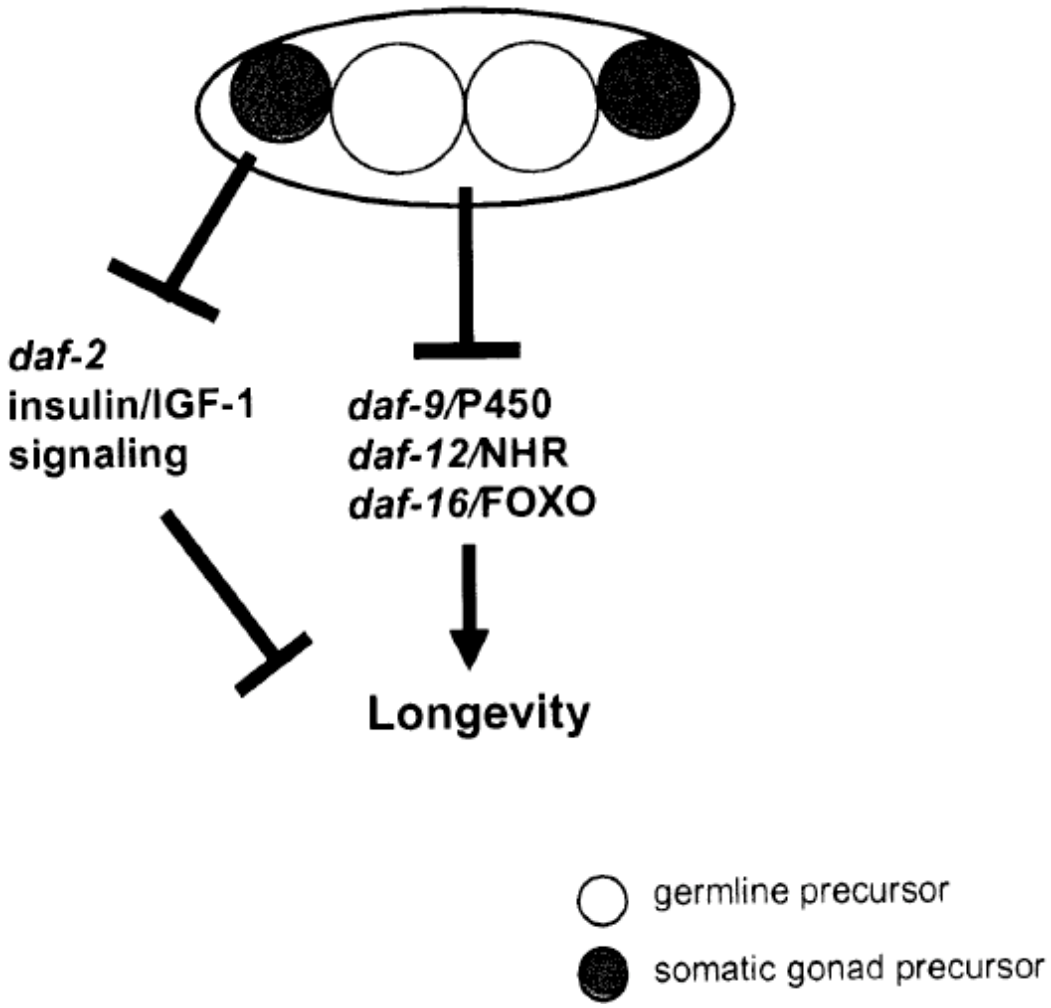


Figure 1.5 A genetic working model for the effects of the reproductive system on aging: For germline ablation to increase lifespan, the animal requires the functions of *daf-9*, a cytochrome P450, *daf-12*, a nuclear hormone receptor, and *daf-16*, a FOXO transcription factor. The germline regulates lifespan independently of the *daf-2*/insulin-like signaling pathway since germline ablation further extends the lifespan of *daf-2* mutants. The somatic gonad cannot act to promote longevity in *daf-2* mutants, indicating that the somatic gonad functions to increase lifespan by antagonizing *daf-2* signaling. An L1 gonad is shown for simplicity, although these pathways have been shown to work during adulthood. Adapted from results from (Hsin and Kenyon 1999; Gerisch, Weitzel et al. 2001).

Thesis Overview

The goal of my research was to investigate whether gene expression regulators *lin-11* and *let-711* regulate aging in *C. elegans.* As discussed in the introduction, it is clear that numerous cellular mechanisms exist that regulate the rate of organismal aging. These pathways influence aging by regulating the activity of a few gene expression regulators, such as DAF-16, HSF-1, SKN-1 and SIR-2.1. In this project, I have characterized the role of two additional gene expression regulators in the regulation of *C. elegans* aging.

lin-11 and *let-711* had previously been characterized as developmental genes and had no identified role in aging regulation. *lin-11* is a LIM homeodomain transcription factor involved in neuronal development and fate specification, as well as vulval development. *let-711* is the NOT1 ortholog of the CCR4/NOT complex, which is involved in gene expression regulation. *let-711* is required in *C. elegans* for proper centrosomal and spindle morphology during early embryogenesis. I became interested in these genes as potential aging regulators, since they showed a strong aging-correlated expression profile in an aging time-course microarray study (Lund, Tedesco et al. 2002). *lin-11* is strongly upregulated in very old worms and *let-711* is progressively downregulated in aging worm populations.

In Chapter 2,data will show that *lin-11* and *let-711* are aging regulators. RNAi knockdown of either gene resulted in a reduced lifespan in *C. elegans.* Microarray studies were then performed to identify the genome-wide targets of *lin-11* and *let-711* in adult worms, to study whether they regulate aging genes. Using two-fold change as the cutoff value, sixteen genes were identified as *lin-11* target candidates and sixty genes as *let-711* target candidates. These genes identified from the microarray experiments were individually knocked down to study their effect on lifespan. I found these gene pools to be highly enriched for aging genes, thus establishing *lin-11* and *let-711* as regulators of aging genes in adult *C. elegans.*

Sixteen genes show at least two-fold change in expression when *lin-11* was knocked down. I then performed lifespan assays with RNAi knockdown of eleven genes from this pool that had an existing RNAi construct (Kamath and Ahringer 2003). RNAi knockdown of four of these genes significantly extended*C. elegans* lifespan (Figures 2.5, 2.6, 2.7), showing that this pool is highly enriched for aging genes and hence providing strong evidence that *lin-11* regulates aging genes in adult *C. elegans.*

RNAi knockdown of *lin-11* also produced a two-fold increase in insulin-related peptide *ins-3* (Figure 2.4b), which suggests that *lin-11* maybe be regulating lifespan in *C. elegans* through the insulin/IGF-1 signaling pathway.I propose that upregulation of *lin-11* in old worms may be contributing to longevity because *lin-11* downregulates *ins-3* in wild-type adult worms, which leads to reduced insulin/IGF-1 signaling, to preserve lifespan.

The pool of differentially expressed genes upon *let-711* RNAi is also highly enriched for aging genes. I knocked down the expression of the 27 most differentially expressed genes when *let-711* was knocked down, to study their effect on lifespan. Individual RNAi of seven of these genes significantly extended lifespan indicating that this pool is highly enriched for aging genes. Thus *let-711* is regulating the expression of aging genes in adult *C. elegans.*

Down regulation of *let-711* in aging worms may be protective because reduced *let-711* activity leads to reduced insulin/IGF-1 signaling. *let-711* RNAi resulted in reduced *pdk-1* expression, which is an integral part of the insulin/IGF-1 pathway. Accordingly, *pdk-1* RNAi significantly extended *C. elegans* lifespan (Figure 2.8). Therefore *let-711* exerts its influence on lifespan by upregulating the insulin/IGF-1 pathway.

The results also show that both gene expression regulators control the expression of aging genes that are not known to be a part of the insulin/IGF-1 pathway. RNAi knockdown of*let-711* in particular, produces downregulated expression of several

germline-related genes, and RNAi of these genes extend lifespan (Figures 2.9, 2.10). Since the germline is known to regulate aging, I further investigate the mechanism of aging regulation by these genes in the next chapter, to see whether *let-711* is also regulating aging through the germline-signaling pathway.

In Chapter 3, further evidence is provided to establish *let-711* as a regulator of aging. Genetic studies were performed with the *let-711*-regulated, germline-related genes to investigate whether the mechanism of aging regulation by these genes agrees with current knowledge of germline regulation of aging. The results demonstrate that the lifespan extension by these genes require *daf-16,* but is independent of *daf-2,* which is characteristic of the germlineregulation of aging. These results indicate that aging *C. elegans* maybe actively restricting germline proliferation through downregulation of *let-711* to preserve longevity.

I also show that lifespan extension due to RNAi of *let-711* regulated gene,*cdc-25.1,* is dependent on both DAF-16 and DAF-2, unlike other germline related genes in this study. This indicates that *cdc-25.1,* being a checkpoint gene, functions upstream of DAF-2 to upregulate the insulin/IGF-1 signaling to influence lifespan.

Based on the results of this study, I propose a model for *let-711* regulation of aging in *C. elegans* (Figure 3.4)*: let-711* influences lifespan determination in adult *C. elegans* by inhibiting DAF-16 activation. It does so in at least three distinct ways: 1. By upregulating expression of *pdk-1,*leading to increased insulin/IGF-1 signaling to inhibit DAF-16 activity. 2. By upregulating expression of germline proliferation genes leading to increased germline signals to inhibit DAF-16 activity (independent of DAF-2). 3.By upregulating expression of *cdc-25.1* leading to inhibition of DAF-16 through cell-cycle checkpoint signaling mechanisms (upstream of DAF-2).

CHAPTER 2: Characterization of early developmental transcription factors *lin-11* and *let-711* as aging regulators.

Introduction

Aging is a complex biological process involving contributions from numerous genes. A systematic RNAi screen for longevity genes in *C. elegans* identified 89 genes, whose inactivation extends lifespan (Hamilton, Dong et al. 2005). Microarray studies identified 164 genes with significant changes in expression as aging occurs (Lund, Tedesco et al. 2002),and Serial Analysis of Gene Expression identifies 120 genes with significant differences in expression between long-lived and normal wild type *C. elegans*(Ruzanov, Riddle et al. 2007). Many of these genes have shown up in multiple studies, and in total, roughly 1% of *C. elegans* genes have been identified as aging genes.

Over the last several decades, it has become clear that genetic pathways exist that regulate the rate of organismal aging and that these pathways can respond to environmental inputs (reviewed in Guarente and Kenyon 2000). The insulin/IGF-1 pathway, germline signaling pathway, and TOR pathway,are some of the cellular signaling pathways with considerable evidence for their role in aging regulation. Through the use of model organisms with a short lifespan, such as *Caenorhabditis elegans, Drosophila melanogaster, Saccharomyces cerevisiae* and even *Mus musculus,* researchers have shown that single gene mutations in these pathways can drastically change the rate of aging and lifespan of the organism (reviewed in (Guarente and Kenyon 2000). These genetic pathways influence the activity of key gene expression regulators that controlthe expression of a multitude of aging genes leading to changes in organismal lifespan. A gene is characterized to be an aging gene, if manipulating the activity of that gene leads to a delay in aging or an extension of lifespan.

DAF-16 is such a master regulatorwith a well-established role in lifespan regulation. The longevity phenotype of mutants in the insulin/IGF-1 pathway is completely dependent on DAF-16/FOXO transcription factor. While microarray studies identify over 1,600 direct and indirect target genes for DAF-16 (McElwee, Bubb et al. 2003; Murphy, McCarroll et al. 2003), a ChIP assay identified 103 direct target sequences (Oh, Mukhopadhyay et al. 2006). As such, DAF-16 directly regulates at least 103 genes, many of which have secondary effects in gene epression or are directly involved in gene expression regulation, resulting in a bigger pool of over 1600 direct and indirect targets.As discussed in chapter one, there are other regulators of aging and there likely are still other genes that we do not know about that regulate aging. Identifying additional regulators of aging gene expressionwould help us decipher the complex mechanisms by which an organism regulates its lifespan. These regulators can act in parallel, upstream or downstream of *daf-16.*

I am particularly interested in two additional gene expression regulators: *lin-11* and *let-711*. Both genes had no known role in aging regulation, but show strong aging-correlated expression profiles: *lin-11* is strongly upregulated in very old worm populations, whereas *let-711* shows progressivelydownregulated expression as the worm population ages. *lin-11* is a LIM homeodomain transcription factor involved in neuronal development, fate specification, and vulval development (Ferguson and Horvitz 1985; Freyd, Kim et al. 1990; Garriga, Desai et al. 1993; Newman, Acton et al. 1999; Sarafi-Reinach, Melkman et al. 2001). *let-711* is the NOT1 ortholog of the CCR4/NOT complex, a complex of nine subunits involved in all stages of gene expression (reviewed in Collart and Timmons 2004). *let-711* is essential in *C. elegans* for proper spindle positioning, microtubule length and centrosome morphology in early embryos (DeBella, Hayashi et al. 2006), and these phenotypes are likely to arise from effects of the CCR4/NOT complex on gene expression.

In this chapter, I identify the genome-wide targets of *lin-11* and *let-711* through microarray analysis and show that their target pools are enriched for aging genes. Therefore,*lin-11* and *let-711* regulate the expression of aging genes to influence the

lifespan in adult *C. elegans.*

Results and Discussion

Individual knockdown of*lin-11* and *let-711* in adult worms shortens lifespan

As mentioned previously,*lin-11* and *let-711* show strong aging expression profiles. To understand whether these gene expression regulators are involved in aging regulation, I started by knocking down each gene in adult worms to look for an effect on adult lifespan. Both genes are required for normal development: loss of *lin-11* results in vulvaless worms leading to "bag of worms" phenotype and a premature death, while loss of *let-711* results in larval lethality. Accordingly, in my experiments, initiating RNAi knock down of *let-711* as the eggs hatch (L1) led to larval lethality and that of *lin-11* let to bagging in adults, indicating effective knockdown of these genes through RNAi (data not shown). Therefore, RNAi was induced starting on the first day of adulthood (day 3) by the feeding method (Timmons and Fire 1998; Timmons, Court et al. 2001) using *rrf-3* worms, and lifespan assay was carried out in four independent trials for each gene (see methods for details). *rrf-3* is a worm strain that is hypersensitive to RNAi (Simmer, Tijsterman et al. 2002).

Gene expression knockdown via RNAi in *C. elegans* is a robust and reliable tool (Fire, Xu et al. 1998; Timmons and Fire 1998). Hence it is not uncommon in *C. elegans* literature, for RNAi knockdowns not to be validated through independent methods such as RT-PCR. This is especially true if the RNAi procedure induces a phenotype, since the level of false postives is extremely low (less than one percent)(Kamath, Fraser et al. 2003). Another variable to take into account when using RNAi is the stability of the protein product of the gene being knocked down. Some proteins are stable over many days, and could still be present even if mRNA is effectively degraded, and it is possible that a small amount of residual protein is sufficient for gene activity. These factors

contribute to the higher level of false negatives at about 30% over all genes (Kamath, Fraser et al. 2003). An antibody can be used to measure the level of protein reduction, but this is less of a concern for this study since I am only interested in positive RNAi results.

lin-11 knockdown in adult *C. elegans*significantly reduced mean lifespan by seven percent from 18.4±0.4 days (n=166) to 17.1±0.3 days (n=169) (Figure2.1). Since *lin-11* knockdown in adult worms reduced lifespan, and it is strongly upregulated in very old worms, it may normally be acting to preserve lifespan in *C. elegans.* Alternatively, *lin-11* may be required for survival and thus the lower lifespan may be due to non-specific deleterious effects of the reduction in its expression.

Knockdown of *let-711,* which is normally, progressively downregulated in *C. elegans* populations older than six days,also resulted in reduced mean lifespan. *let-711* RNAi in adult worms reduced mean lifespan by fifteen percent, from 18.4±0.4 days, n=166 to 15.6±0.3 days, n=166 (Figure 2.1). This suggests that downregulated expression of *let-711* in aging worms may be contributing towards their deaths.

Since neither experiment was able to extend lifespan, they cannot be characterized as aging regulators from these experiments alone. It is possible that these genes maybe causing cellular disruptions that are unrelated to aging, leading to a shortened mean lifespan of the population. To continue the investigation of whether *lin-11* and *let-711* are involved in aging regulation, I wanted to identify their genome-wide targets to find out if these genes regulate the expression of known aging genes.

It is interesting to note that in the microarray study conducted by Lund et al., there is no progressive upregulation of *lin-11* as the *C. elegans* population ages. Instead, it has a strong spike in expression levels in the last 10% of the population still alive. It is unlikely that normal worms upregulate *lin-11* expression just before death, for whatever reason, because we should be able to see such upregulation as the survival curve turns sharply past midlife. It is possible that worms are not upregulating *lin-11* expression to combat aging, in which case, you would expect a progressive upregulation as the

population ages. Instead, about 10% of the population may be stochastically expressing *lin-11* at a higher than normal level, which enables them to outlive others in their population. Such stochastic expression levels would only show up in the microarray data when most of the worms that express *lin-11* at a normal level are dead. Testing whether a subset of the worm population stochastically expresses*lin-11* at a higher level, and whether such higher expression levels correlate to longer lifespan is discussed in the future studies section of this thesis.

Genome-wide target identification of *lin-11* and *let-711* in adult worms utilizing microarray analysis

To identify the genome-wide targetsof *lin-11* and *let-711*,in an effort to understand their functionsin the adult worm,microarray experiments were performed to compare genome-wide expression profiles of *lin-11* or *let-711* knocked down worms against a control population of *rrf-3* worms. *rrf-3* worms were maintained at 20°C and fed bacteria expressing dsRNA against *lin-11* or *let-711* from the first day of adulthood (3 days old) and total RNA was collected from these populations on day7, four days later, for microarray analysis. Total RNA was extracted on day 7 to minimize sampling of embryonic genes since worms have typically finished laying eggs by that time. Extracted RNA was hybridized to the AffymetrixGeneChip: *C. elegans* Genome Array.Probe-set level data were subjected to quality control by examining pair-wise M vs A plots among replicate GeneChipsto examine agreement among replicates for each experiment (Figure 2.2) (Bolstad, Collin et al. 2005). M is the sample to control intensity ratio: $M= \log_2 R- \log_2 G$. A is the average intensity: $A=1/2*(\log_2 R+ \log_2 G)$. One *lin-11* RNAi sample was discarded upon visual inspection of the chip image. Data normalization and analysis were performed as described in the methods.

Genes with strong and consistent expression differences between the control and experimental samples were identified. Applying a False Discovery Rate(Benjamini and Hochberg 1995)analysis with a cutoff of $p = 0.05$,further selection was done to identify

probe-sets that were up or down-regulated by at least two fold, a conservative filter that only includes genes with strong expression level change. A total of sixty-nine probe-sets yielded significantly different hybridization intensities (transcript abundance) from the control (Table 2.1). Sixteen genes displayed at least two-fold change in their expression levels when *lin-11* was knocked down (Figure 2.4b, Table 2.1b) andsixty genes were differentially expressed by at least two fold when *let-711* was knocked down (Figure 2.4c, Table 2.1c). Seven genes show significant change in expression in both *lin-11* and *let-711* knockdowns (by two-fold in at least one experiment). Four genes were upregulated and three downregulated in both microarray experiments (Figure 2.4a, Table 2.1a).

lin-11 knockdownincreased expression of an insulin-related peptide

RNAi knockdown of *lin-11* in adult worms (day 3) produced a two-fold increase in the expression of *ins-3* by day 7 (Figure 2.4b). While it is clear that *daf-2* is the insulin-like receptor in *C. elegans* that activates the insulin/IGF-1 pathway to prevent nuclear localization of *daf-16* (leading to reduced lifespan), the identity of the ligand that activates *daf-2* receptor is unclear. INS-3 is one of thirty-seven insulin related peptides and it is expressed in some amphid sensory neurons (www.wormbase.org version WS219; (Pierce, Costa et al. 2001). Increased INS-3 levels may activate the insulin-like signaling pathway to shorten lifespan. If so, the increased expression of *lin-11* in very old worms may be acting to extend lifespan by down-regulating *ins-3* (and hence the Insulin-like Signaling Pathway). I did not test *ins-3* effect on lifespan further as it was not available in the *C. elegans* RNAi library (Kamath and Ahringer 2003; Kamath, Fraser et al. 2003). Elucidating the role of*ins-3*in aging regulation is discussed further in the future studies section.

lin-11 target pool is enriched for aging genes

RNAi knockdown of *lin-11* resulted in a slight reduction in mean lifespan of *C. elegans*(Figure 2.1).This does not help clarify whteher*lin*-11 has a role in aging regulation because the phenotype could be due to aging unrelated deleterious effects.Ithen wanted to see if *lin-11* is regulating the expression of aging genes, thereby influencing *C. elegans* aging. I knocked down the expression of the microarray identified *lin-11* target genes inorder to identify aging genes in *lin-11's* target pool. If knocking down a particular gene leads to an extension in lifespan, it would indicate that gene to be an aging gene.

I performed lifespan assays for RNAi knockdowns of eleven of the *lin-11* targets identified through microarray analysis (see methods). The remaining five targets did not have dsRNA construct in the *C. elegans* RNAi library (Kamath and Ahringer 2003; Kamath, Fraser et al. 2003) and were not studied further. *rrf-3* worms were fed bacteria expressing dsRNA constructs from the time of hatching for all following lifespan studies. RNAi knockdown of four of these eleven genes: K11E4.2, R53.5, C49A9.2 and Y82E9BR.5, significantly increased lifespan in *C. elegans* (Figure 2.4b).

C49A9.2 and Y82E9BR.5 9are upregulated when *lin-11* is knocked down (Figure 2.4b). C49A9.2 and Y82E9BR.5 are predicted genes, partially confirmed by cDNAs (www.wormbase.org version WS219), and have no known RNAi phenotype generated from genome-wide RNAi studies (Maeda, Kohara et al. 2001; Kamath, Fraser et al. 2003; Rual, Ceron et al. 2004; Sonnichsen, Koski et al. 2005). C49A9.2 knockdown increased mean lifespan from 15.3±0.1 days, n=235 to 17.7±0.3 days, n=144 which is a sixteen percent extension (Figure 2.6). C49A9.2 RNAi also increased the maximum lifespan by thirty percent from 20 days to 26 days. Y82E9BR.5 RNAi extended mean lifespan by nine percent to 16.7±0.2 days, n=203 from 15.3±0.1 days, n=235 (Figure 2.6). Knocking down Y82E9BR.5 also increased the maximum lifespan by fifty percent form 20 days to 30 days. As mentioned, both genes are upregulated upon *lin-11* knockdown. Therefore, in old worms, when *lin-11* is upregulated, these genes should be downregulated thereby

contributing to extending lifespan and opposing the deterioration of aging.

In contrast, R53.5 is downregulated when *lin-11* is knocked down (Figure 2.4b).Yet, RNAi knockdown of R53.3 resulted in a significant increase in mean lifespan from 16.3±0.2 days, n=185 to 17.5±0.3 days, n=178 which is an increase of seven percent (Figure 2.7). R53.5 RNAi also increased maximum lifespan by seventeen percent from 24 days to 28 days.

R53.5 is widely expressed in larval and adult *C. elegans* including ventral nerve cord, intestines, muscles and hypodermis (McKay, Johnsen et al. 2003; Hunt-Newbury, Viveiros et al. 2007; Reece-Hoyes, Shingles et al. 2007). RNAi of R53.5 has not produced any phenotype in genome-wide RNAi studies (Maeda, Kohara et al. 2001; Kamath, Fraser et al. 2003; Rual, Ceron et al. 2004; Sonnichsen, Koski et al. 2005) but R53.5 mutants generated by National Bioresource Project of Japan are sterile and lethal (www.wormbase.org version WS219).Perhaps reduced expression of R53.5 is beneficial for longevity, but a complete loss results in lethality.

The effectiveness of knockdown through RNAi for R53.5, as for all other RNAi knockdown in this study, is assumed and not validated. Therefore, it is possible that the dsRNA construct used for the knockdown of R53.5 is ineffective, or interferes with the expression of other gene/genes to produce the longevity phenotype. RT-PCR experiments are needed to validate the effective reduction in mRNA levels of R53.5 and all tested genes. Also, scrutiny of the dsRNA sequence may provide indications as to whether the construct may be inhibiting the expression of other genes.

The *lin-11* target poolis highly enriched for aging genes as four of the eleven tested genes extended lifespan upon RNAi knockdown. Genome-wide studies for aging genes in *C. elegans* identify less than one percent of the genome as gerontogenes (Lund et al. 2002, Hamilton et al. 2005). Therefore,*lin-11* is very likely involved in regulation of aging, since 36.4% of its microarray-identified targets tested, are capable of increasing lifespan. Three of these genes are upregulated when *lin-11* is knocked down, and

knockdown of *lin-11* itself reduces lifespan. This suggests that elevated expression of *lin-11* is beneficial for longevity, as these genes would then be downregulated and contributing to organismal longevity. R53.5 on the other hand, is downregulated in our microarray study and its RNAi extends lifespan. Howeverthe R53.5 mutant is lethal and short-lived. Nonetheless, it is clear that knockdown of *lin-11* results in altered expression of genes that regulate lifespan in adult *C. elegans. lin-11* may also be acting to extend lifespan in adult worms by downregulating the insulin-like signaling pathway through down-regulation of *ins-3.*

let-711 interaction with the Insulin-like signaling pathway

RNAi knockdown of *let-711* in adult worms resulted in nearly four-fold reduction in the expression of *pdk-1* (Figure 2.4c, Table 2.1c),an integral part of the insulin/IGF-1 signaling pathway (Figure 1.1). PDK-1 is activated AGE-1 (PI3 kinase), and activated PDK-1 phosphorylates AKT and SGK kinases within the insulin/IGF-1 signaling pathway (Alessi, James et al. 1997; Paradis, Ailion et al. 1999). Inhibition of this conserved insulin/IGF-1 signaling during development in *C. elegans* results in constitutive dauer formation (Morris, Tissenbaum et al. 1996; Kimura, Tissenbaum et al. 1997; Paradis and Ruvkun 1998). Inhibition of this pathway, after the dauer-arrest decision-point, increases organismal lifespan, and this lifespan increase is dependent on*daf-16*(Kenyon, Chang et al. 1993; Larsen, Albert et al. 1995; Morris, Tissenbaum et al. 1996). *pdk-1* mutants are longlived and require *daf-16* for their longevity ((Paradis, Ailion et al. 1999).Therefore, as expected, RNAi knockdown of *pdk-1* resulted in extension of mean lifespan by 19% from 14.1±0.2 days, n=205 to 16.8±0.2 days, n=175 (Figure 2.8).*pdk-1* RNAi also increased the maximum lifespan by, 27% from 22 days to 28 days. This suggests that progressive reduction of *let-711* expression in aging worms may be beneficial for the worm. Reduced *let-711* expression may extend lifespan through a reduction in*pdk-1* expression, thereby downregulating insulin/IGF-1 signaling and resulting in lifespan extension.

let-711 RNAi also resulted in nearly a three-fold decrease in *daf-7* expression (Figure 2.4c, Table 2.1c). RNAi knockdown of *daf-7* in our study did not extend lifespan and *daf-7* mutants are not long lived (Larsen, Albert et al. 1995). *daf-7* is one of five TGF-β-related genes in *C. elegans,* and it is involved in the regulation of dauer formation as the ligand for the TGF-β-related pathway (Ren, Lim et al. 1996). DAF-7 is expressed in ASI chemosensory neurons under favorable environmental conditions. Dauer-inducing environmental conditions repress its expression, resulting in loss of TGF-β-related signaling and entry into the dauer stage (Ren, Lim et al. 1996; Schackwitz, Inoue et al. 1996). As discussed earlier, the insulin/IGF-1 pathway is also involved in regulation of dauer formation. *let-711* regulation of *daf-7* suggests that *let-711* may have a role in dauer formation through both the TGF-β and the insulin/IGF-1 pathways.

let-711 target pool is enriched for aging genes that maybe contributing to lifespan extension by downregulating germline signaling

As was done for *lin-11* target pool, I knocked down microarray-identified*let-711* targets to look for their effects on aging. Twenty-three other differentially expressed *let-711* targetsthat have an existing RNAi construct were tested for their effect on lifespan. RNAi of five of these genes: *meg-1, cdc-25.1, grsp-4, ooc-3* and *gna-2* significantly extended mean lifespan in *C. elegans.* RNAi knockdown of *grsp-4* increased mean lifespan by eleven percent from 14.1±0.2d, n=205 to 15.7±0.2d, n=158 and *meg-1* did so by thirteen percent from 14.1±0.2d, n=205 to 15.9±0.1d, n=189 (Figure 2.9). Individual RNAi of *cdc-25.1, ooc-3* and *gna-2* increased mean lifespan by twenty percent, thirteen percent and twenty-four percent respectively [Control: 14.1±0.1d, n=213. *cdc-25.1:* 17.0±0.2d, n=183. *ooc-3:* 15.9±0.1d, n=190. *gna-2:* 17.5±0.3d, n=195](Figure 2.10).RNAi of *cdc-25.1, gna-2* and *grsp-4* also increased maximum life span by 20%, 40% and 9% respectively. *meg-1* and *ooc-3* RNAi did not result in any increase in maximum lifespan from the wild type control.

Four of these five genes: *cdc-25.1, meg-1, ooc-3* and *gna-2* are known to be involved in germline specification and/or proliferation, and they are all downregulated when *let-11* is knocked down. This is of interest because germline cells have been implicated previously in lifespan regulation. As described earlier, laser ablation of germline precursor cells extends *C. elegans* lifespan in a *daf-16* dependent and *daf-2* independent manner (Hsin and Kenyon 1999; Arantes-Oliveira, Apfeld et al. 2002). Removing the entire gonad does not extend lifespan, indicating that this longevity is not a simple consequence of sterility. Rather, germ cells appear to actively signal *daf-16* to suppress longevity. Specific roles of these *let-711* targets in germline proliferation and lifespan regulation through germline signaling shall be discussed in greater detail in the next chapter.At this point, the results of my experiments indicate that *let-711* influences lifespan determination in adult *C. elegans* by upregulating*pdk-1*expression and thereby the insulin/IGF-1 signaling pathway. These results also indicate that *let-711* regulates germline related genes that are capable of extending lifespan. This leads me to speculate that *let-711* may be regulating aging upstream of both the insulin/IGF-1 and the germline signaling pathways.

lin-11 and *let-711*coregulated genes

As mentioned, seven genes show significantly different expression levels in both microarray studies (Figure 2.4a, Table 2.1) indicating overlap in the genes regulated by the two gene expression regulators. Four genes were upregulated and three genes downregulated in both microarray experiments. It is surprising that these genes change expression in the same direction, since *lin-11* and *let-711* display opposite expression patterns as a function of age.

One hypothesis is that the two regulators are acting on these genes in different directions during aging, either to temporally regulate a change in expression or to keep them fairly constant overall. Genes that are regulated in one direction during early aging due to reduced *let-711*expression, might be regulated in the opposite direction by the

increased expression of *lin-11* in very old worms. If so, it suggests that *lin-11* and *let-711*may actin a reciprocal feedback loop (or as components of a regulatory network) and not as independently acting regulators.

K11E4.2, a common target in both microarray experiments, extends lifespan

Of the seven common targets identified by the microarray experiments, five have a corresponding RNAi construct available in the Ahringer RNAi library (Kamath and Ahringer 2003; Kamath, Fraser et al. 2003). I knocked down each of them individually via RNAi to study effects on lifespan. K11E4.1 is the only gene from this group whose RNAi led to an increased lifespan. K11E4.2 is an unannotated gene confirmed by cDNA analysis (www.wormbase.org version WS219) whose expression is upregulated upon *lin-11* or *let-711* knockdown in our microarray studies. This implies that in wild type worms, both *lin-11* and *let-711* suppress expression of K11E4.2. RNAi of K11E4.2 significantly increased mean lifespan by nine percent, from 16.1±0.1, n=233 to 17.6±0.2, n=188 days (Figure 2.5). Knocking down K11E4.2 also increased the maximum lifespan by twenty percent, from 22 days to 26 days. Therefore, K11E4.2 downregulation by increased activity of *lin-11* in old worms seems to be beneficial to lifespan extension. The same could not be said of *let-711* regulation since decreased *let-711* expression in aging worms would upregulate K11E4.2 expression.

One RNAi screen implicates K11E4.2 in synaptic function (Sieburth, Ch'ng et al. 2005), which may be relevant since the nervous system has been implicated as a central regulator of organismal lifespan (Apfeld and Kenyon 1999; Wolkow, Kimura et al. 2000). Not enough is known about the gene at this point to make any speculations as to how it may be affecting lifespan or the net effect of the conflicting influences of *lin-11* and *let-711* on its expression during early and extreme stages of aging.

Conclusions

lin-11 regulates aging genes in *C. elegans*

lin-11 had no previously known function in regulating aging. This study is the first to show *lin-11* regulating genes that influence aging in *C. elegans.* The microarray studiesshow that *lin-11* knockdown decreases*ins-3* expression. This indicates *lin-11*could be downregulating the insulin/IGF-1 signaling pathway in adult *C. elegans.* Therefore, the highly upregulated expression of *lin-11* in very old wormscould promote longevity by inhibiting insulin/IGF-1 signaling.

A role for*lin-11* in aging regulation is further suggested by the finding that RNAi of fourother *lin-11* regulated genes extended lifespan in *C. elegans.* RNAi ofK11E4.2, R53.5, C49A9.2 and Y82E9BR.5 were able to extend mean and maximum lifespan in *C. elegans*(Figures 2.5 – 2.7). As with*lin-11,* none of these *lin-11* regulated genes have any known function in aging, and hence, aging regulation is a novel function for these genes established through this study.Not enough is known about the function of these genes to speculate how *lin-11* may be regulating aging through these genes.

Since C49A9.2, Y82E9BR.5 and *ins-3* are all upregulated upon *lin-11* RNAi, *lin-11*may function to downregulate their expression in wild type worms. RNAi of C49A9.2 and Y82E9BR.5 extended both mean and maximum lifespan suggesting that the strong upregulation of *lin-11* in very old worms may be acting to promote longevity through its effect on these genes. Although RNAi of R53.5 (downregulated by *lin-11*) also increased lifespan in my experiments, R53.5 mutant worms are sterile and lethal (www.wormbase.org version WS219). Thus,while reduced expression of R53.5 appears to be beneficial for lifespan, complete loss of gene function results in lethality.

let-711 is an aging regulator in *C. elegans*

This project also indicates a role for*let-711* in aging regulation. Knocking down *let-711* produced an almost four-fold reduction in *pdk-1*expression(Figure 2.4c), which indicatesan interaction between *let-711* and theinsulin/IGF-1 pathway. RNAi knockdown of *pdk-1* resulted in extension of mean and maximum lifespan (Figure 2.8). These results suggest that decreased expression of *let*-711 during aging promotes longevity by inhibiting insulin/IGF-1 signaling pathway.

Further evidence of *let-711*'s involvement in aging regulation is provided by the finding thatRNAi of five additional *let-711*-upregulated genes extend mean lifespan (Figures 2.9 and 2.10). Four of these genes: *meg-1, cdc-25.1, ooc-3* and *gna-2*, have previously been implicated in germline specification and proliferation. Signals from the germline act antagonistically to lifespan preservation in a DAF-16 dependent, but DAF-2 independent manner(Hsin and Kenyon 1999; Arantes-Oliveira, Apfeld et al. 2002). This leads to speculation that *let-711* may also be regulating lifespan in adult worms by regulating germline proliferation, and hence, germline signals to DAF-16.

Materials and Methods

RNA interference

RNAi by feeding was performed as described (Kamath, Martinez-Campos et al. 2001). RNAi clones were inoculated overnight at 37°C in LB plus carbenicillin at 25μg/ml (10μg/ml tetracycline for the control with empty vector pL4440). This culture was then seeded onto NGM plates with 25μg/ml carbenicillin (10μg/ml tetracycline for the control) and 0.2mM IPTG for RNAi induction overnight at 37°C. *rrf-3* worms were transferred to *lin11* and *let-711* RNAi plates on the first day of adulthood at day 3 while RNAi knockdown of all other genes were started during L1 stage on day 1.

Lifespan Analysis

Lifespan assays were conducted, in general, as described previously (Hansen, Hsu et al. 2005). *lin-11* and *let-711* lifespan assays were performed at 20°C, while all other lifespan assays were performed at 25°C. Each trial consisted of three plates per RNAi strain with 20 worms per plate, which was repeated three times for each lifespan assay. The animals were transferred to RNAi plates that contained 5mg/ml FuDR to inhibit sample contamination by F1 worms. The numbers of live and dead worms were scored every other day, and the worms were transferred to fresh RNAi plates on a weekly basis. Animals that crawled off the plate, exploded, bagged or became contaminated were censored. R software was used to calculate mean lifespans and perform statistical analyses. P values were determined using Logrank (Mantel-Cox) statistics.

Microarray Analysis

Synchronized populations of worms were generated and allowed to develop into adults feeding on HT115 bacteria containing the empty vector pL4440. The worms were transferred to RNAi plates containing 5mg/ml FuDR to prevent F1 contamination, on the first day of adulthood (day3). 30,000 worms were transferred per plate and three plates were used for each RNAi strain. The animals were allowed to age at 20°C and total RNA was extracted from them on day 7 with TRIzol according to the manufacturer's protocol.RNA preparations were further purified using a QiagenRNeasy mini column. Total RNA extracted from day 7 *rrf-3* worms that were fed HT115 bacteria containing the empty vetor pL4440 was used as control. UV spectrophotometry and a 2100 Agilent Bioanalyzer were used to quantify and check the quality of RNA preparations. At least three high quality RNA isolations from each treatment (pL4440, *lin-11, let-711*) were used for reverse-transcriptase polymerase chain reaction (RT-PCR) analysis and to make pools of biotin labeled cRNA probes. Each pool was subsequently hybridized to separate*C. elegans* Genome ArrayGeneChips (Affymetrix). The University of Kentucky

Microarray Core Facility generated cRNA probes and performed hybridizations according to standard Affymetrix protocols.

Background subtraction, normalization and expression summarization were performed using the GC-Robust Multiple-array Average (GC-RMA) algorithm ((Irizarry, Ooi et al. 2003). A False Discovery Rate (FDR) witha threshold of 0.05 was used to identify probe-setsshowing significant differences in hybridization intensity between the control and experimental samples (Benjamini and Hochberg 1995). Hybridization intensities were averaged across each GeneChip replicate of the control for all further analyses.Cluster/Treeview 2.11 software (Eisen, Spellman et al. 1998) was subsequently used to cluster differentially expressed genes based on their expression patterns. This clustering was conducted on log2-ratio data and was done using hierarchical clustering algorithm.

Figure 2.1: *lin-11* RNAi and *let-711* RNAi effects on lifespan: Individual RNAi of *lin-11* and *let-711* in adult *C. elegans* significantly reduced mean lifespan at 20°C. *rrf-3* worms were fed HT115 bacteria, expressing specific dsRNA or the empty vector L4440 as control, starting from the first day of adulthood (day3). Individual RNAi knockdown of both genes reduced mean lifespan. L4440 control, n=166, m=18.4±0.4 days; *let-711* RNAi, n=166, m=15.6±0.3 days, p=3.3e-07 and *lin-11* RNAi, n=169, m=17.1±0.3 days, p=0.003.

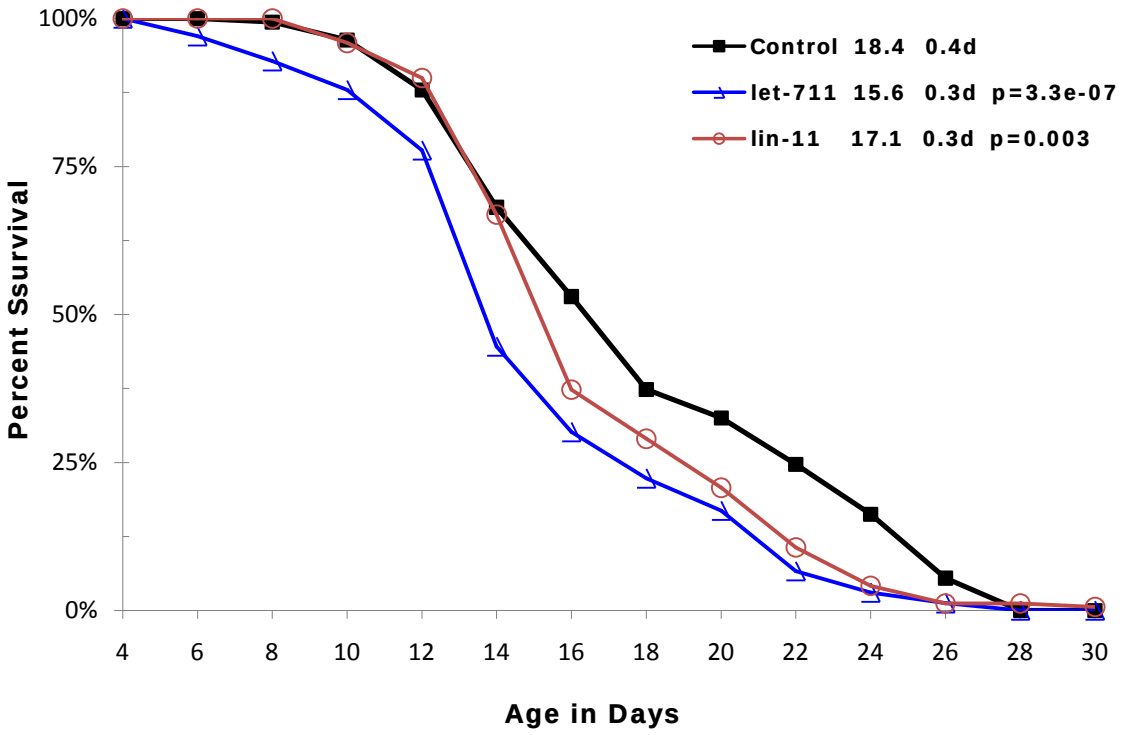
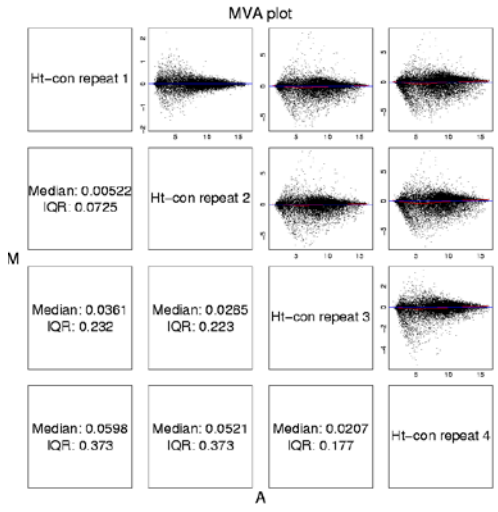
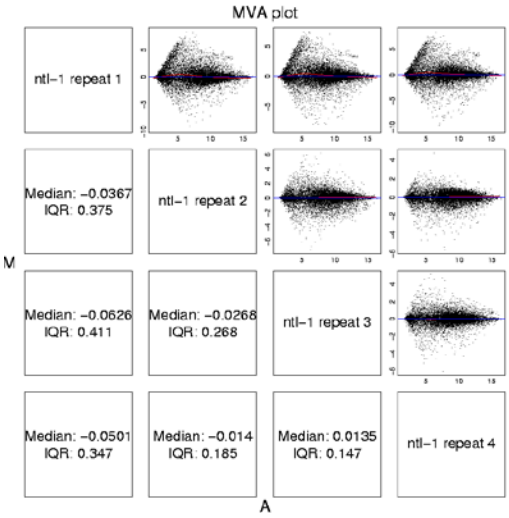


Figure 2.2: M vs A plots show good agreement among replicate GeneChips. The expression ratio M on the y-axis, is plotted against the average expression level A on the x-axis. M is the sample(R)/control(G) intensity ratio: M= $\log_2$R- $\log_2$G. A is the average intensity: A=1/2*($\log_2$R+ $\log_2$G). Comparison among replicate GeneChips for a) empty vector L4440 (HT115), b) *let-711 (ntl-1)*and c) *lin-11* indicate good agreement among replicates since data points show good centration on the midline with low scatter between replicates. Only three *lin-11* GeneChips are included in our study as one Chip was discarded upon visual inspection of the Chip image. All four Genechips were used for *let-711* and control.

a)



b)



c)

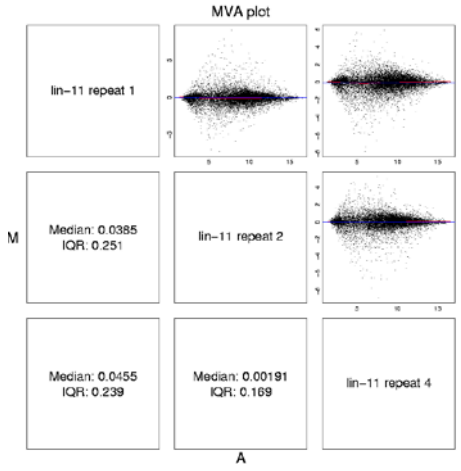


Figure 2.3: Venn Diagram summarizing the results of the microarray analyses. Sixteen genes were differentially expressed upon *lin-11* RNAi and 60 upon *let-711* RNAi. Seven genes were expressed differentially in both experiments.

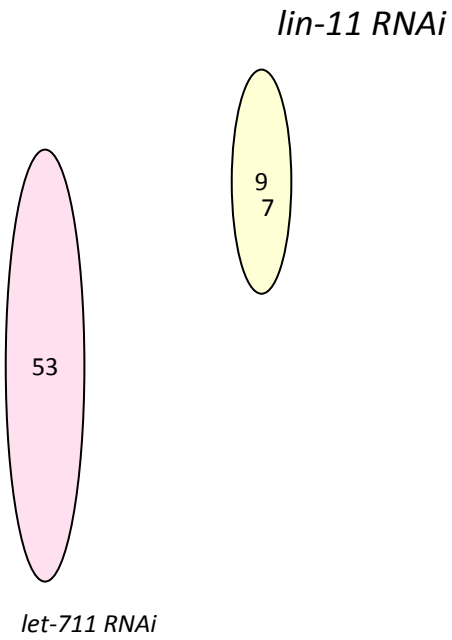
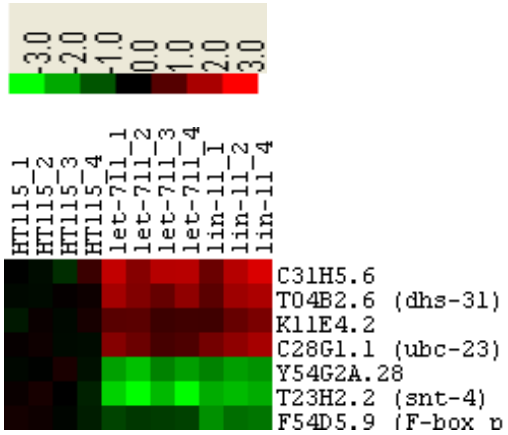
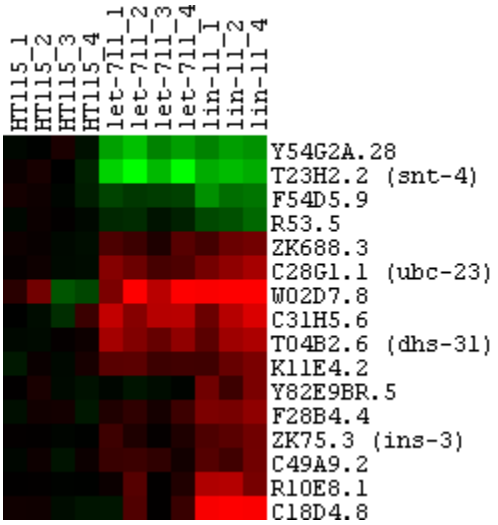


Figure 2.4. Expression profiles of the genes that were differentially expressed in the microarray experiments: Cluster/Treeview software (Eisen, Spellman et al. 1998) was used to cluster the genes that were differentially expressed in the microarray experiments. This clustering was conducted on log_2-transformed data using the heirarchical clustering algrithm. Data is normalized to the average expression of the control chips, red indicates increase expression and green indicates reduced expression. a) Seven genes were differentially expressed in both experiments, four were upregulated and three were downregulated in both experiments. b) Sixteen genes showed greater than two-fold change in expression when *lin-11* was knocked down and c) sixty genes did when*let-711*was knocked down.

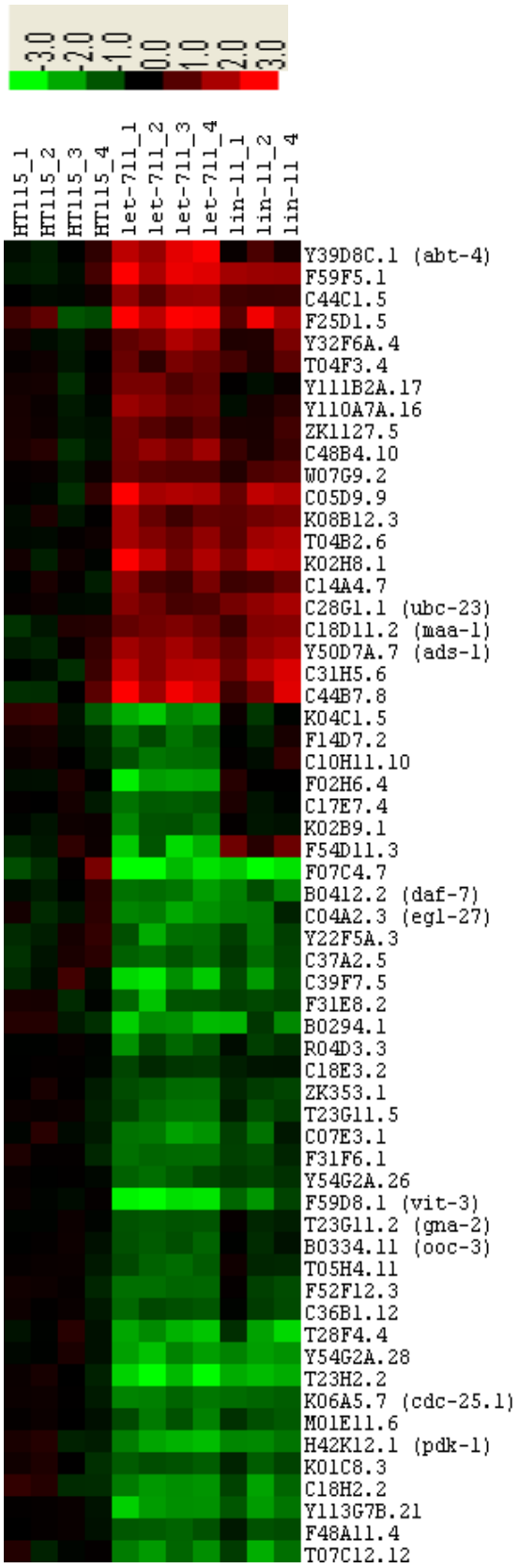
a)



b)



c)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-1.0
0.0
1.0
2.0
3.0
HT115_1
HT115_2
HT115_3
HT115_4
let-711_1
let-711_2
let-711_3
let-711_4
lin-11_1
lin-11_2
lin-11_4
Y39D8C.1 (abt-4)
F59F5.1
C44C1.5
F25D1.5
Y32F6A.4
T04F3.4
Y111B2A.17
Y110A7A.16
ZK1127.5
C48B4.10
W07G9.2
C05D9.9
K08B12.3
T04B2.6
K02H8.1
C14A4.7
C28G1.1 (ubc-23)
C18D11.2 (maa-1)
Y50D7A.7 (ads-1)
C31H5.6
C44B7.8
K04C1.5
F14D7.2
C10H11.10
F02H6.4
C17E7.4
K02B9.1
F54D11.3
F07C4.7
B0412.2 (daf-7)
C04A2.3 (egl-27)
Y22F5A.3
C37A2.5
C39F7.5
F31E8.2
B0294.1
R04D3.3
C18E3.2
ZK353.1
T23G11.5
C07E3.1
F31F6.1
Y54G2A.26
F59D8.1 (vit-3)
T23G11.2 (gma-2)
B0334.11 (ooc-3)
T05H4.11
F52F12.3
C36B1.12
T28F4.4
Y54G2A.28
T23H2.2
K06A5.7 (cdc-25.1)
M01E11.6
H42K12.1 (pdk-1)
K01C8.3
C18H2.2
Y113G7B.21
F48A11.4
T07C12.12

TABLE 2.1: Seven genes showed significant change in expression in both*lin-11* and *let-711* microarray experiments.

a. Significantly extended lifespan upon RNAi knockdown

GENE	NAME	ANNOTATION	Fold change upon *lin-11* RNAi	Fold change upon *let-711* RNAi
C28G1.1	*ubc-23*	Predicted conjugating enzyme (UBCs/E2s) of the ubiquitin-conjugation system.	3.20	2.22
C31H5.6			3.98	4.16
F54D5.9		Paralog of PROM-1 that suppresses excess embryonic cell division.	-2.76	-1.67
K11E4.2[a]			2.20	1.86
T04B2.6	*dhs-31*	Predicted short chain-type dehydrogenase	3.17	3.02
T23H2.2	*snt-4*	Ca2+ dependent phospholipid-binding protein Synaptotagmin, required for synaptic vesicle and secretory granule exocytosis	-4.27	-6.45
Y54G2A.28			-3.28	-3.73

TABLE 2.2: Sixteen genes were differentially expressed by at least two-fold when *lin-11* gets knocked-down.

a. Significantly extended lifespan upon RNAi knockdown

GENE	NAME	ANNOTATION	Fold change
C18D4.8			3.98
C28G1.1	*ubc-23*	Predicted conjugating enzyme (UBCs/E2s) of the ubiquitin-conjugation system.	2.19
C31H5.6			2.20
C49A9.2[a]			2.14
F28B4.4			2.87
F54D5.9[a]		Paralog of PROM-1 that suppresses excess embryonic cell division.	-4.27
K11E4.2[a]			8.97
R10E8.1			2.04
R53.5[a]			-3.28
T04B2.6	*dhs-31*	Predicted short chain-type dehydrogenase	3.66
T23H2.2	*snt-4*	Ca2+ dependent phospholipid-binding protein Synaptotagmin, required for synaptic vesicle and secretory granule exocytosis	-2.02
W02D7.8			13.40
Y54G2A.28			-2.76
Y82E9BR.5[a]			3.17
ZK688.3			2.24
ZK75.3	*ins-3*	Insulin-related peptide. Expressed in some amphid sensory neurons.	3.20

TABLE 2.3: Sixty genes that showed at least two-fold change in expression in response to *let-711* RNAi.

a. Significantly extended lifespan upon RNAi knockdown

GENE	NAME	ANNOTATION	Fold Change
B0294.1			-4.00
B0334.11[a]	*ooc-3*	abnormalOOCyte formation. Maternally required for establishing polarity in early embryos.	-2.05
B0412.2	*daf-7*	abnormalDAuer Formation. Member of the transforming growth factor beta superfamily that affects dauer larvae formation, fat metabolism, egg laying and feeding behavior in a TGF-beta-mediated pathway.	-2.78
C04A2.3	*egl-27*	EGg Laying defective. Required for embryonic viability.	-3.22
C05D9.9			4.92
C07E3.1	*stip-1*	Predicted Septin and Tuftelin Interacting Protein.	-3.05
C10H11.10	*kca-1*	Predicted Kinesin Cargo.	-2.31
C14A4.7			2.26
C17E7.4			-2.19
C18D11.2	*maa-1*	Membrane Associated Acyl-CoA binding protein. Required for endosomal recycling and for proper endosomal shape and morphology.	2.49
C18E3.2			-2.03
C18H2.2			-3.19
C28G1.1	*ubc-23*	Predicted conjugating enzyme (UBCs/E2s) of the ubiquitin-conjugation system.	2.22

TABLE 2.3 Continued

C31H5.6			4.16
C36B1.12	*imp-1*	Predicted IntraMembraneProtease(IMPAS) family member.	-2.02
C37A2.5	*pqn-21*	Predicted Prion-like-(Q/N-rich)-domain-bearing protein.	-2.22
C39F7.5			-5.08
C44B7.8	*pmp-1*	Peroxisomal Membrane Protein related. ATP-binding cassette (ABC) transporter involved in peroxisome organization and biogenesis.	6.26
C44C1.5			2.96
C48B4.10			2.96
F02H6.4			-4.37
F07C4.7[a]	*grsp-4*	Glycine Rich Secreted Protein	-8.35
F14D7.2			-2.22
F25D1.5			11.45
F31E8.2	*snt-1*	Synaptotagmin 1. Required for synaptic vesicle recycling (endocytosis) synaptic vesicle exocytosis and neurotransmitter release.	-2.57
F31F6.1			-2.40
F48A11.4			-2.09

TABLE 2.3 Continued

F52F12.3	*mom-4*	MAP kinase kinase kinase-related protein. Positive regulator of the Wnt pathway signaling that governs embryonic anterior/posterior (A/P) polarity	-2.40
F54D11.3			-4.03
F59D8.1	*vit-3*	Vitellogenin, a major yolk component and likely functions redundantly with other vitellogenins to provide essential nutrients to the developing embryo.	-7.03
F59F5.1			7.09
H42K12.1[a]	*pdk-1*	3-Phosphoinositide-Dependent Kinase 1. A component of the DAF-2/insulin receptor-like signaling pathway and accordingly, functions to regulate such processes as dauer larvae formation, longevity, and salt chemotaxis learning.	-3.85
K01C8.3	*tdc-1*	Predicted Tyrosine DeCarboxylase.	-2.03
K02B9.1[a]	*meg-1*	Maternal Effect Germ-cell defective. Maternally required for germline specification. Also required for germline proliferation and normal levels of fertility.	-2.28
K02H8.1	*mbl-1*	Muscleblind splicing regulator required in adults for normal muscle dense body organization, locomotion, and vulval morphogenesis.	4.53
K04C1.5			-3.81
K06A5.7[a]	*cdc-25.1*	CDC25 phosphatase homolog that affects embryonic viability, meiosis, mitosis, proliferation of the intestine (E cell lineage), and germ line proliferation.	-2.67

TABLE 2.3 Continued

K08B12.3			2.38
M01E11.6	*klp-15*	Predicted Kinesin-Like Protein	-2.29
R04D3.3			-2.26
T04B2.6	*dhs-31*	Predicted Short chain-type Dehydrogenase	3.02
T04F3.4			2.17
T05H4.11			-2.14
T07C12.12			-2.91
T23G11.2[a]	*gna-2*	Glucosamine 6-phosphate N-Acetyl transferase for synthesis of UDP-N-acetylglucosamine (UDP-GlcNAc), N-acetylgalactosamine (UDP-GalNAc), alpha-GalNAc-modifications of mucin-like proteins and chitin. GNA-2 affects cytokinesis, embryonic viability, and fertility.	-2.01
T23G11.5	*rlbp-1*	Predicted RaL Binding Protein	-2.28
T23H2.2	*snt-4*	SyNapTotagmin; Ca2+ dependent phospholipid-binding protein required for synaptic vesicle and secretory granule exocytosis	-6.45
T28F4.4			-4.03
W07G9.2	*glct-6*	Predicted GLuCuronosylTransferase-like	2.05
Y110A7A.16	*elpc-1*	Predicted ELongator complex Protein Component.	2.76

TABLE 2.3 Continued

Y111B2A.17	*elpc-2*	Predicted ELongator complex Protein Component.	2.34
Y113G7B.21	*mdt-17*	Predicted subunit of Mediator complex.	-3.81
Y22F5A.3	*ric-4*	SNAP-25 component of SNARE complex associated with synaptosomes, required for axonal growth.	-2.61
Y32F6A.4			2.99
Y39D8C.1	*abt-4*	Predicted ATP-Binding cassette (ABC) Transporter that couples energy to transport of various molecules across membranes.	5.31
Y50D7A.7	*ads-1*	Alkyl-Dihydroxyacetonephosphate Synthase required for normal larval development.	3.35
Y54G2A.26			-2.13
Y54G2A.28			-3.73
ZK1127.5			2.02
ZK353.1			-2.17

Figure 2.5: K11E4.2 RNAi extended lifespan. Lifespan curves were obtained as described in Material and Methods. Synchronized *rrf-3* L1 animals were transferred to RNAi plates and raised at 25°C. K11E4.2 was significantly upregulated in both microarray experiments and its knockdown by RNAi extends mean lifespan by nine percent and maximum lifespan by twelve percent. Control L4440: n=233, max=22 days;K11E4.2 RNAi:n=188, max=26 days.

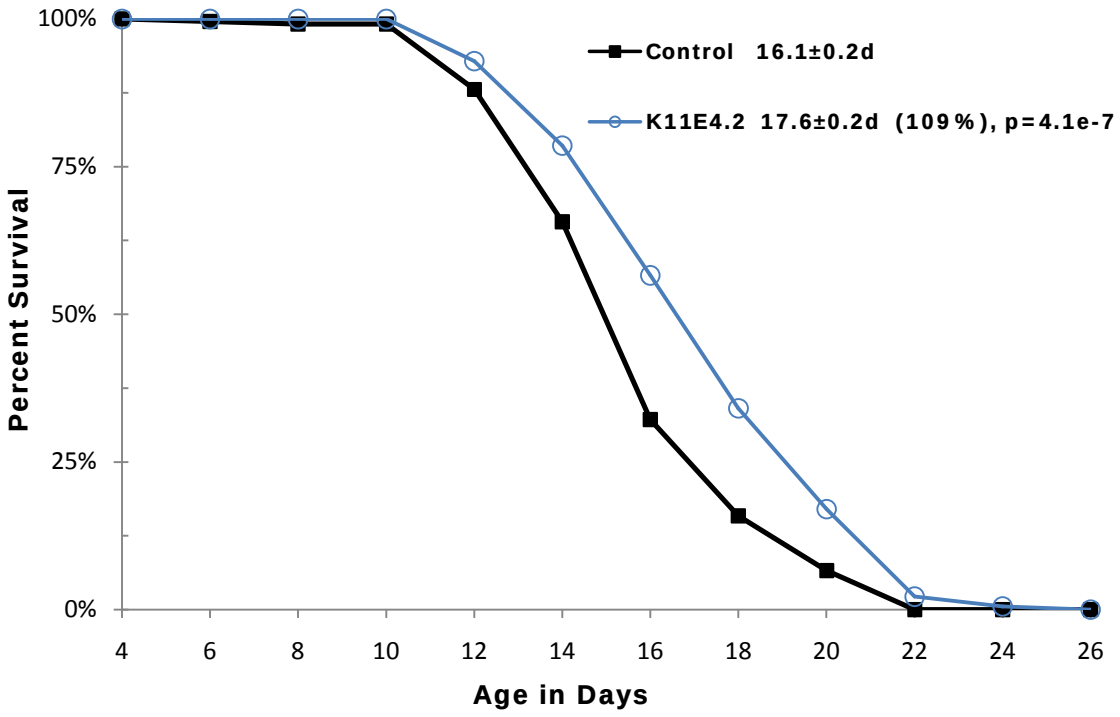


Figure 2.6: RNAi of up-regulated *lin-11* targets,C49A9.2 and Y82E9BR.5, extended lifespan. Lifespan curves were obtained as described in Material and Methods. Synchronized *rrf-3* L1 animals were transferred to specific RNAi plates and raised at 25°C. RNAi of C49A9.2 and Y82E9BR.5 individually extended mean lifespan by sixteen percent and nine percent respectively, and maximum lifespan by thirty percent and fifty percent respectively. L4440 control: n=253, max=20 days;C49A9.2 RNAi: n=144, max=26 days; Y82E9BR.5 RNAi: n=203, max=30 days.

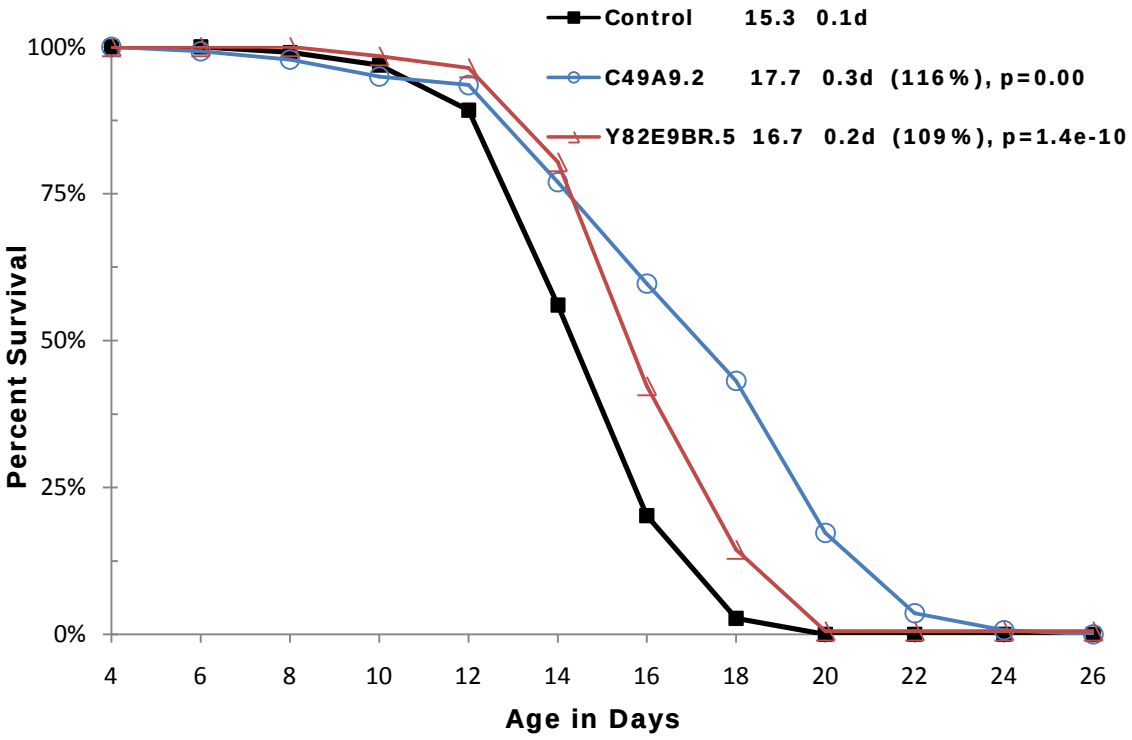


Figure 2.7: RNAi of down-regulated *lin-11* target R53.5 extended lifespan.In the same experiment described in Figure 2.6, knockdown of *lin-11* target,R53.5 by RNAi extended mean lifespan by seven percent and maximum lifespan by seventeen percent. L4440 control: n=185, max=24 days; R53.5 RNAi: n=178, max=28 days.

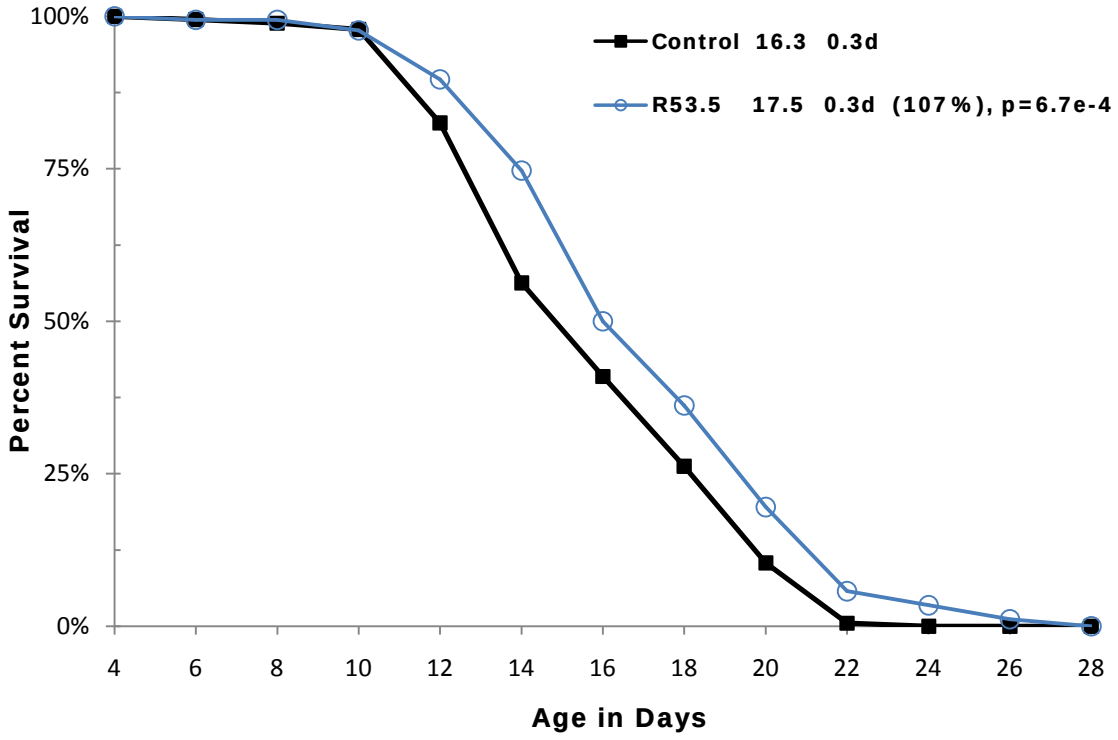


Figure 2.8: RNAi of let-711 target*pdk-1* extended lifespan.Lifespan curves were obtained as described in Material and Methods. Synchronized *rrf-3* L1 animals were transferred to RNAi plates and raised at 25°C. Knocking down *pdk-1* expression extended mean lifespan by nineteen percent and maximum lifespan by 27%. L4440 control: n=205, max=22 days; *pdk-1* RNAi: n=175, max=28 days.

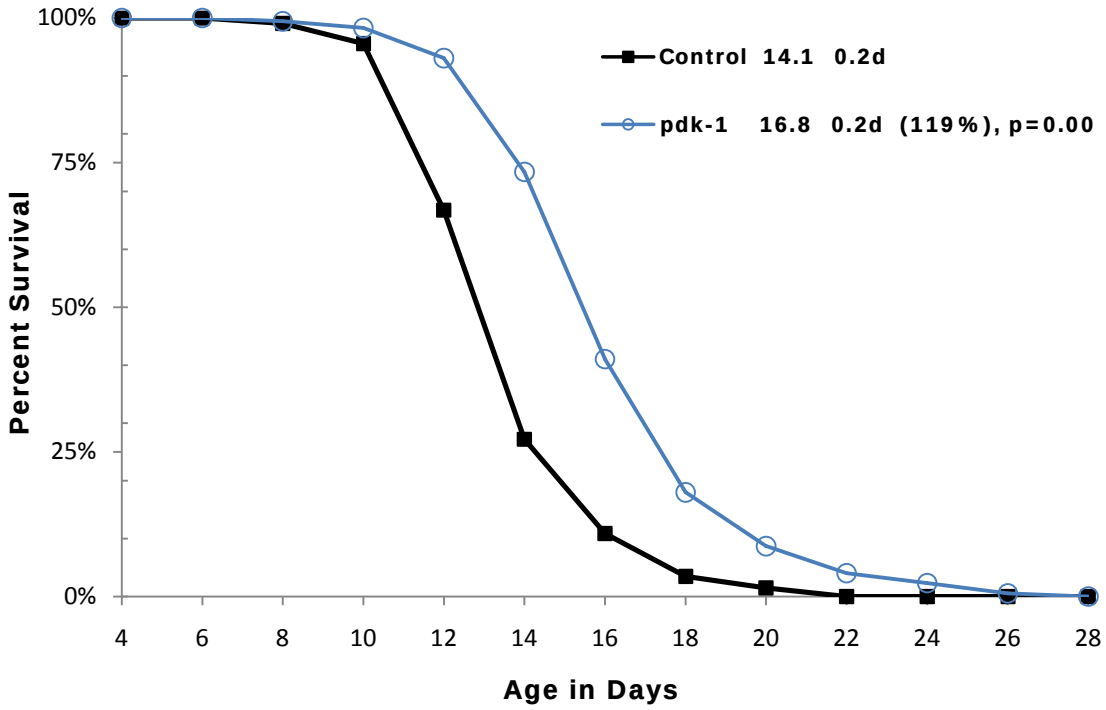


Figure 2.9: *grsp-4* RNAi and *meg-1* RNAi extended lifespan.Lifespan curves were obtained as described in Material and Methods. Synchronized *rrf-3* L1 animals were transferred to specific RNAi plates and raised at 25°C. RNAi knockdown of *grsp-4* increased mean lifespan by eleven percent and maximum lifespan by nine percent.*meg-1* RNAi extended mean lifespan by thirteen percent with no change in maximum lifespan. L4440 control: n=205, max=22 days; *grsp-4* RNAi: n=158, max=24 days; *meg-1* RNAi: n=189, max=22 days.

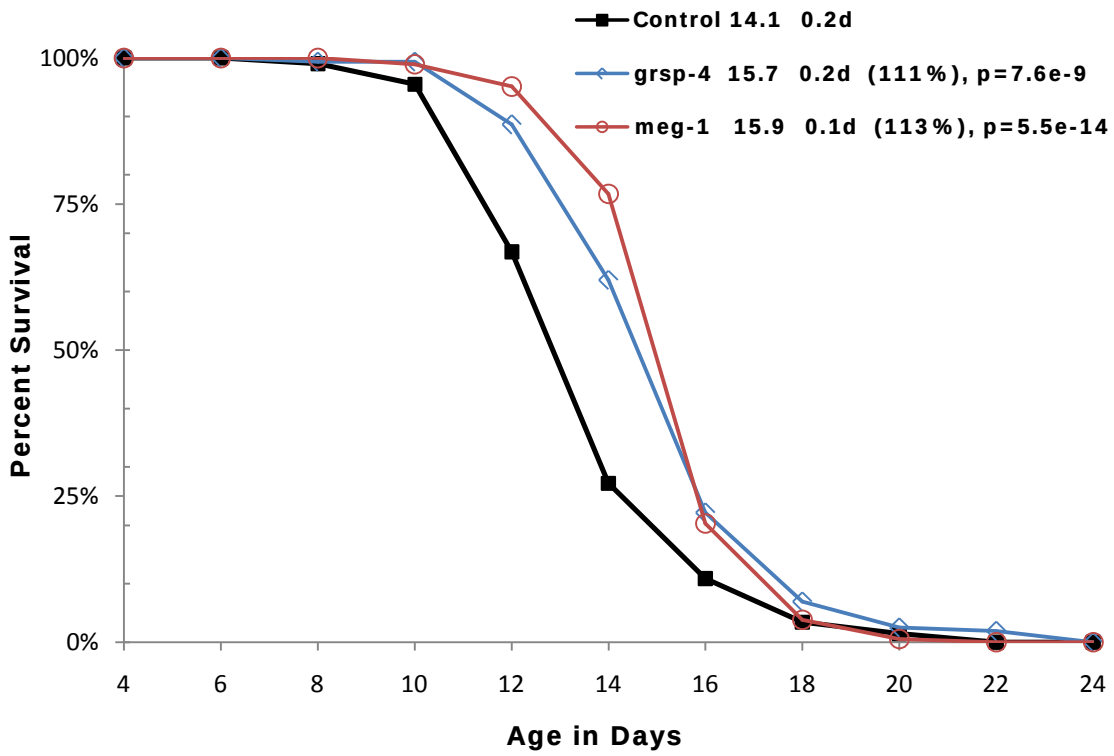
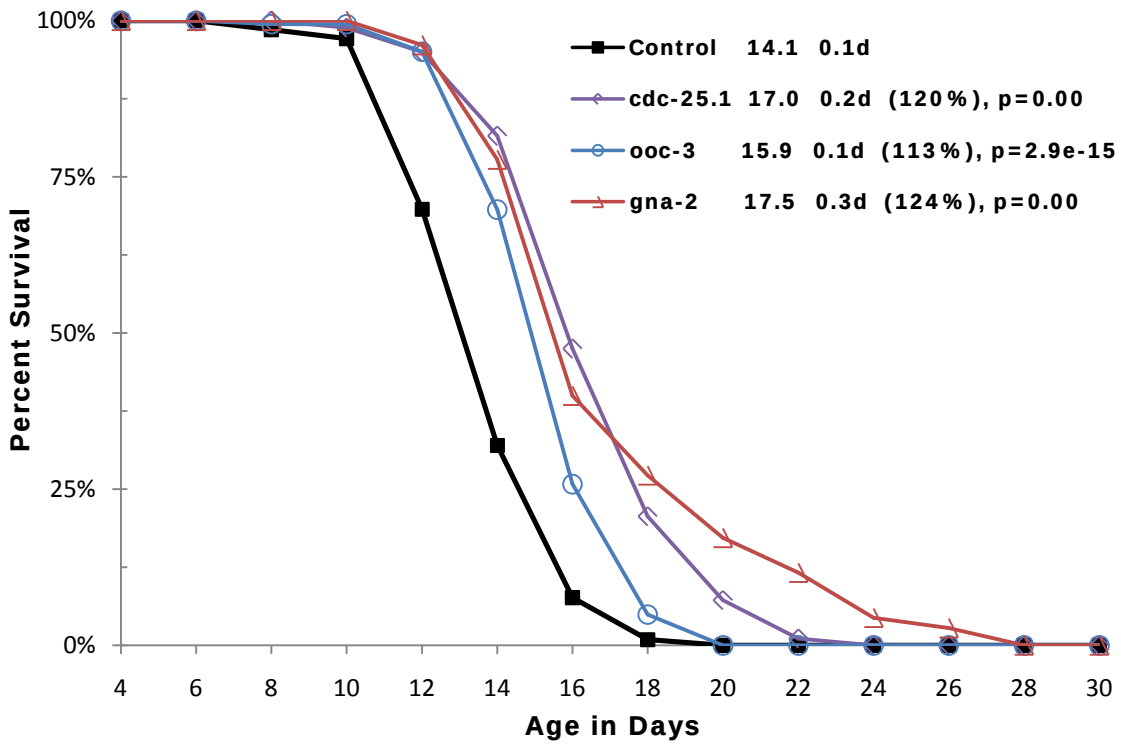


Figure 2.10: RNAi of *cdc-25.1, ooc-3* and *gna-2* individually extended lifespan.In the same experiment described in Figure 2.9, *cdc-25.1* RNAi extended mean lifespan by Twenty percent,*ooc-3* RNAi increased mean lifespan by thirteen percent and*gna-2*RNAi extended mean lifespan by 24%.RNAi of *cdc-25.1* and *gna-2* also increased maximum lifespan by twenty percent and forty percent respectively. L4440 control:max=20 days, n=213; *cdc-25.1* RNAi: max=24 days, n=183; *ooc-3* RNAi:max=20 days, n=190; *gna-2* RNAi:max=28 days, n=195.



CHAPTER 3: *let-711* Regulates Aging Through the Germline Signaling Pathway

Introduction

Regulation of lifespan by the germline

As discussed in Chapter 1, removing the germline of *C. elegans* by laser ablation of the germline precursor cells extends lifespan by up to 60% (Hsin and Kenyon 1999; Arantes-Oliveira, Apfeld et al. 2002). Similarly,*Drosophila* mutants lacking a germline also live longer than wild type flies by up to 50% (Flatt, Min et al. 2008). Modulation of lifespan by the reproductive tissue has also been shown in mammals: transplanting ovaries from young, sexually mature mice into middle-aged overiectomized mice increased the life span of the older recipients by up to 60% (Cargill, Carey et al. 2003). Even in humans, removal of the ovaries from women increases all-cause mortality during aging (Parker, Broder et al. 2009).

In *C. elegans*, removal of the germline extends lifespan while removal of the entire reproductive tissue does not extend lifespan (Hsin and Kenyon 1999). This indicates that the longevity of germline-deficient animals is dependent on the somatic gonad, and that sterility alone is insufficient to lengthen lifespan. Furthermore, it has been shown that it is the proliferating germline stem cells (rather than differentiated sperm or oocytes), which must be absent to produce an extended lifespan (Arantes-Oliveira, Apfeld et al. 2002). Therefore, in wild type *C. elegans*, proliferating germline stem cells inhibit longevity.

The germline regulates lifespan by inhibiting DAF-16 activity, independent of the *daf-2*/insulin-like signaling pathway

Germline ablation in *daf-16* null mutants fails to generate long-lived *C. elegans* showing that the *daf-16*/FOXO transcription factor is essential for this extension of lifespan (Hsin and Kenyon 1999). DAF-16/FOXO is best known for its ability to extend lifespan in response to inhibition of the insulin/IGF-1 signaling. When the insulin/IGF-1 pathway is inhibited in worms, DAF-16 localizes to the nuclei of tissues throughout the animal (Henderson and Johnson 2001; Lee, Hench et al. 2001; Lin, Hsin et al. 2001), where it alters the expression of a variety of genes that influence lifespan (Lee, Lee et al. 2003; McElwee, Bubb et al. 2003; Murphy, McCarroll et al. 2003; Oh, Mukhopadhyay et al. 2006). Removal of the germline precursor cells produces a different pattern of DAF-16 nuclear localization: DAF-16 accumulates primarily in the nuclei of one tissue, the intestine (Lin, Hsin et al. 2001). Furthermore, expression of *daf-16* specifically in the intestine also fully restores the longevity phenotype in germline ablated *daf-16* animals (Libina, Berman et al. 2003). Therefore, DAF-16 functions in various tissues to extend lifespan in response to the inhibition of insulin/IGF-1 signaling, but it appears to function primarily in the intestine to extend lifespan in response to loss of the germline. In addition, germline ablation in *daf-2* mutants shows a synergistic effect and further extends the lifespan of these long-lived mutants (Hsin and Kenyon 1999). These results indicate clearly that germline regulates aging by inhibiting the activity of DAF-16, independently of DAF-2 and the insulin/IGF-1 pathway.

To summarize, it seems that removal of the germline results in the removal of an inhibitory signal to DAF-16, which leads to DAF-16 nuclear localization in the intestinal cells to upregulate expression of longevity genes. This germline-signaling pathway is acting in parallel to the *daf-2*/insulin-like pathway to regulate DAF-16 activity,and DAF-16 signaling is not maximized or saturated when it receives signals from either source alone.

let-711 regulated genes that influence germline survival and proliferation

Sixty genes were differentially expressed by at least two-fold when *let-711* expression was knocked down in adult *C. elegans* (Figure 2.4c, Table 2.3). I studied the effect of twenty-seven of the most differentially expressed genes on the worm lifespan. RNAi knockdown of seven of these twenty-seven genes produced an increase in lifespan, establishing them as aging genes. Four of these aging genes: *cdc-25.1*, *gna-2*, *meg-1* and *ooc-3* are germline related genes: they are involved in various stages of germline specification, maintenance and proliferation. All four genes showed decreased expression upon *let-711* RNAi. This led me to speculate that *let-711* may inhibit longevity by promoting germline proliferation in wild type adult *C. elegans.* Below is a short description of each of these *let-711*-regulated, germline-related genes.

***cdc-25.1*, a cell cycle regulator required for embryonic viability and germline proliferation:** *cdc-25.1* is a cell-cycle regulator that affects cell division, embryonic viability and germline proliferation. It is one of four cdc25 genes in the *C. elegans* genome and encodes a CDC25 phosphatase that acts at the G2/M transition during cell cycle (Ashcroft, Kosinski et al. 1998; Kostic and Roy 2002). CDC25 dephosphorylates cyclin-dependent kinase/cyclin complexes to drive the cell division forward from the G2 phase to the M phase.

cdc-25.1 is essential for embryonic viability,and isnexpressed primarily in the germline.It is localized in the nuclei of oocytes, and nuclei and cell cortex of early embryos (Ashcroft, Srayko et al. 1999; Clucas, Cabello et al. 2002). During early embryonic development, CDC-25.1 is asymmetrically enriched in the anterior cytoplasm in a PAR protein dependent manner (Rivers, Moreno et al. 2008). Disruption of CDC-25.1 in just-fertilized eggs using RNA mediated interference produces multiple defects in the first few cell divisions including mispositioning of meiotic spindle as the fertilized egg completes meiosis, defects in polar body extrusion and chromosome segregation, and abnormal cleavage furrows, ultimately leading to an unviable embryo (Ashcroft, Srayko et al. 1999). This suggests that *cdc-25.1* is required maternally for proper establishment

of cell polarity and asymmetric embryonic divisions, and thus, embryonic viability. *cdc-25.1* homozygous mutant embryos, derived from a heterozygous parent, hatch but develop into sterile adults showing that thelethality is a maternal-effect phenotype(Ashcroft and Golden 2002). Presumably, the first generationmutants do not display any embryonic defects because of maternally contributed *cdc-25.1* mRNA.

Germline precursors in these*cdc-25.1* null mutants divide slowly and undergo only three to four rounds of germ-cell divisions before they die during L3 and L4 larval stages (Ashcroft and Golden 2002; Kim, Lee et al. 2009). These mutants have reduced number of germ cells but show no noticeable somatic cell-division defects, which indicate that *cdc-25.1* functions predominantly in the germline cells in post-embryonic *C. elegans.* It also suggests that *cdc-25.1* activity is probably not required (or isredundant with other CDC25 phophatases) in the somatic lineages.

***meg-1* is an embryo-specific P-granule component required for germline development:** *meg-1* and its paralog *meg-2,*are novel *C. elegans* genes identified through a microarray experiment to identify genes enriched in the *C. elegans* germline (Leacock and Reinke 2006). In situ hybridization experiments reveal that MEG-1 is expressed specifically in the proximal germline of adult hermaphrodites. Beginning at the 4 to 8 cell embryonic stage, MEG-1 proteins localize to P granules until the germline precursor cells Z2 and Z3 are born at the 100-cell stage (Leacock and Reinke 2008). Once the germline precursor cells are born, *meg-1* expression begins to fade and is not observed anymore.

meg-1 is required maternally for proper germline development and fertility, because inhibition of maternal *meg-1* via RNAi resulted in sterile adults with severely underproliferated germline (Leacock and Reinke 2008). *meg-1* mutants also exhibit maternal-effect sterility. Homozygous mutants born of heterozygous hermaphrodites are fertile, but their progeny are sterile. This sterility phenotype displays incomplete penetrance and is also temperature sensitive. *meg-1(vr10) and meg-1(vr11)* are severe loss-of-function mutants, yet only 1-15% of the progeny are sterile at 20° and 45-80% of the progeny are sterile at 25°C (Leacock and Reinke 2008). The sterility in the progeny of

meg-1 mutants approaches complete penetrance when combined with *meg-2* RNAi, whereas*meg-2* RNAi alone in wild-type animals produces no phenotype (Leacock and Reinke 2008). This result suggests that *meg-2* may be genetically redundant with *meg-1* and compensates for the loss of *meg-1*.

Immunofluorescence experiments tracking germline development in larvae revealed that *meg-1* mutants fail to proliferate germ-cells during the L3 and L4 stages when extensive germline proliferation occurs in wild-type animals (Leacock and Reinke 2008), leading to sterile adults with a severely underproliferated germline. The same experiments also show mis-segregation of P-granules during embryonic development, indicating a requirement for*meg-1* in proper P-granule segregation. It is possible that maternal *meg-1* is essential for germ cell specification through proper segregation of P-granules and larval *meg-1* for germline proliferation during L3 and L4 larval stages.

ooc-3,* a novel ER transmembrane protein required for germline fate specification:ooc-3* was initially identified in a screen for maternal-effect lethal mutations in *C. elegans* (Kemphues, Kusch et al. 1988).In the P lineage (germline) of the *C. elegans* early embryo, establishment of polarity and spindle orientation, leading to cleavage axes is mediated by a 90° rotation of the centrosome-nucleus complex (Albertson 1984; Hyman 1989). Further analysis of *ooc-3* embryos through time-lapse microscopy revealed that these mutants are defective for rotation of the centrosome-nucleus complex in the P1 blastomere (Basham and Rose 1999; Pichler, Gonczy et al. 2000), leading to spindle disorientations, defects in chromosome segregation and aberrant cell divisions from the two-cell stage. *ooc-3* mutant embryos also display mislocalized polarity proteins (PAR) and P-granules in the P1 cells (Basham and Rose 1999; Pichler, Gonczy et al. 2000). This further establishes the role of*ooc-3* in the maintenance of germ cell identity of the P lineage, since localization of P granules defines the P lineage from the somatic precursor cells.

Sequence analysis suggests that OOC-3 is a novel *C. elegans* protein with a signal sequence, three transmembrane domains and two putative PEST sequences common to

rapidly degraded proteins (Pichler, Gonczy et al. 2000). Antibody staining indicates that *ooc-3* is localized to the Endoplasmic Reticulum in these early embryos, and displays dynamic distribution through the cell cycle (Pichler, Gonczy et al. 2000). *ooc-3*,therefore,appears to be an ER transmembrane protein that is required for proper localization of PAR proteins and P granules for maintenance of germ cell identity of the P lineage in the *C. elegans* early embryo.

***gna-2* is involved in maintaining the germline identity of P lineage:** *gna-2* encodes one of two *C. elegans* glucosamine phosphate N-acetyl transferases expressed in the larval and adult pharynx (McKay, Johnsen et al. 2003; Hunt-Newbury, Viveiros et al. 2007). *gna-2* mRNA in the gonads is first detected in germ cells as they enter meiosis, and its abundance increases as they progress though meiosis in the distal arm of the gonads. *gna-2* mRNA levels decrease as the oocyte develops in the proximal gonadal region but re-accumulates in the most proximal mature oocytes (Lee and Schedl 2004). During embryogenesis, *gna-2* is required for synthesis of UDP-N-acetylglucosamine (UDP-GlcNAc), the substrate for eggshell chitin synthesis (Hunt-Newbury, Viveiros et al. 2007). Secretion of the chitinous eggshell begins in the one cell embryo, very early in embryogenesis. RNAi of *gna-2* in genome-wide studies produced phenotypes including defects in embryonic osmotic integrity, sterility, maternal sterility, lethality and embryonic lethality(Simmer, Moorman et al. 2003; Rual, Ceron et al. 2004; Sonnichsen, Koski et al. 2005). While *gna-2* homozygous mutant males are fertile, hermaphrodites are viable but 100% penetrant for a maternal-effect embryonic lethal phenotype (Johnston, Krizus et al. 2006), indicating the importance of its role in oocyte viability and survival.

Apart from its role in eggshell synthesis and osmotic integrity(or proper eggshell development), *gna-2* is also involved in the completion of meiosis upon fertilization, establishing polarity and asymmetric segregation of cell fate determinants in the one-cell embryo (Johnston, Krizus et al. 2006). *gna-2* embryos display errors in chromosome segregation and polar body extrusion. They fail to properly associate the sperm pronucleus-centrosome complex with the early embryonic cortex, which is important in establishing the AP axis of the embryo. They are also defective in posterior localization

of PAR-2, P granules and PIE-1 prior to the first embryonic cell division (Johnston, Krizus et al. 2006), which are all essential for maintaining germline identity of the P lineage.

To test whether the lifespan extension produced by RNAi of these germline related genes fits the known mechanism of aging-regulation by the germline, I knocked down each gene in *daf-16* and *daf-2* mutants. Germline ablation studies indicate that lifespan extension by loss of germline is dependent on DAF-16, but independent of DAF-2. If these genes are influencing aging in a similar manner, then RNAi knockdown of these genes should extend the lifespan of *daf-2* mutants but not be able to extend the lifespan of *daf-16* mutants.

Results and Discussion

daf-16 mutation suppresses lifespan extensions produced by RNAi of germline-related *let-711*-regulated genes

Previous studies have shown that lifespan extension via germline ablation requires *daf-16* activity (Hsin and Kenyon 1999; Arantes-Oliveira, Apfeld et al. 2002). To test if the genes identified through our microarray study function in a similar manner, these germline-related *let-711*-regulated genes were knocked down via RNA mediated interference in a *daf-16* background. The *C. elegans* strain IU10 (Hamilton, Dong et al. 2005) was used, which carries a *daf-16* loss-of-function mutation (*mgDf47)*I, as well as the *rrf-3 (pk1426)*IImutation making it hypersensitive to RNAi (Simmer, Tijsterman et al. 2002). Worms were maintained at 25°C, and feeding of dsRNA expressing bacteria was initiated at the L1 stage. The *daf-16* mutation was able to suppress the lifespan extension phenotype of all four genes. All four RNAi experiments produced a mean lifespan of about nine days at 25°C, which is not significantly different from the control *daf-16; rrf-3* (IU10)worms (Figure 3.1, Table 3.1).

The results support the notion that lifespan extension through removal of germline is mediated through *daf-16* activity. *let-711*-regulated genes: *cdc-25.1, meg-1, ooc-3* and *gna-2,* are all involved in germline development and proliferation.Losing the function of *cdc-25.1* or *meg*-1 are also known to severely restrict germline proliferation. Individual RNAi of each of these genes significantly extended lifespan but that lifespan extension was completely reverted in the absence of *daf-16.* These results agree with the results observed in germline ablated or *glp-1* mutants and strengthen the notion that in wild type animals, germline signals inhibit the activity of DAF-16, thereby suppressing longevity.

meg-1, ooc-3 and *gna-2* regulate lifespan independently of *daf-2*

As discussed earlier, lifespan extension via germline ablation requires DAF-16, but is independent of DAF-2. Our previous experiments showed that lifespan extension by these germline-related *let-711*-regulated genes also requires functional *daf-16.* To check whether *let-711* affects lifespan via the insulin-like signaling pathway or independently of it, I knocked down these *let711*-regulated genesin CF1814 worms, which are *daf-2(e1370)*III*;rrf-3(pk1462)*IImutants.

CF1814 (*daf-2;rrf-3)* mutants are long-lived with a mean lifespan of 23.9±0.5 days and a maximum lifespan of 44 days at 25°C. Individual RNAi of three *let-711*-regulated genes, *meg-1, ooc-3* and *gna-2,* further increased the mean and maximum lifespans of these long-lived *daf-2* worms (Figure 3.3, Table 3.1), indicating that they regulate lifespan in a *daf-2* independent manner. *meg-1* RNAi resulted in a mean lifespan increase by 28.5% to 30.7±0.8 days, it also increased maximum lifespan by 13.6% to 50 days. RNAi of *ooc-3* produced mean and maximum lifespan increases by 14.6% to 27.4±0.7 days and 18.2% to 52 days respectively. Knocking down *gna-2* increased mean lifespan by 18.4% to 28.3±0.7 days and maximum lifespan by 4.5% to 46 days (Figure 3.3). These results also support that germline signaling to DAF-16 is independent of DAF-2 and the insulin-like signaling pathway.

Lifespan extension by *cdc-25.1* is dependent on *daf-2*

RNAi of *cdc-25.1* failed to produce any significant mean-lifespan extension in CF1814 worms; it produced a mean lifespan of 23.9±0.7 days (Figure 3.2, Table 3.1). This indicates that the mean-lifespan extension due to loss of *cdc-25.1*, unlike that of other germline-related genes,requires *daf-2* activity. Knocking down *cdc-25.1* however, increased maximum lifespan by 18.2% to 52 days (Figure 3.2) in *daf-2* mutants indicating that this gene is capable of influencing maximum lifespan independently of the insulin/IGF-1 pathway. Therefore, while lifespan extension due to RNAi of *cdc-25.1* requires DAF-16, its relation with DAF-2 is more complicated. While *cdc-25.1* RNAi requires DAF-2 to increase mean lifespan of the population, it functions independently of DAF-2 to extend maximum lifespan in *C. elegans.*

The survival curve of the *daf-2* population subjected to *cdc-25.1* RNAi (Figure 3.2) indicates a higher death rate for these worms during early adulthood, but a strongly reduced death rate during late life, compared to the control. This produces the increased maximum lifespan of the population. Could this be indicating that*cdc-25.1* has antagonistic plieotropic functions based on age of the organism? This biphasic nature of the survial curve is only observed in a *daf-2* background, whereas *cdc-25.1* knockdown in wildtype worms increases both mean and maximum lifespan (Figure 2.10). Exactly how *cdc-25.1* interacts with *daf-2* in regulating aging in *C. elegans* is unclear at this point.

Conclusions

let-711 regulates lifespan through the germline signaling pathway

The observation that four of *let-711* regulated genes that extended lifespan when knocked down through RNAin my studies were germline related, led me to speculate that *let-711* may be regulating lifespan through the germline signaling pathway. Previous studies have shown that proliferating germline cells inhibit longevity by inhibiting DAF-16 activity independently of the DAF-2/insulin-like signaling pathway (Hsin and Kenyon 1999).

The results of my experiments show that the genetic mechanism of aging regulation by these genes fits the existing model of lifespan regulation by the germline. Individual RNAi of each of these *let-711*-regulated genes: *cdc-25.1, gna-2, meg-1* and *ooc-3*, significantly extended lifespan (Figures 2.9 and 2.10), and those lifespan extensions were completely reverted in the absence of *daf-16* (Figure 3.1). Three of the germline-related *let-711*-regulated genes: *meg-1, ooc-3* and *gna-2* were also able to extend lifespan synergistically with the loss of *daf-2* (Figure 3.3). These results strengthen the notion that in wild type animals, germline signals suppress longevity by inhibiting the activity of DAF-16, independently of DAF-2 in the insulin-like signaling pathway. Since these germline-related genes are downregulated upon *let-711* RNAi, *let-711* normally functions to promote germline proliferation in wild type worms, thereby inhibiting longevity.

let-711 regulates aging through *daf-2* dependent check-point signaling mechanisms

cdc-25.1 RNAi was unable to produce a significant change in the mean lifespan of *daf-16* or *daf-2* mutants, but it increased maximum lifespan to a similar degree in *daf-2* mutants (18.2%) (Figure 3.2) as it did in wildtype worms (20%) (Figure 2.10). Therefore,

while lifespan extension due to RNAi knockdown of *cdc-25.1* requires DAF-16, its relation with DAF-2 is more complicated. *cdc-25.1* RNAi requires DAF-2 to extend mean lifespan of the population, but it seems to function independently of DAF-2 to affect maximum lifespan in *C. elegans.*

The extension of mean lifespan due to *cdc-25.1* RNAi requires both *daf-2* and *daf-16* (Figures 3.1, 3.2). At first glance this seems to be in contrast to the idea that germline signals to DAF-16 are completely independent of DAF-2. However, we have to consider the dual molecular nature of *cdc-25.1*. While *cdc-25.1* is a gene required primarily for germline proliferation in *C. elegans,* it is also a cell cycle regulator, and part of the checkpoint mechanisms that arrest cell division in response to DNA damage or stalled replication forks. The role of checkpoint genes in aging regulation will be discussed further below. For now, our results show that mean lifespan extension due to loss of *cdc-25.1* requires both *daf-16* and *daf-2* activity and hence functions upstream of the insulin/IGF-1 pathway, possibly through checkpoint signaling mechanisms.

Model for *let-711* regulation of lifespan in adult *C.elegans*

let-711 regulates lifespan through two pathways: the insulin/IGF-1 pathway and through the germline signaling pathway. Knocking down *let-711* expression produced an almost four-fold reduction in *pdk-1* expression (Table 2.3, Figure 2.4c), which indicates crosstalk between the regulation of lifespan by the insulin/IGF-1 pathway and that of *let-711.* Four other *let-711*-regulated genes whose individual RNAi resulted in extension of lifespan in *C. elegans* are germline-related genes. These four genes: *cdc-25.1, gna-2, meg-1* and *ooc-3* all show downregulated expression when *let-711* expression is knocked down. Lifespan extensions generated by the knockdown of these genes require functional *daf-16. cdc-25.1* is the only gene in this group whose effect on lifespan is also dependent on *daf-2.* So I propose a model for regulation of aging by *let-711* (Figure 3.4)*: let-711* influences lifespan determination in adult *C. elegans* by inhibiting DAF-16 activation. It does so in at least three distinct ways: (1) By upregulating expression of *pdk-1* leading to

increased insulin/IGF-1 signaling to inhibit DAF-16 activity,(2) By upregulating expression of germline proliferation genes leading to increased germline signals to inhibit DAF-16 activity (independent of DAF-2). 3.By upregulating expression of *cdc-25.1* leading to inhibition of DAF-16 through cell-cycle checkpoint signaling mechanisms (upstream of DAF-2).

Materials and Methods

Strains and maintenance

The following *C. elegans* strains were used in this study: *rrf-3 (pk1426).* IU10: *daf-16(mGdf47);rrf-3(pk1426).* CF1814: *daf-2(e1370);rrf-3(pk1426).* These were obtained from Caenorhabditis Genetics Center (CGC) and maintained as described (Sulston and Hodgkin 1988).

RNA interference

RNAi by feeding was performed as described (Kamath, Martinez-Campos et al. 2001).RNAi clones were inoculated overnight at 37°C in LB plus carbenicillin at 25μg/ml (10μg/ml tetracycline for the control with empty vector pL4440). This culture was then seeded onto NGM plates with 25μg/ml carbenicillin (10μg/ml tetracycline for the control) and 0.2mM IPTG for RNAi induction overnight at 37°C. *rrf-3* worms were transferred toRNAi plates on day 1, during the L1 stage.

Lifespan Analysis

Lifespan assays were conducted in general as described previously (Hansen, Hsu et al. 2005). Lifespan assays were performed at 25°C and feeding of dsRNA expressing

bacteria was initiated at the L1 stage. Each trial consisted of three plates per RNAi strain with 20 worms per plate, which was repeated three times for each lifespan assay. Since the first trial of lifespan assays with the *daf-16* mutants failed to produce any significant results, the assay was not repeated for a second and a third trial. The animals were transferred to RNAi plates that contained 5mg/ml FuDR to inhibit sample contamination by F1 worms. The numbers of live and dead worms were scored every other day and the worms were transferred to fresh RNAi plates on a weekly basis. Animals that crawled off the plate, exploded, bagged or became contaminated were censored.

The R statistics package was usedto calculate mean lifespans and perform statistical analyses. TheLogrank (Mantel-Cox) test in theR survival library was used to calculatep-values and determined survivalstatistics on the censored individual worm survival data.

Figure 3.1: The *daf-16* gene is required for the longevity of*cdc-25.1, meg-1, ooc-3* and *gna-2* RNAi. Lifespan curves were obtained as described in Material and Methods. Synchronized IU10 [*daf-16(mGdf47)I;rrf-3(pk1426)II*]L1 animals were transferred to specific RNAi plates and raised at 25°C. Mean lifespans were 9.05±0.20 days for IU10(n=60); 8.67±0.15 days for *cdc-25.1* RNAi (n=60), 9.52±0.22 for *meg-1* RNAi (n=60), 9.19±0.20 days for *ooc-3* RNAi (n=60) and 9.59±0.18 days for *gna-2* RNAi (n=60). None of the mean lifespans were significantly different from that of the control population.

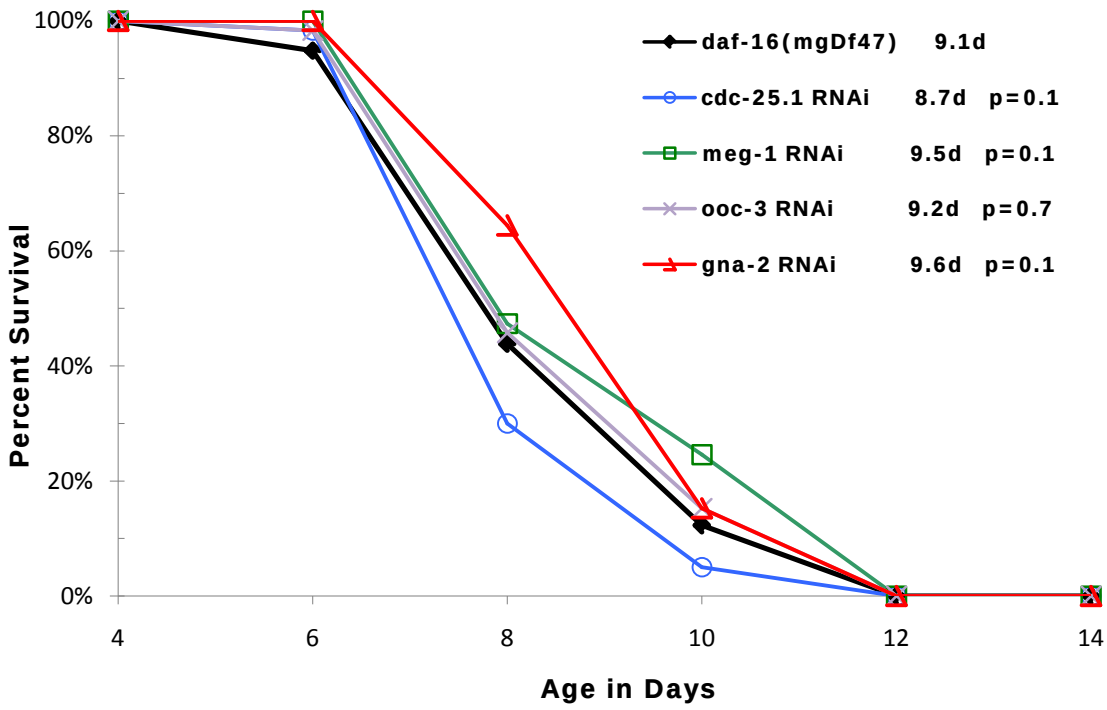


Figure 3.2: Loss of *daf-2* suppressed the lifespan extension of *cdc-25.1* RNAi. Lifespan curves were obtained as described in Material and Methods. Synchronized CF1814 [*daf-2(e1370)III;rrf-3(pk1426)II]* L1 animals were transferred to specific RNAi plates at 20°C, then moved to 25°C as young adults. The mean lifespan of *cdc-25.1* RNAi population is not significantly different from that of the control CF1814population. CF1814 [*daf-2(e1370)III;rrf-3(pk1462)II*] control: n=180, m=23.9±0.5 days. *cdc-25.1* RNAi: n=180, m=23.9±0.7 days, p=0.91. *cdc-25.1* RNAi increase maximum lifespan by 18.2%, from 44 days to 52 days.

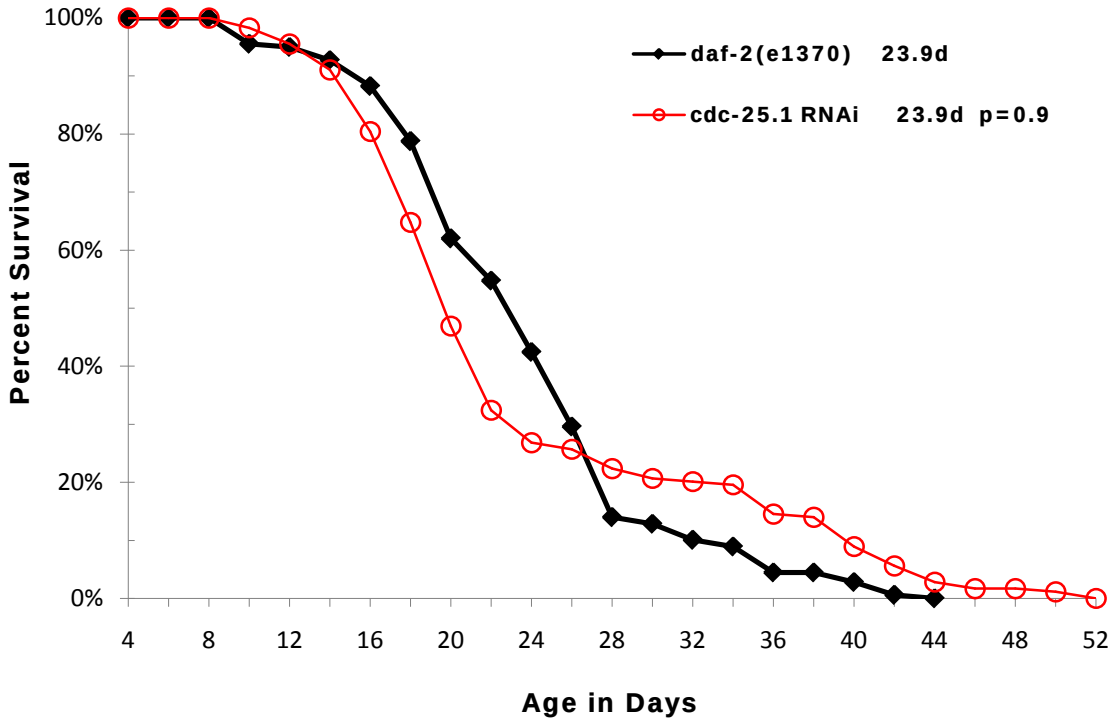


Figure 3.3: Lifespan extensions of individual *meg-1*, *ooc-3* and *gna-2* RNAi were independent of *daf-2*. In the same experiment described in Figure 3.3, individual RNAi of *meg-1*, *ooc-3* and *gna-2* further increased the mean and maximum lifespan of CF1814 [*daf-2(e1370)III;rrf-3(pk1426)II]* worms. CF1814control: n=180, m=23.9±0.52 days, max=44 days; *meg-1* RNAi: n=180, m=30.7±0.8 days, p=2.8e-15, max=50 days; *ooc-3* RNAi: n=180, m=27.4±0.7 days, p=6.7e-06, max=52 days; *gna-2* RNAi: n=180, m=28.3±0.7 days, p=7.8e-08, max=46 days.

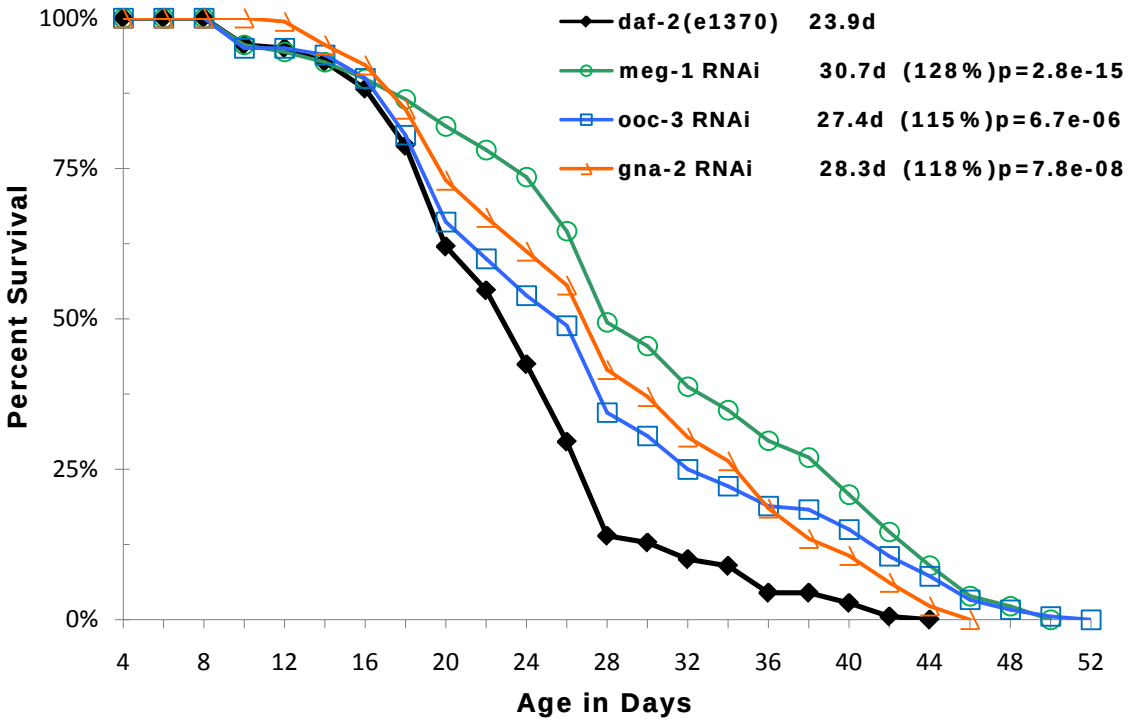


Figure 3.4: A model for *let-711* regulation of lifespan in adult *C. elegans*.
let-711 influences lifespan determination in adult *C. elegans* by inhibiting DAF-16 activation. It does so in at least three distinct ways: 1. By upregulating expression of *pdk-1* thereby upregulating the insulin-like signaling pathway (Shown in blue). 2. By upregulating expression of germline proliferation genes leading to increase germline signals to inhibit DAF-16 activation (Shown in red). 3. By upregulating expression of *cdc-25.1* leading to DAF-16 inhibition through check-point/stress-response pathway (Shown in green).

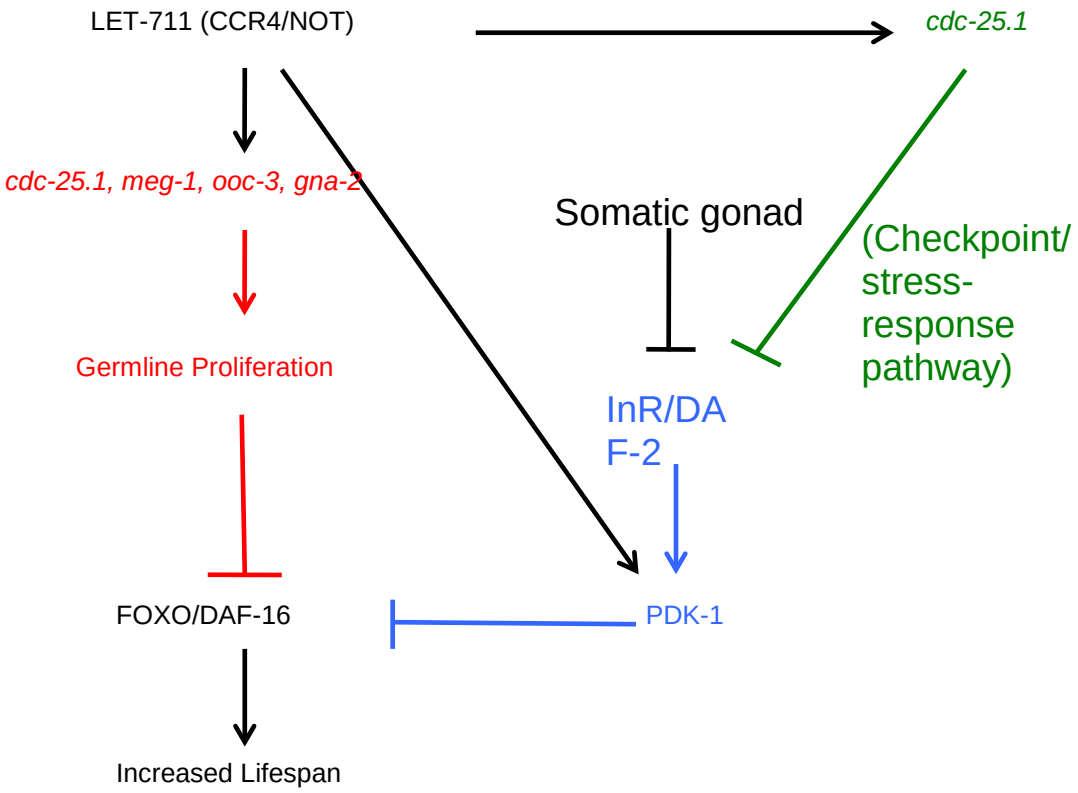


Table 3.1: Summary of life-span assays. Log-rank *P* values from comparisons between worms fed RNAi bacteria with and without the indicated fragment inserted in the feeding vector (pL4440). *n* indicatesnumber of deaths recorded.ns indicates the result was not significant.

Strain	Survival (mean ± SE days)	n	Log-rank test P value	Temp.	RNAi induction
Experiment I				20°C	L4/Adult
rrf-3(pk1426)	18.4 ± 0.4	166			
rrf-3(pk1426) lin-11 RNAi	17.1 ± 0.3	169	0.0003		
rrf-3(pk1426) let-711 RNAi	15.6 ± 0.3	166	3.29e-07		
Experiment II				25°C	L1
rrf-3(pk1426)	16.1 ± 0.2	233			
rrf-3(pk1426) K11E4.2 RNAi	17.6 ± 0.2	188	4.06e-07		
Experiment III				25°C	L1
rrf-3(pk1426)	15.3 ± 0.1	253			
rrf-3(pk1426) C49A9.2 RNAi	17.7 ± 0.3	144	0.00		
rrf-3(pk1426) Y82E9BR.5 RNAi	16.7 ± 0.2	203	1.39e-10		
Experiment IV				25°C	L1
rrf-3(pk1426)	16.3 ± 0.3	185			
rrf-3(pk1426) R53.5 RNAi	17.5 ± 0.3	178	6.74e-04		
Experiment V				25°C	L1
rrf-3(pk1426)	14.1 ± 0.2	205			
rrf-3(pk1426) pdk-1 RNAi	16.8 ± 0.2	175	0.00		
Experiment VI				25°C	L1
rrf-3(pk1426)	14.1 ± 0.2	205			
rrf-3(pk1426) grsp-4 RNAi	15.7 ± 0.2	158	7.64e-09		
rrf-3(pk1426) meg-1 RNAi	15.9 ± 0.1	189	5.45e-14		
Experiment VII				25°C	L1
rrf-3(pk1426)	14.1 ± 0.1	213			
rrf-3(pk1426) cdc-25.1 RNAi	17.0 ± 0.2	183	0.00		

rrf-3(pk1426) gna-2 RNAi	17.5 ± 0.3	195	0.00		
rrf-3(pk1426) ooc-3 RNAi	15.9 ± 0.1	190	2.87e-15		
Experiment VIII				25°C	L1
IU10 [*daf-16(mGdf47); rrf-3(pk1426)*]	9.1 ± 0.2	60			
IU10 *cdc-25.1* RNAi	8.7 ± 0.1	60	ns		
IU10 *gna-2* RNAi	9.6 ± 0.2	60	ns		
IU10 *meg-1* RNAi	9.5 ± 0.2	60	ns		
IU10 *ooc-3* RNAi	9.2 ± 0.2	60	ns		
Experiment IX				25°C	L1
CF1814 [*daf-2(e1370); rrf-3(pk1426)*]	23.9 ± 0.5	180			
CF1814 *gna-2* RNAi	28.3 ± 0.7	180	7.79e-08		
CF1814 *meg-1* RNAi	30.7 ± 0.8	180	2.78e-15		
CF1814 *ooc-3* RNAi	27.4 ± 0.7	180	6.73e-06		
CF1814 *cdc-25.1* RNAi	23.9 ± 0.7	180	ns		

CHAPTER 4: Conclusions and Future Directions

This project was initiated to study whether gene-expression regulators*lin-11* and *let-711* play a role in the regulation of aging in adult *C. elegans.* Although both genes had no known role in aging and are characterized as early developmental genes, they show strong aging-correlated expression profiles(Lund, Tedesco et al. 2002). Knockdown of either gene reduced lifespan in our studies (Figure 2.1). Weidentifiedgenome-wide targets of *lin-11* and *let-711* in the adult worm, to study if their targets are capable of influencing lifespan. Through lifespan studies of worms in which these putative target genes were knocked down, we establish that both *lin-11* and *let-711* do indeed influence expression of aging genes (Table 3.1). We propose that *lin-11* activity acts to extend lifespan by downregulating the insulin/IGF-1 pathway. *let-711* activity, on the other hand, may be detrimental to longevity as it upregulates the insulin/IGF-1 pathway in two distinct manners: by upregulating *pdk-1* expression, which is an integral part of the insulin/IGF-1 pathway, and by upregulating *cdc-25.1*, thereby possibly upregulating the insulin/IGF-1 pathway through checkpoint mechanisms. *let-711* also seems to be promoting germline proliferation, which inhibits longevity independently of the insulin/IGF-1 receptor, *daf-2*. Therefore,we propose that progressive downregulation of *let-711* in aging worm populations promotes longevity by downregulating the insulin/IGF-1 signaling pathway and by downregulating the germline signaling pathway.

lin-11 regulation of aging

The microarray experiments identified sixteen genes that show differential expression by at least two fold with*lin-11* knockdown. RNAi of four of these*lin-11* affectedgenes: K11E4.2, R53.5, C49A9.2 and Y82E9BR.5,were able to significantly extend lifespan in *C. elegans,*showing that *lin-11* does indeed have a role in the expression of longevity genes. How these genes, and hence *lin-11,* regulate aging in *C.*

elegans is unknown at this point. *lin-11* seems to have a complex effect on lifespan, since RNAi of both upregulated (C49A9.2, Y82E9BR.5) and downregulated (K11E4.2, R53.5) genes can extend lifespan in *C. elegans.*

While *lin-11* expression is upregulated in very old worms, knocking down the expression of *lin-11* in young adults resulted in a significantly shortened lifespan. This suggests a role for *lin-11* activity in promoting longevity. If *lin-11* activity is indeed promoting longevity, overexpression of *lin-11* in adult worms should lead to lifespan extension. Genetic analysis of these long-lived worms would be needed to decipher whether *lin-11* influences lifespan through the insulin/IGF-1 pathway.

Ipropose that*lin-11* influences the insulin/IGF-1 pathway, because RNAi knockdown of *lin-11* produced a strong increase in the expression of the insulin-like ligand *ins-3* (Figure 2.4b, Table 2.2). There are more than thirty insulin-like ligands in the *C. elegans* genome, which are hypothesized to bind the single *C. elegans* insulin/IGF-1 receptor, DAF-2, to activate this pathway. Under normal conditions, *lin-11* presumeablyfunctions to downregulate *ins-3* expression, which may downregulate the insulin/IGF-1 pathway,resulting in an increased lifespan. If so, over-expressing *lin-11* should have no effect on lifespan of *daf-2* mutants. If *lin-11* regulates aging independently of the insulin/IGF-1 pathway, loss of *daf-2* should further extend the lifespan of *lin-11* overexpressing worms. If *ins-3* influences lifespan through the insulin/IGF-1 pathway, RNAi of *ins-3* should extend lifespan in wild-type worms but not in *daf-2* mutants.

Since *lin-11* is expressed in the vulval cells, as well as some neurons (Gupta, Wang et al. 2003; Reece-Hoyes, Shingles et al. 2007), it would be interesting to test whether neuronal and vulval *lin-11* have discreet functions in lifespan regulation. Many sensory neurons produce insulin-like peptides that influence longevity through the insulin/IGF-1 pathway (Apfeld and Kenyon 1999; Pierce, Costa et al. 2001; Li, Kennedy et al. 2003; Murphy, McCarroll et al. 2003; Alcedo and Kenyon 2004). Therefore, it is

reasonable to propose that neuronal *lin-11* plays a part in regulating the aging insulin/IGF-1 pathway.

Strong upregulation of *lin-11* is reported in the final 10% of *C. elegans* populations when 90% have succumbed to death by old age. It is possible that instead of *lin-11* being upregulated in very old worms, 10% of the population stochastically express *lin-11* to a higher degree than the rest of the population, enabling them to outlive the others. If so, LIN-11 could be a molecular marker for aging protection of susceptibility. To test if LIN-11 could be an aging marker, transgenic worms can be created that express a GFP-tagged LIN-11 and such populations scanned through fluorescent microscopy to see if individuals within this isogenic population express higher levels of GFP during early adulthood. If so lifespans of higher GFP expressers can be compared with those that express GFP at a normal level to see if these high GFP expressers have a longer lifespan.

let-711 regulation of aging

In wild type worms, *let-711* is progressively downregulated in aging populations. RNAi of several genes that are downregulated upon loss of *let-711: pdk-1, cdc-25.1, gna-2, meg-1, ooc-3* and *grsp-4*, all lead to an extended lifespan in *C. elegans*, suggesting that *let-711* normally functions to inhibit longevity. Yet, RNAi of *let-711* significantly reduces the worm lifespan. *let-711*, expressed broadly in the gonads (Kohara 2005), is the ortholog of NOT1. NOT1 itself has no active or DNA binding domains. It is instead, the scaffold for the CCR4/NOT complex that is involved in regulating various aspects of gene-expression such as transcription, mRNA deadenylation and protein ubiquitination (reviewed in (Collart and Timmers 2004). There are nine subunits to this complex, many subunits with unique domains. Since NOT1 is the scaffold, it is possible that its loss results in failure to assemble the complex, leading to general deleterious effects on cellular function that result in premature death. It would therefoe, be interesting to test whether the loss of another subunit of this complex can extend lifespan. Perhaps one or more of the subunits function in the adult worm to regulate aging, while others regulate

genes involved in more basic cellular functions. Knocking down the expression of each subunit through RNAi would allow identification of subunits that inhibit longevity in wild type *C. elegans.*

The results of this study indicate that *let-711* inhibits longevity by upregulating germline proliferation as well as by upregulating *pdk-1* of the insulin/IGF-1 pathway. Consistent with previous studies, lifespan extension due to loss of germline proliferation requires DAF-16 but is independent of DAF-2 (Figure 3.1, 3.3). Although the germline signal is received by DAF-16, it is not known whether DAF-16 receives this signal directly or if this signal is relayed to DAF-16 through an intermediary protein. Since *let-711* RNAi strongly reduces *pdk-1* expression, I hypothesize that the germline signal is relayed through PDK-1. If the signal is relayed through PDK-1, the lifespan extension seen in germline deficient worms should be reverted to wild type levels in *pdk-1* mutants, just as in *daf-16* mutants. If PDK-1 is not involved in the germline pathway, then loss of *pdk-1* would further extend the lifespan of germline deficient worms or it produces no change.

let-711 RNAi also reduced expression of *cdc-25.1* and subsequent RNAi of *cdc-25.1* extended mean lifespan in *C. elegans* (Figure 2.10). Although *cdc-25.1* is primarily expressed in the gonads and its major function is germline proliferation, it affects lifespan differently than other germline-related genes. Unlike other germline genes, the extension in mean lifespan due to *cdc-25.1* RNAi was dependent on DAF-2 (Figure 3.2), in addition to its dependence to DAF-16 (Figure 3.1). This indicates that *cdc-25.1* functions upstream of DAF-2 in regulating aging, which is unlike how other germline genes regulate aging.

cdc-25.1 is a cell cycle regulator that functions in an evolutionarily conserved cell-cycle checkpoint mechanism. Checkpoint signaling mechanisms upregulate DNA repair mechanisms and cause increased stress resistance and lifespan in *C. elegans* (Olsen, Vantipalli et al. 2006). Many of the genes upregulated by inhibition of the insulin/IGF-1 pathway (increased DAF-16 activity) are also involved in combating DNA-

damage inducing stressors, such as oxidative stress and UV stress (Honda and Honda 1999; McElwee, Bubb et al. 2003; Murphy, McCarroll et al. 2003; Halaschek-Wiener, Khattra et al. 2005; Wolff, Ma et al. 2006). Furthermore, upregulation of individual DAF-16 targeted stress resistance genes can extend lifespan in wild type *C. elegans* (Back, Matthijssens et al. 2010). Individual RNAi of other checkpoint genes: *cdc-25.3, cid-1* and *chk-1,* also produced increased thermotolerance and extended lifespanin *C. elegans* (Olsen, Vantipalli et al. 2006).

Although *cdc-25.1* mutants develop into sterile adults with no apparent somatic defect, two independent gain-of-function mutants induce extra divisions in the intestine and hyperplasia of intestinal cells (Clucas, Cabello et al. 2002; Kostic and Roy 2002). This suggests that *cdc-25.1* is also active in the intestinal cells. It is thought that *cdc-25.1* mutants do not show any somatic defects because other CDC25 family members may be compensating for the loss of *cdc-25.1* in the intestinal cells. Also, RNAi of other members of the CDC25 family;*cdc-25.2* and *cdc-25.3,* which are not expressed in the germline, extend lifespan (Olsen, Vantipalli et al. 2006).

Our results show that mean lifespan extension due to loss of *cdc-25.1* requires both *daf-16* and *daf-2* activity and hence functions upstream of the insulin/IGF-1 pathway. It is therefore possible that *cdc-25.1* signals as a cell-cycle regulator which requires *daf-2* activity, whereas*meg-1, ooc-3* and *gna-2* signal through the germline pathway that is independent of *daf-2* activity. It would be interesting to study whether the increase in lifespan due to loss of other checkpoint genes also requires DAF-2 activity to test whether these functionally related genes act in a single pathway upstream of the DAF-2 receptor.

Germline proliferation decreases in aging organisms, but it is not known how this reduction is germline proliferation is regulated. This study indicates that *let-711* may be part of the mechanism that regulates this reduction in germline proliferation. In wildtype worms, *let-711* expression starts to decline just as worms have exhausted their sperm reserve and laid their final eggs (day 6-9 cohort) (Lund, Tedesco et al. 2002). RNAi

knockdown of *let-711* strongly reduces the expression of genes, like *cdc-25.1* and *meg-1*,which are required for proliferation of the germline. This suggests post-reproductive *C. elegans* may be actively downregulating *let-711* expression in an effort to restrict germline proliferation and preserve lifespan.

In this study, I have identified potential genome-wide targets of *lin-11* and *let-711* through microarray studies. By knocking down these genes and through subsequent lifespan assays, I show evidence, for the first time, that gene expression regulators *lin-11* and *let-711* are required for wildtype expression of aging genes. I then performed further genetic studies to propose mechanisms for how these gene expression regulators may be controlling the rate of aging and lifespan in *C. elegans.* I propose that *lin-11* contributes to increased lifespan by downregulating the longevity-inhibiting insulin/IGF-1 signaling pathway. *let-711,* on the other hand, functions against increased lifespan by inhibiting DAF-16 activity through the insulin/IGF-1 pathway as well as the germline signaling pathway. This study also provides evidence to suggest the involvement of *let-711*in the active shutdown of germline proliferation in post-reproductive wildtype*C. elegans* to promote longevity.

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Vita

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EDUCATION

B.A., Biology, Berea College, 2002.

APPOINTMENTS AND AWARDS

Teaching Assistant, University of Kentucky (2004 – 2011)

Biology Rep., Graduate Student Council (2009)

Biology Faculty Search Committee, University of Kentucky (2008 - 2009)

President, Biology Graduate Student Association (2007 – 2008)

University of Kentucky, Ribble Scholarship(2007)

Teaching Assistant, Berea College (2002)

KBRIN Undergraduate Summer Research Fellowship (2002)

Undergraduate Academic Scholarship, Berea College (1998 - 2002)

RESEARCH EXPERIENCE

Doctoral Research (2005 – 2010).

University of Kentucky, Biology Department.

Advisor: Jim Lund Ph.D.

Characterizing the role of transcription factors *lin-11* and *let-711* in aging regulation, using *C. elegans* as a model organism: Research involved identification of genome-wide targets of these two transcription factors using microarray analysis. RNAi knockdowns of their targets toobserve effect on lifespan and genetic studies to understand the molecular signaling pathways these genes modulate to regulate *C. elegans* lifespan. Other techniques employed include, IPTG induction of dsRNA constructs, qRT-PCR to validate effective mRNA knockdowns, western

blots to validate loss of target proteins, total RNA extraction, RNA quality analysis and microarray data analysis using the R statistical package.

Development of a ChIP-SAGE method to identify genome-wide direct targets of tagged transcription factors: Performed western blot to validate antibody specificity, Chromatin immunoprecipited DNA fragments bound to tagged transcription factor, and generated SAGE-like tags for sequencing and genome mapping to identify direct targets. Also created tagged constructs of other aging transcription factors of interest using the Gateway cloning system. Other techniques employed: Bacterial cloning, *C. elegans* transformation (biolistic bombardment method), PCR, Gel electrophoresis, DNA digestion, DNA ligation.

Undergraduate Research Assistant (2002).

University of Kentucky, Biomedical Engineering Center.

Advisor: David Puleo, Ph.D.

Assisted in the development of a biological coating for implantable medical devices to enhance wound healing and reduce incidence of chronic inflammatory response. Performed flourospectrophotometric assays to determine protein content and retention of enzymatic activity, and quantify release of fluorescently labeled molecules of DNA, as a function of formulations.

ORAL PRESENTATIONS

INVITED PRESENTATIONS

Biology Graduate-Research Symposium (2009) **Aging and Stress Response in *C. elegans*.** (U. Kentucky)

Tri-State Worm Meeting (2006) **A Novel ChIP-SAGE Method to Characterize Direct Targets of Transcription Factors in *C. elegans*.**(Purdue, Indiana)

POSTER PRESENTATIONS

INTERNATIONAL MEETINGS

International *C. elegans* Meeting (2009) **Aging Transcription Factors *lin-11* and *let-711* in *C. elegans*.**(UCLA, California)

International *C. elegans* Meeting (2007) **Characterization of Aging Transcription Factors in *C. elegans*.**(UCLA, California)

LOCAL MEETINGS

*C. elegans*Aging, Metabolism, Stress, Pathogenesis, and Small RNA Meeting (2010) **Early-developmental Transcription Factors *lin-11* and *let-711* are Involved in Regulating Lifespan in Adult *C. elegans*. (U. Wisconsin, Madison)**

*C. elegans*Aging, Stress, Pathogenesis and Heterochrony Meeting (2008) **Identifying Direct Targets of *daf-16* using a Modified ChIP-SAGE protocol in *C. elegans*.** (U. Wisconsin, Madison)

Tri-State Worm Meeting (2006) **A Novel ChIP-SAGE Method to Characterize Direct Targets of Transcription Factors in *C. elegans*.**(Purdue, Indiana)