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Jui Atul Bhingarde Date

**Indoles Derived from Commensal Gut Bacteria Regulate Host Somatic and Reproductive Aging via the Interferon Pathway**

By

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Environmental Health

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By:

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Bachelor of Science
University of Georgia
2014

Thesis Committee Chair:
Dr. Daniel Kalman, Ph.D.

An abstract of
the thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Environmental Health
2018

## Abstract

**Background:**

At the cusp of the 20th century, public health advances have contributed rapid increase in life expectancy to global populations by almost 30 years (Christensen et al. 2009). An increase in lifespan does not imply better health and decrease in healthcare cost, but increased longevity without health span improvement can lead to higher expenditures (Wouterse et al. 2015). Healthspan is broadly defined as the length of time that an individual remains healthy and free of age-related infirmities (Tissenbaum et al. 2012, Tatar et al. 2009). Gradual exposure to environmental stressors and lifestyle factors lead to deterioration of the body (Figure S1). Although there have been several studies identifying lifespan mechanisms, health span has received much less attention. Health span has often been convolved with lifespan, and extended health span has been associated with slowed onset of normal age-related changes (e.g. sarcopenia). Here the research objective is to further evaluate evolutionarily conserved pathways that may be involved in extending health span.

**Methods:**

The study was conducted using lifespan experiments on a well-studied *Caenorhabditis elegans* animal model, while statistical analysis was performed using PRISM to give survival analysis curves. Genetic mutants were exposed to indoles from E. coli bacterial strains and were tested against controls to see whether they exhibit different phenotypes.

**Results:**

The results showed a series of type I interferon pathway genes with elevated exposure when *C. elegans* worms lacking certain genes exposed to indoles showed similar response to worms not exposed to indoles. These genes are crucial in the type I interferon pathway and provided further evidence to study the pathway and how indoles interacts with the genes.

**Conclusions:**

In conclusion, the study reported genes in the Type 1 interferon pathway that were upregulated by indole through a current unknown mechanism. These results not only indicate the likelihood that the aging mechanism is modulated by the immune system but aging itself is a very complex phenomenon. These implications for the public health studies, show that more information can be gathered through population genomic studies for understanding how they age.

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Thank you Aai and Baba for being wonderful parents who have supported my relentless pursuit of my dreams. You have always given me courage to follow my heart and stand up for my decisions. Aai and baba, your passion for your work has always inspired me to follow my own calling. Thank you for always feeding me delicious food

Thank you, Hemant, for always being a supportive, encouraging, and understanding companion throughout my research endeavors. Thank you for always making me laugh even when the data didn't seem to emit any humor.

*In memory of my beloved grandmother, Shaila Berde. No amount of words can express how much I miss you. (1946-2011)*

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### Chapter II

# Chapter I

**Introduction**:

At the cusp of the 20th century, public health advances have contributed rapid increase in life expectancy to global populations by almost 30 years (Christensen et al. 2009). An increase in lifespan does not imply better health and decrease in healthcare cost, but increased longevity without health span improvement can lead to higher expenditures (Wouterse et al. 2015). Healthspan is broadly defined as the length of time that an individual remains healthy and free of age-related infirmities (Tissenbaum et al. 2012, Tatar et al. 2009).

Gradual exposure to environmental stressors and lifestyle factors lead to deterioration of the body (Figure S1). Current research indicates that the immune system may play a significant role in contributing to age related infirmities, (Reale M 2014). Environmental stressors and genetics promote the age-related inflammation or hinder the processes that mitigate inflammation (Reale M 2014). We are faced with stressors on a daily basis from different sources that lead to progressive damage to our bodies through production of reactive oxygen species (ROS). ROS can lead to chronic inflammation through several different mechanisms.

Although there have been several studies identifying lifespan mechanisms, health span has received much less attention. Health span has often been convolved with lifespan, and extended health span has been associated with slowed onset of normal age-related changes (e.g. sarcopenia). Here the research objective is to further evaluate evolutionarily conserved pathways that may be involved in extending health span.

The model organism, *Caenorhabditis elegans* is advantageous to study aging and health span due to its short generation times, easy visualization, easy culturing, and quick reproductive rates. Because 60-80% of human homologues have been identified in *C. elegans*, it is a preferred tool for genetic research in humans (Kaletta & Hengartner 2006). *C. elegans*, are microscopic nematodes that grow up to 1-2 mm in length. They transit from hatched larva to adult in approximately 2 days in 20° C. With an average lifespan of 2-3 weeks after adulthood, *C. elegans* serve as an important tool for studying aging. Their transparent bodies make them easy visual tools.

Commensal microbiota facilitates nutrient metabolism, augment integrity of the intestinal epithelial barrier, enhance host immunity, and limit pathogen colonization (Mazmanian et al. 2005, Lee et al. 2014, Fukuda et al. 2011). Shifts in commensal microbiota occur with aging and may contribute to infirmity (Langille et al. 2014). However, little is known about the mechanisms by which the microbiota regulates health span and frailty. Using *C. elegans* as a biosensor, we identified indole and several metabolites (e.g. indole-3-carboxaldehyde (ICA), indole acetic acid (IAA)) as molecules secreted by *E. coli* that induce hormetic protection against stressors in worms, an effect mediated by factors controlling innate immunity and lifespan (Anyanful et al. 2009, Bommarius et al. 2013, Anyanful et al. 2005, Jones et al. 2015). In mammals, indoles derived from plant-based dietary sources, or produced by intestinal microbiota via tryptophanase (TnaA)-mediated catalysis of dietary tryptophan, attain millimolar concentrations in the intestinal tract, and derivatives can be detected throughout the body (Lee et al. 2015). Indoles regulate virulence in pathogenic bacteria, protect hosts from

infection, and limit colitis induced by pathogens or chemical stressors (Bommarius et al. 2013, Zelante et al. 2013, Shimada et al. 2013).

We wanted to investigate whether indoles from commensal bacteria extend health span in diverse species and the genetic players responsible in this phenomenon.

**Materials and Methods:**

**Bacterial strains.**

An E. coli strain variant resistant to nalidixic acid and streptomycin was selected as bacterial lawn due to its ability to efficiently colonize murine intestinal tract. The E. coli strain, MG1655* (called K12) while a MG1655*ΔtnaA was constructed after deleting tryptophanase gene (K12ΔtnaA) using the lambda-red-recombinase system.

**C. elegans strains**

*Caenorhabditis elegans* strains were attained from University of Minnesota Caenorhabditis Genetics Center (CGC). They have been maintained in medium size plates (size 60 mm diameter) with NGM agar medium inside each plate. The NGM plates were prepared using NaCl, Agar, Peptone, 4mb/ml cholesterol in ethanol (EtOH), 1 M $KPO_4$ buffer, 1 M $MgSO_4$, $H_2O$, petri dishes, and auto pipets with tips (Stiernagle et al. 1999). They can be maintained on small petri dishes as well as in conical tube solutions. Strains attained from CGC, wild-type Bristol strain N2, drh-1, rde-1, skn-1, and dcr-1. All strains from the CGC were maintained in the Nematode Growth Medium (NGM) plates at 16°C using standard culturing techniques (Sulston 1988).

**NGM plates.**

The plates used to grow the C. elegans worms were purchased from VWR International (plate sizes 100x15mm & 60x15mm). The Nematode Growth Medium (NGM) was developed by using 15 g Bacto Agar (BD, Becton, Dickinson Company), 3 g Sodium Chloride (Sigma Aldrich), 2.5 g Bacto Peptone (BD, Becton, Dickinson Company) in a 1000 mL (1 L) glass container. The container was filled to 1000 mL mark with distilled water and then autoclaved for 45 minutes. The NGM agar was removed from autoclaved

and placed into hot water bath. After waiting till the agar reached luke warm temperature, 1.0 mL of cholesterol, 0.5mL of 1M $CaCl_2$, 1.0 mL of 1M $MgSO_4$, 25.0 mL of $KPO_4$ were sequentially added to the agar. The NGM agar was then poured into petri dishes for the assays.

**Methods:**

**Lifespan experiments.**

Bacterial strains used for the lifespan assay were grown overnight in Luria-Bertani (LB) broth at 37°C to an $OD_{600}$ of 0.8 – 1.0 to seed plates on which the worms were maintained. The lifespan assays were performed at 16°C on NGM plates. *C. elegans* adult worms were grown at least through two generations to separate effects of the different bacterial strains. To ensure a synchronized experiment, the gravid *C. elegans* adult worms were transferred to NGM assay plates spotted with bacteria and were allowed to lay eggs for 4-5 hours at room temperature. NGM plates with the synchronized eggs were maintained at 16°C. The lifespan assays were conducted without the use of 5-fluorodeoxyuridine (FUdr) because as research has stated, the compound may impact lifespan itself (Anderson et al. 2016). Due to the *C. elegans* adult worm's rapid reproductive rate were transferred daily for the first 8-10 days to avoid generation mixing.

Our research seeks to identify cellular pathways by which indoles extend health span through immune modulation. Our eventual goal is to use indoles as interventions or therapies that will allow humans to live better for longer after being subjected to environmental stressors.

**Results:**

**Indoles derived from commensal *E. coli* extend healthspan of *C. elegans.*** To investigate effects of indoles secreted by commensal bacteria on survival, wild type *C. elegans (*N2) were grown on plates seeded with either an *E. coli* K12 variant (called K12) that produces and secretes indole and indole derivatives, or an isogenic mutant *E. coli* strain that contains a deletion in the tryptophanase gene (*tnaA*), which is required to convert tryptophan into indole (called K12ΔtnaA). K12 was selected for its capacity to colonize the intestinal tract and grow as a commensal in mice (16). Growing *C. elegans* on K12ΔtnaA, as compared to K12, shifted the Kaplan-Meier survival curve to the left (Fig. 1a) but was without significant effect on maximal lifespan (Fig. 1b). Growth of animals on K12ΔtnaA supplemented with 250□M indole, as compared to K12ΔtnaA supplemented with vehicle (methanol) shifted the Kaplan-Meier survival curve to the right (Fig. 1c) but was without significant effect on maximal lifespan (Fig. 1b). The shift of the population survival curves with K12 or indole, even without an attendant increase in maximal lifespan, raised the possibility that indole produced by K12 augments healthspan. Notably, growth of animals on K12 supplemented with 100□M indole further shifted the survival curve to the right compared to animals grown on K12 alone, and marginally extended maximal lifespan, suggesting that exogenous indole may provide a therapeutic effect even beyond that of commensal bacteria (Fig. 1d).

Motility, pharyngeal pumping and resistance to thermal stress were thus assessed over the lifespan of animals grown on K12ΔtnaA supplemented with either indole or vehicle. Exposure to indole increased survival of both young (2 day) and old (18 day) adult worms following thermal stress (Fig. 1e). Moreover, brief pre-exposure (72 hrs) of young

(1d) adults to indole in the absence of K12*ΔtnaA* still rendered animals more resistant to heat stress compared to vehicle-treated controls (Fig. 1f), suggesting that indole acts directly on the host rather than via an intermediate produced by bacteria. Worms grown until d8 on K12*ΔtnaA* with or without indole exhibited similar rates of thrashing motility and pharyngeal pumping, and exhibited no paralysis. However, differences were evident after d15; animals grown with indole exhibited more thrashing motility and pharyngeal pumping, and exhibited delayed onset of paralysis, compared to animals grown without indole (Figs. 1g-i). As a means of quantifying overall healthspan, the percentage of animals displaying at least 50% of the maximal response for each parameter at each time point were counted as healthy, whereas those falling below this mark were considered frail (Fig. 1j;(1)). When normalized to maximum lifespan, these data indicate that indole increased healthspan, and concomitantly decreased frailspan (Fig. 1k). Taken together, these data suggest that indole provided either exogenously, or via K12, extends healthspan of *C. elegans*.

**Indoles extend healthspan via DRH-1 interferon pathway in C. elegans**

The preliminary results provided a hint of the possibility that indoles may be modulating the aging mechanism through type I interferon pathway genes. The gene ontology (GO) results were divided into Group A and B (Figure S3). Out of the 52 genes identified in the interferome analysis of Group B, 36 genes (69%) were found to be associated with Type 1 Interferon (IFN1) antiviral response. The Group B genes included genes with orthologs in *C. elegans* which could be further studied to determine functionality.

The *C. elegans* strain N2 had been initially evaluated to get a reference point of how the wildtype *C. elegans* strain interacts with indoles when assessed for life span parameter. Although the N2 (wildtype) *C. elegans* strain did not show a significant improvement in lifespan of the worm, the strain did show a difference in length of healthy life. This observation served as basis for evaluating how the two survival curves depicted (Figure 2) differ in their health span.

**Figures:**

**Figure 1**

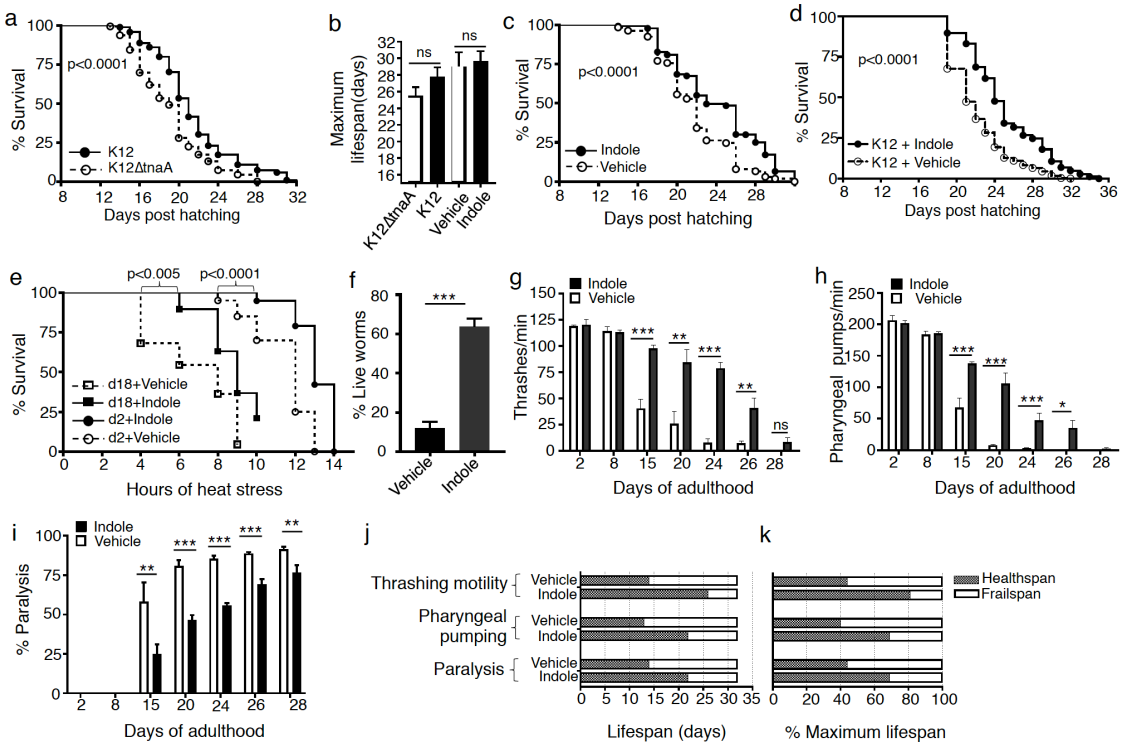
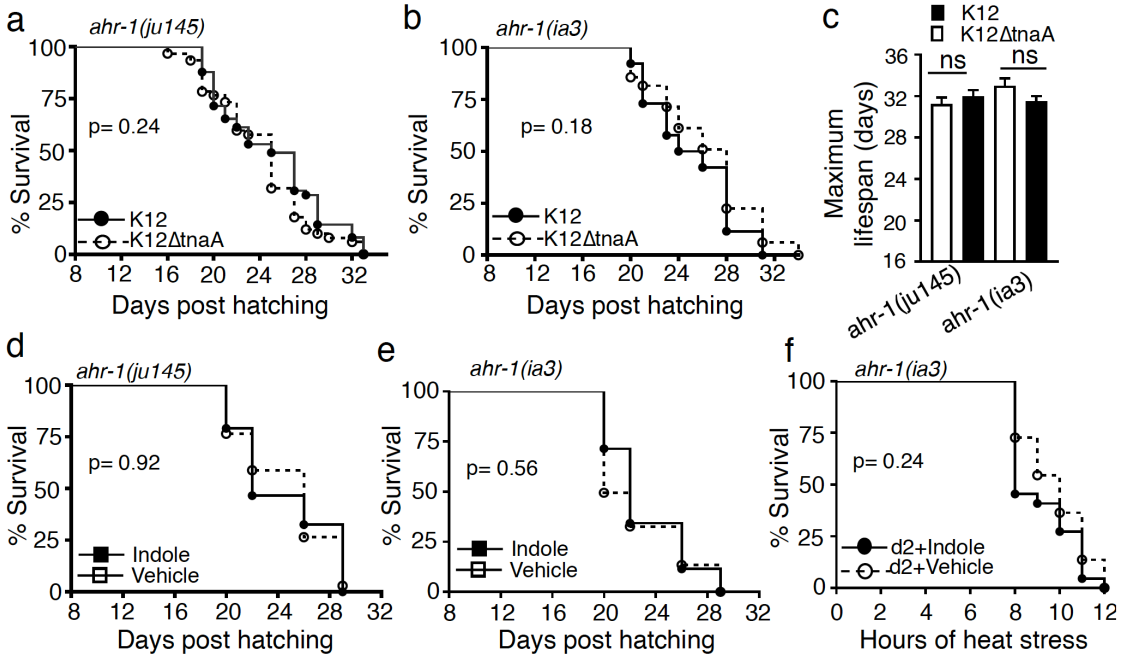


**Figure 2**


a
ahr-1(ju145)
p= 0.24
K12
-K12ΔtnaA
% Survival
Days post hatching
b
ahr-1(ia3)
p= 0.18
K12
-K12ΔtnaA
% Survival
Days post hatching
c
K12
K12ΔtnaA
ns
ns
Maximum lifespan (days)
ahr-1(ju145)
ahr-1(ia3)
d
ahr-1(ju145)
p= 0.92
Indole
Vehicle
% Survival
Days post hatching
e
ahr-1(ia3)
p= 0.56
Indole
Vehicle
% Survival
Days post hatching
f
ahr-1(ia3)
p= 0.24
d2+Indole
d2+Vehicle
% Survival
Hours of heat stress

# Chapter II

## Introduction:

Current research indicates that a compromised or altered immune system in frail individuals may play a significant role in aberrant inflammation (called inflammaging), and compromised responses to infection (Reale M 2014). Aging humans are faced with stressors on a daily basis from different sources that lead to progressive damage to many cells, including those of the immune system through production of reactive oxygen species (ROS). The attendant tissue damage particularly in the intestinal tract can disrupt epithelial barrier integrity, resulting in systemic dissemination of bacteria or bacterial products which can drive inflammation. Such inflammation can in turn cause additional damage creating a positive feedback cycle that results in hyperinflammation and tissue damage. Such inflammatory processes contribute to frailty and decreased health span, the time an organism remains healthy.

A curative therapy, allogenic bone marrow transplantation (allo-BMT) is used for patients with hematologic diseases. In approximately 40% of the transplant patients, the transplant procedure induces deadly hyper-inflammatory response called Graft vs. Host Disease (GvHD) (Jagasia et al. 2012). This reaction would greatly benefit new treatments that mitigate the deadly inflammatory response. Indole's ability to modulate immune response therefore serves as a consideration in forming new therapies.

In our lab's previous work, our data suggests that microbiota derived indoles mitigate damage from GvHD (Swimm et al manuscript in review) Indole-3-aldehyde (ICA) regulates expression of genes in the Type I interferon and circadian rhythm

pathways. ICA acts via IFNAR1 to limit radiation damage and promote epithelial regeneration (Swimm et al, manuscript in review).

Type I interferon pathway is important in activating innate and adaptive immunity in mammals. Type I interferons as polypeptides serve to: induce antipathogenic states that reduce pathogen spread, modulate anti-inflammatory mechanisms while promoting pathogen presentation, and promote innate and adaptive immune responses (Ivashkiv et al. 2014). Retinoic acid-inducible gene I (RIG-I)-like receptors are intracellular viral sensors that help the innate immune system respond to RNA virus infections as part of the interferon I pathway (Coffman et al. 2017). While Type I interferons (IFNs) play an important role in viral recognition, they still present elusive roles in bacterial infections and autoimmune diseases (Trichieri et al. 2010).

However according to another study dsRNA from commensal bacteria play a significant role in anti-inflammatory responses and in development of adaptive immune responses (Kawashima et al. 2013). Although *C. elegans* lack the interferon pathway, RNA interference (RNAi) is a major antiviral response. The Orsay virus, known and described to infect *C. elegans* triggers an antiviral response using Dicer-related helicase 1 (DRH-1), a conserved helicase related to RIG-I in mammals (Felix et al. 2011).

According to latest research, it is unclear whether Dicer-related helicase 1 (DRH-1) initiates the antiviral RNAi sensing in *C. elegans*. Studies suggest mammalian RLRs and interferon signaling may an important role in production of viral siRNAs (Coffman et al. 2017). In this section of the study, the data suggests indoles may upregulate a pathway similar to type I interferon pathway in mammals, to modulate health span against environmental stressors.

Based on preliminary results, type 1 interferon pathway gene expression was upregulated in the gene ontology results (Figure S3). This data suggested a possibility of indole regulating functions in mammalian systems through interferon pathway.

**Statistical analysis.**

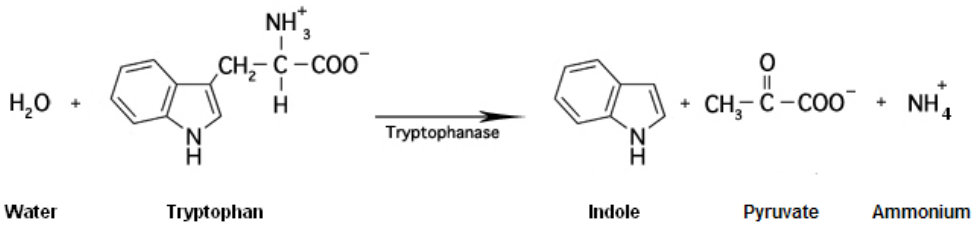
The Kaplan-Meier lifespan curves used log rank Mantel-Cox test for analysis. For comparisons, experiments were repeated at least three times. The three comparisons were analyzed further using a T-test (ANOVA). A p-value less than 0.05 was considered to show significance.

**Results:**

The initial life span assay results showed that *C. elegans* N2 wildtype strain showing a significant difference in the survival analysis curves. The N2 *C. elegans* showed a strong tolerance to aging if exposed to indoles from the K12 strain (Figure 2). The gap between the survival curves between worm strain exposed to K12 and K12Δ*tnaA* can be expressed as health span (length of healthy life). To further evaluate which genes are likely involved in increasing health span, previous gene ontology (GO) results were used as reference point to further evaluate future research avenues. The GO results indicated type I interferon pathway genes with increased gene expression when exposed to indoles. This indicated a likely chance that *C. elegans* worms lacking type I interferon gene homologues could show significant increase in health span.

Since N2 wildtype *C. elegans* strain showed a gap in the survival curves, a gene involved in the type I interferon pathway would not show a similar gap in the curves. The N2 *C. elegans* strain has not been genetically modified and thus has all functioning genes in its genetic makeup. The survival analysis curve from the N2 strain show a reference curve in which N2 with K12 strain shows an increased health span, while N2 with K12Δ*tnaA* show a lower quality of healthy life among the worms. The tested *C. elegans* mutants had the noted gene removed from their genetic makeup. Thus, those genes would not be involved in displaying the noted phenotype, increased health span. The survival analysis curves from these mutants suggest that without these genes, indole is unable to regulate health span in *C. elegans*.

**Figure 1: Tryptophan metabolism**




The mechanism above depicts how indoles are derived from tryptophan by the enzyme tryptophanse (tnaA). Indole production according to several studies depends on exogenous tryptophan amount (Li et al. 2013). According to the same study high levels of indole production by E. coli is dependent on TnaA and TnaB proteins, but final yield of indole was determined by exogenous supply of tryptophan (Li et al. 2013). Image was obtained from a website Dewaswala 2012.

**Figure 2: Life span assay of N2 (wildtype) in *C. elegans* mutant.** The figure below describes the life span assay results in a figure. The figure shows the N2 (wildtype) strain exposed to K12 and K12ΔtnaA and the worm lifespan assessed over the course of 30 days. The N2 strain exposed to K12ΔtnaA (bacterial strain not producing indole) showed a difference in the quantity of healthy life the worms survived, while the worms exposed to indoles through K12 showed a better health span.

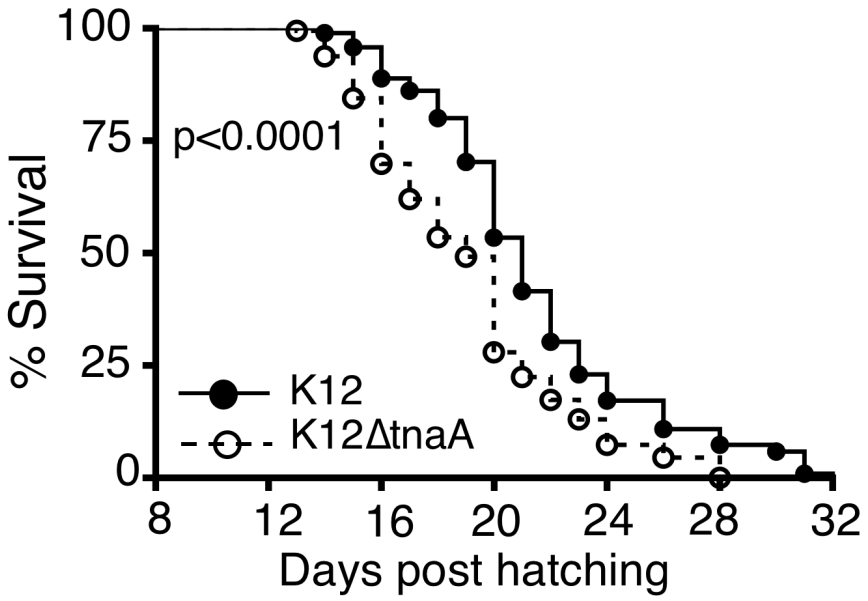
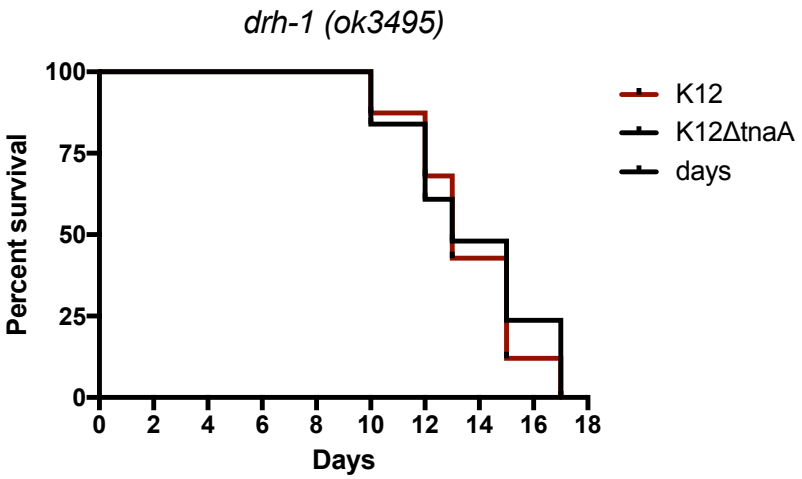
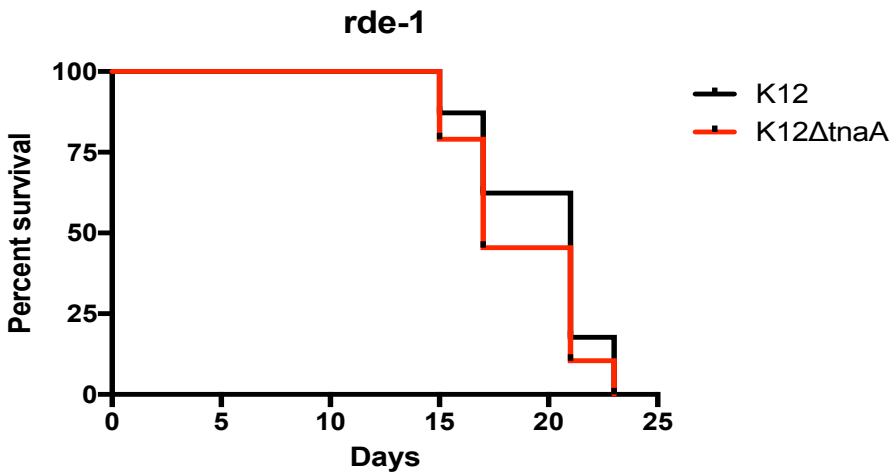


**Figure 3: Life span assays of *C. elegans* mutants.** We evaluated the effects of indoles on loss of function alleles in genes that are known to modulate immunity (Coffman et al. 2017) in mammalian systems. These included drh-1 (ok3495) (Figure 3a), rde-1 (Figure 3b), and the Nrf-2 homologue skn-1(Figure 3d). We compared the survival curves of N2 worms in K12 and K12Δ*tnaA* as a control, to loss of function alleles mutants. The drh-1 (ok3495) mutants exposed to indoles from K12 bacterial strain did not show significant increase in life span. However, the drh-1 (ok3495) mutant worm exposed to indoles from K12 bacterial strain showed survival curve similar to curve from mutant worms exposed to K12Δ*tnaA* bacterial strain. This presented a possibility that indole produced by K12 bacterial strain may play an important role in regulating health span in *C. elegans*. NF-E2-related factor (Nrf)/ CNC are a group of transcription regulators, well-known for their function in stress response. New evidence suggests, that Nrf2 may be a central regulator in metabolic functions in mammalian systems (Blackwell et al. 2015). In *C. elegans* Nrf/CNC proteins are represented by SKN-1, a functional ortholog. Due to their evolutionarily conserved functional similarities in mammals and *C. elegans*, Nrf/CNC and SKN-1 were suggested as important aspect to study in aging.

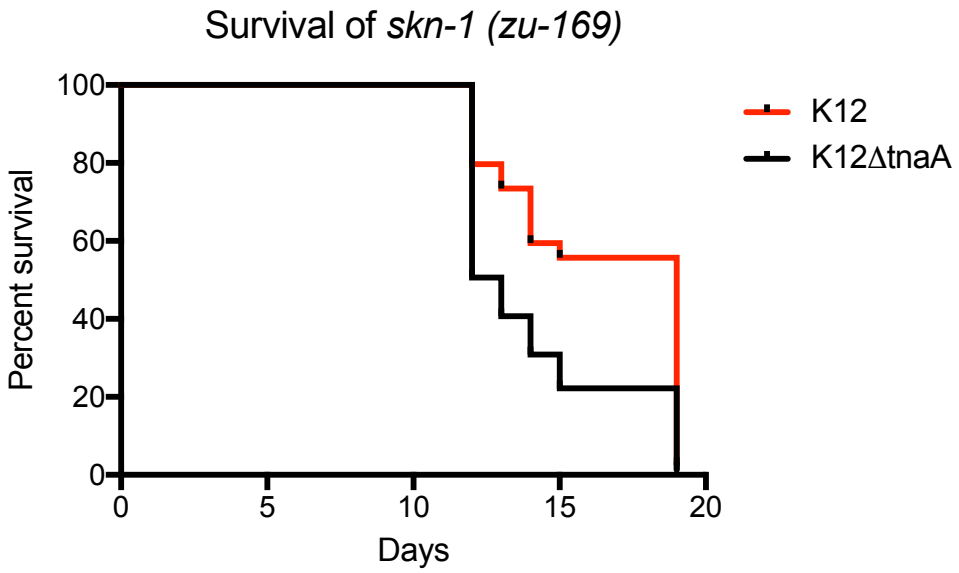
**A. Lifespan Assay of drh-1 (ok3495) in *C. elegans* mutant**




**B. Lifespan assay of rde-1 in *C. elegans* mutant.**



C. **Lifespan assay of Nrf-2 homolog, skn-1 (zu169).**




**Discussion:**

Environmental exposures vary from solar radiation, chemical exposures, to food intake. The ability to tolerate the stress, repair damage, and continue to survive with minimum impairment serves as an important marker for health span (Richardson et al 2016, Lithgow et al 2000, and Huffman et al. 2016). As a living system continues to age and continually become exposed to different environmental stressors, health diminishes while frailty increases. The novelty about this research examines the possibility of an evolutionarily conserved pathway that gut microbiota may be using to modulate host immune system. Exogenous administration of indole or ICA can complement microbiota-provided indole, in mice and worms, and modulate health span (Zelante et al. 2013). The study aimed to observe the phenotypes in *C. elegans* as the nematode animal model is easy to manipulate and characterize. Current research has aimed to identify genes involved in the life span, but this study chose to identify genes involved in health span. Health span, length of healthy life, studies will help scientists understand how

environmental stressors impact the human health and how the human body responds to them.

Evaluating prospective research on the effects of environmental stressors in *C. elegans* and mammals will lead to a better understanding how biological systems protect from damage across species. While data presented here shows that the *C. elegans* dicer related helicase interferon pathway modulates health span further research is needed to identify genetic pathway in the mammalian systems. In mammalian systems, the retinoic acid-inducible gene I (RIG-I) is a crucial pathogen receptor that recognizes viruses, RNA viruses, while promoting DNA virus recognition (Kell et al. 2015). In research, we have not been able to address a direct role that the interferon pathway in mammalian systems may play in promoting health span because the interferon-mutant mice show reduced reproductive capacity (Sonowal et al. 2017). Further research into understanding how the indoles may modulate mammalian aging through the interferon pathway may clarify how biological systems help us protect ourselves in face of exposure to environmental stressors. Prospective studies addressing whether administration of indoles may help damaged or inadequate gut microbiota tolerate damage from exposure to environmental stressors and augment health span, may be useful.

Future avenues can help identify how aging occurs and how we can mitigate its effects through several different pathways. Aging a complex phenomenon can be occurring through several different pathways. In the field of immunology, identifying a pathway through which gut microbiota-derived molecules may be affecting the aging mechanism is a future goal that may help science better understanding aging and the broader role that immune system plays in mitigating the effects of environmental

stressors. Current data suggestions that the interferon pathway involvement may require substantiation using the animal murine model. An *in vivo* assessment of interferon response pathway genes will help confirm the hypothesis. Indole production may be common among several bacteria and its function in many animals can further enlighten how indoles display similar functional responses to several environmental stressors. Indoles such as indole-3-carboxaldehyde (ICA) and indole acetic acid (IAA) are produced by cruciferous vegetables, such as kale and broccoli (Weijers et al. 2016). If indoles are implicated in regulating certain pathways in mitigating the effects of aging, then indole-derived therapies may be a future possibility. In a broader public health perspective, this research will help future studies better target populations with different genetic background for therapies diminishing the effects of aging. Public health currently has several studies attempting to understand how different environmental stressors may impact human health, but this research will help scientists evaluate how the mammalian systems respond to damage from environmental stressors and ultimately mitigate the effects of aging.

**Supplementary Information:**

**C. elegans strains.** C. elegans strains were obtained from *Caeborhabditis* Genetics Center (CGC) from University of Minnesota, College of Biological Sciences. Strains attained from CGC, wild-type Bristol strain N2, drh-1, rde-1, skn-1, and dcr-1. All strains from the CGC were maintained in the Nematode Growth Medium (NGM) plates at 16°C using standard culturing techniques (Sulston 1988).

**NGM plates.** The plates used to grow the C. elegans worms were purchased from VWR International (plate sizes 100x15mm & 60x15mm). The Nematode Growth Medium (NGM) was developed by using 15 g Bacto Agar (BD, Becton, Dickinson Company), 3 g Sodium Chloride (Sigma Aldrich), 2.5 g Bacto Peptone (BD, Becton, Dickinson Company) in a 1000 mL (1 L) glass container. The container was filled to 1000 mL mark with distilled water and then autoclaved for 45 minutes. The NGM agar was removed from autoclaved and placed into hot water bath. After waiting till the agar reached luke warm temperature, 1.0 mL of cholesterol, 0.5mL of 1M $CaCl_2$, 1.0 mL of 1M $MgSO_4$, 25.0 mL of $KPO_4$ were sequentially added to the agar. The NGM agar was then poured into petri dishes for the assays.

**Figure S1. Environmental Stressors and their impacts.** The figure below obtained from MitoQ, describes the different environmental exposures and the likely impact inside cells. Exposures such as microbes, food intake, chemical pollution and other environmental factors can change the cell biochemistry.

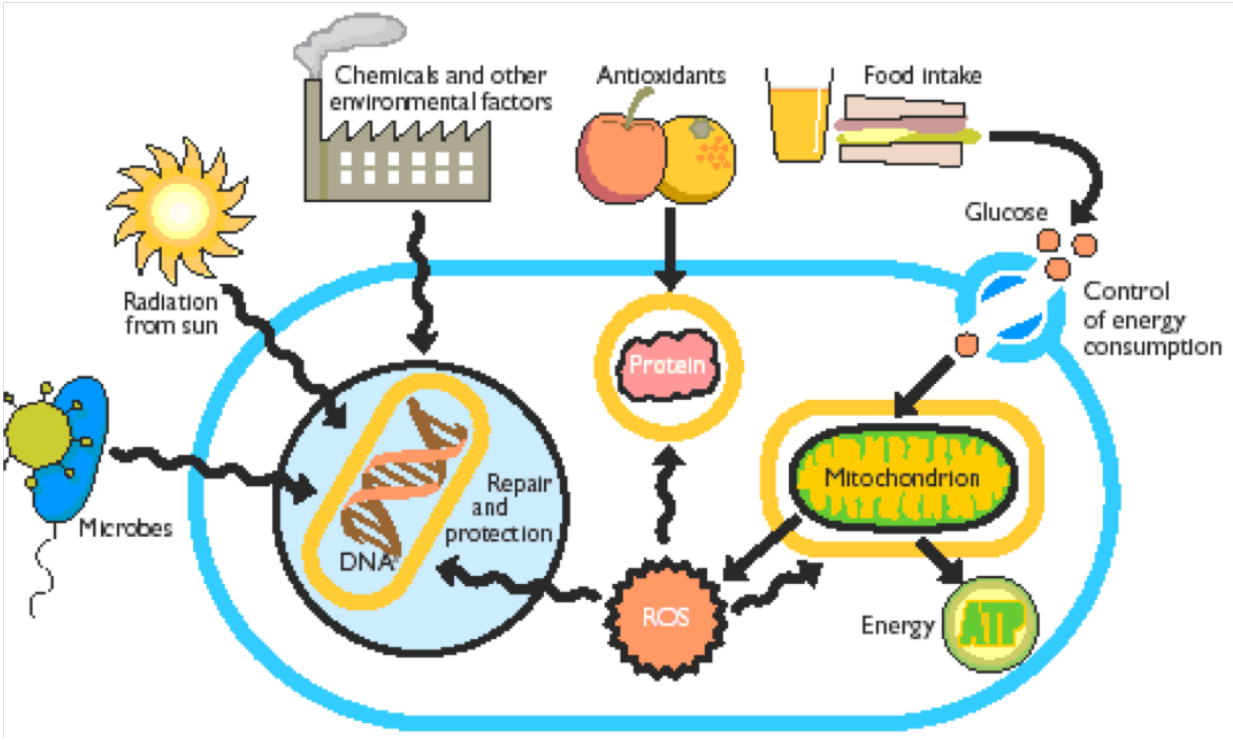


**Figure S2. *Caenorhabditis elegans* life cycle:**

The cycle represents the *C. elegans* life cycle. It is important to note the worm growth from the larvae life stage, through embroyonic development, into initial molting stages, to the L4 stage and finally to the adult worm stage. The worms used in the reproductive span assay were chosen from the L4 stage. The L4 marks the stage right before the egg-laying stage in the *C. elegans* life cycle. The worms used in the life span assays were chosen from eggs (ex utero development).



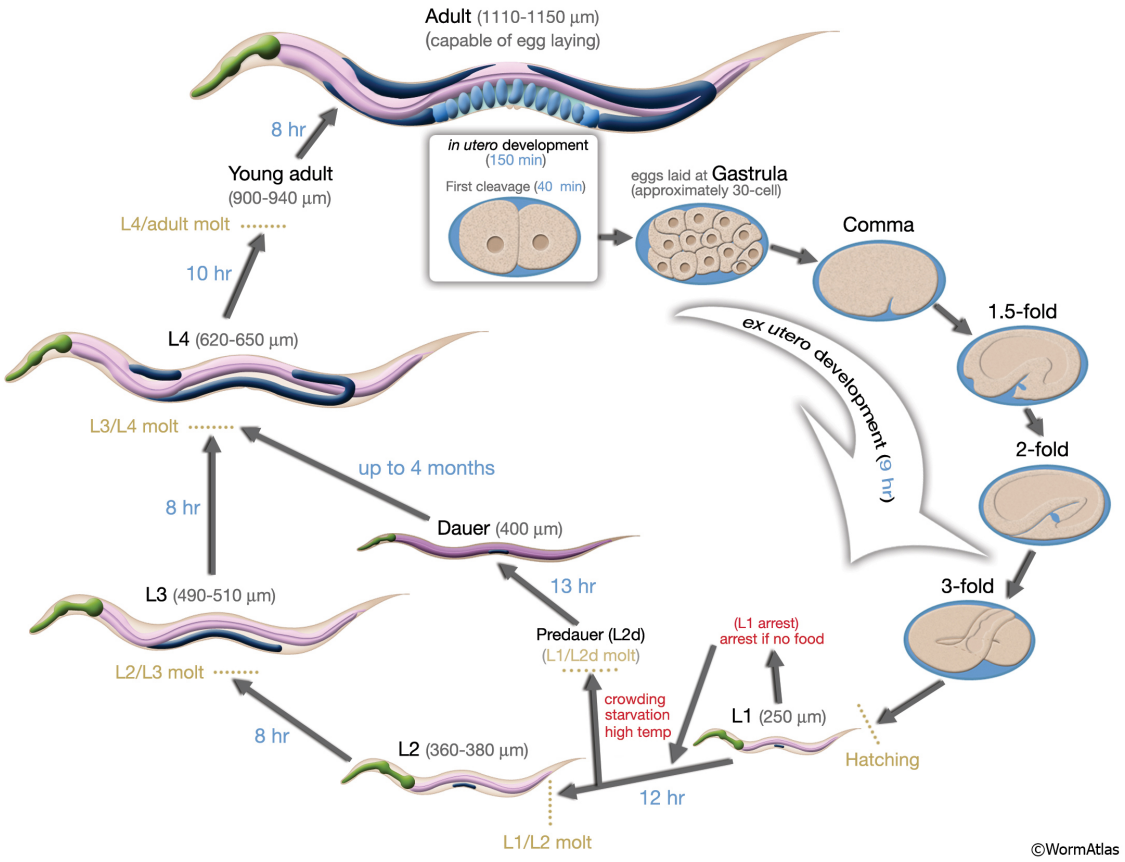


**Figure S3: Indoles regulate expression of Type I interferon gene pathways.** Genetic data was collected from gene ontology (GO) analysis that studied ICA dependent genes *(Swimm et al. Manuscript in review*). The data from this study led to further hypothesize type 1 interferon gene pathways are involved in the health span phenomenon. Using this data, RIG-I homologues such as DRH-1 were analyzed using the life span curves as well.

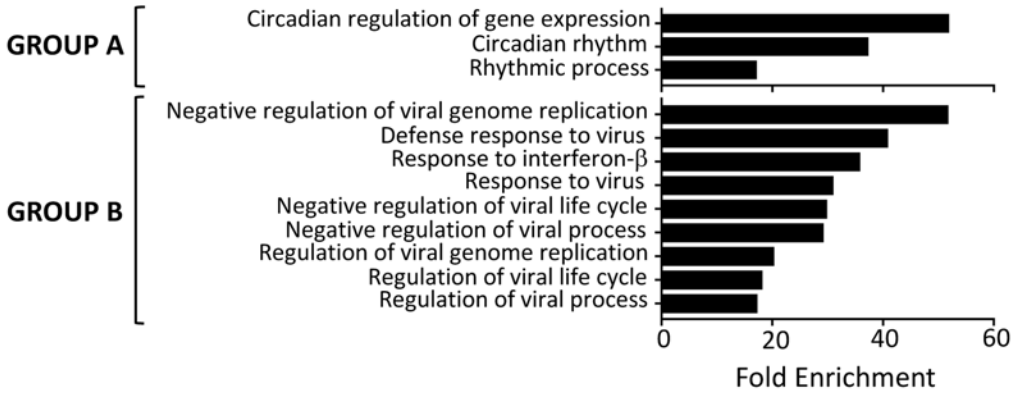
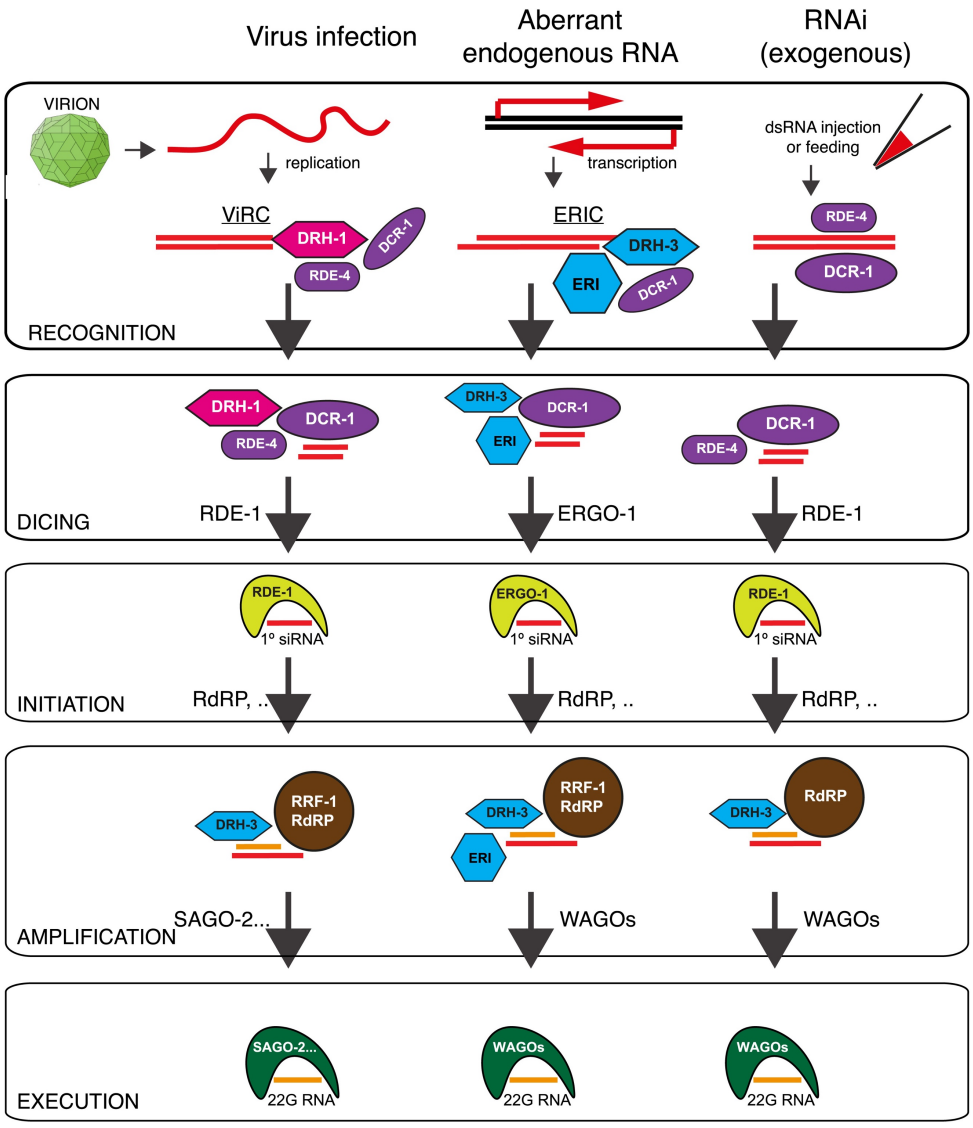


**Figure S4: Hierarchal pathway derived from DRH-1.** The figure obtained from a recent study describes a complex of DRH-1, DCR-1 and RDE-4 shown to recognize endogenous viral transcripts in *C. elegans* (Ashe et al. 2013). This diagram may help identify genes upstream and downstream of initially identified genes involved in increasing health span in *C. elegans* when exposed to indoles.



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