

Annu. Rev. Genet. 2013. 47:X-X  
doi: 10.1146/annurev-genet-111212-133352  
Copyright ©2013 by Annual Reviews. All rights reserved

KIM

<DOI>10.1146/ANNUREV-GENET-111212-133352</DOI>

HOST-MICROBE INTERACTIONS AND AGING

## BACTERIA AND THE AGING AND LONGEVITY OF *CAENORHABDITIS ELEGANS*

---

Dennis H. Kim

*Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; email: [dhkim@mit.edu](mailto:dhkim@mit.edu)*

□ **Abstract** The genetic analysis of longevity of *Caenorhabditis elegans* has yielded fundamental insights into the molecular mechanisms of aging in animals. Recent studies suggest that interactions between *C. elegans* and its microbial environment may shape and influence aging and longevity of the host. Experimental evidence supports a role for bacteria in affecting longevity through distinct mechanisms---as a nutrient source, as a potential pathogen that induces double-edged innate immune and stress responses, and as a coevolved sensory stimulus that modulates neuronal pathways regulating longevity. Motivating this review is the anticipation that the molecular genetic dissection of the integrated host immune, stress, and neuroendocrine responses to microbes in *C. elegans* will shed light on basic insights into the cellular and organismal physiology that governs aging and longevity.

**Keywords** longevity, innate immunity, immunosenescence, microbiota, pathogen, host-microbe interactions

### AGING IN A MICROBIAL ENVIRONMENT: AN OVERVIEW

The isolation of wild strains of *Caenorhabditis elegans* has revealed a diversity of microbes in its natural environment (22). By contrast, the laboratory cultivation of *C. elegans* and, specifically, experimental assays of organismal life span, have commonly involved propagation on agar plates seeded with a monoaxenic culture of *Escherichia coli* OP50 (9). Three principal modes of interaction between the *C. elegans* and bacteria may affect the aging and longevity of the host organism (Figure 1). First, bacteria serve as the food source for *C. elegans*, and differences in nutritional quality and alterations in the production of

bacterial metabolites may influence host aging and longevity. Second, bacteria may cause pathogenic infection, which may contribute to mortality in aging animals. Pathogenic infection induces innate immune and stress responses, which might be anticipated to promote survival, but these host responses, and not just microbial toxicity, may also contribute to tissue damage and host aging. Third, neuronal responses to bacteria, detected as food and/or pathogen, may be integrated with endocrine signaling pathways that regulate organismal longevity. Here, I discuss the experimental studies in support of each of these mechanisms through which microbes may influence the longevity of *C. elegans*. I anticipate that understanding how microbes, innate immunity, and cellular and organismal stress physiology are integrated may yield fundamental insights into the molecular genetic basis of aging and longevity.

<COMP: PLEASE INSERT FIGURE 1 HERE>

**Figure 1 Bacteria can influence aging and longevity through distinct modes of interaction with the *Caenorhabditis elegans* host.**

## **DUAL NATURE OF BACTERIA DURING AGING OF *CAENORHABDITIS ELEGANS***

The relationship between diet and longevity is an important consideration in the interpretation of assays of *C. elegans* life span that involve alterations of the bacterial food source. Nutrients are essential for survival, and yet dietary restriction confers well-documented extension in life span in evolutionarily diverse species (65). *C. elegans* is a bacteriovore, and optimal laboratory growth and development of *C. elegans* requires live bacteria. Compared with the cultivation of *C. elegans* in the presence of bacterial food, growth in liquid axenic culture results in reduced rates of growth and progeny production, and notably, marked extension in life span (19). These observations are consistent with a contribution from dietary restriction due to suboptimal nutrition or perhaps the absence of toxic or pathogenic components of bacteria that are detrimental to life span. However, defining the relative contributions of these possible contributors is not straightforward, and additional studies have examined how changing the bacterial lawn on which *C. elegans* feeds influences life span.

Propagation of *C. elegans* on heat-killed *E. coli* OP50 or on *E. coli* OP50 that has been treated with antibiotics to inhibit bacterial proliferation results in an extension of the life

span compared with propagation on standard live *E. coli* OP50 (24, 27). These studies are consistent with extension in life span being due to the attenuation of bacterial pathogenicity and toxicity. Altering the agar media may also shorten life span, possibly acting through the induction of increased virulence of *E. coli* OP50 (26). However, different methods of killing *E. coli* OP50, inhibiting its proliferation, or altering the growth media may produce variable effects on nutritional quality. Notably, even different strains of *E. coli*, OP50 and HT115, although not causing appreciable differences in *C. elegans* growth and progeny production, may result in markedly different metabolic profiles (10).

More difficult to interpret have been the effects of different species of bacteria on the *C. elegans* life span. Variability in nutritional content and quality and/or pathogenicity among bacterial species likely underlies the wide range of abilities of different species of bacteria to support growth and development as assessed by differences in growth rate and progeny production (17, 80). Propagation of *C. elegans* on *Bacillus subtilis* results in extended longevity compared with propagation on *E. coli* OP50, an observation that has been interpreted in terms of the relatively diminished pathogenicity of *B. subtilis* (26). A lack of difference in *C. elegans* growth rate or progeny production is suggestive of comparable nutrition, indicating that the life-span extension is not due to dietary restriction (26, 30). However, more subtle metabolic effects from differences in nutritional content are difficult to exclude. Interestingly, a recent study has suggested that the production of nitric oxide by *B. subtilis* leads to the induction of stress-activated signaling pathways, which may contribute to the extended life span of *C. elegans* when feeding on *B. subtilis* (30).

Recent work has shown that *C. elegans* exhibits altered developmental rate and life span when propagated on the soil bacterium *Comamonas* DA1877 (51). The persistence of the effect on developmental rate even when *Comamonas* is diluted with *E. coli* has been interpreted in terms of a likely bacterial signal that can modulate development. The shortened life span of *C. elegans* on *Comamonas* relative to *E. coli* OP50, which is also observed on killed bacterial species, is not observed upon diluting the *Comamonas* with *E. coli*, raising the possibility that differences in nutritional content or pathogenicity underlie the observed difference in *C. elegans* life span on the respective bacterial species (51).

The bacterial lawn represents a genetically modifiable nutritional source that has been utilized to explore nutritional determinants of longevity. Mutants of *E. coli* OP50 have been

isolated that are deficient in specific metabolites that lead to altered metabolism. For example, recent genetic analysis determined that increased folate biosynthesis contributes to a shortened life span of *C. elegans* (90). Importantly, changes in life span were found in the absence of differences in growth or development of *C. elegans* or changes in intestinal bacterial proliferation, which might suggest altered bacterial pathogenicity. Notably, the effect of the drug metformin on *C. elegans* life span has also recently been shown to result from drug-induced alterations in bacterial folate metabolism (11).

Complexities in the analysis of the contribution of bacterial molecules to *C. elegans* life span are illustrated by the characterization of the mechanism by which coenzyme Q affects *C. elegans* longevity. *E. coli* deficient in coenzyme Q were shown to confer an extension in life span relative to wild-type *E. coli* (44). Subsequent characterization revealed that *E. coli* that not only lacked coenzyme Q but were also deficient in respiration resulted in an extended life span without apparent alteration of the nutritional content of the bacterial food (73). More recently, respiration-deficient bacteria have been shown to accumulate in the intestines of aging worms to a delayed extent, suggesting that what initially appeared to be a consequence of the lack of a single nutrient may have exerted effects on *C. elegans* life span through alterations in bacterial fitness and ability to proliferate in the *C. elegans* intestine (28). These data illustrate challenges in decoupling experimental effects on nutritional and pathogenic properties of bacteria in studies of *C. elegans* longevity.

The strongest evidence for a role for bacterial pathogenesis in the mortality of aging *C. elegans* has come from direct observations of intestinal accumulation of ostensibly nonpathogenic *E. coli* OP50 in aging animals. The pattern of intestinal accumulation of *E. coli* OP50 observed in aging animals (24) is similar to what is observed in younger *C. elegans* larvae and *C. elegans* adults that are infected with highly pathogenic bacteria, such as *Pseudomonas aeruginosa* PA14 (38, 83). These similarities suggest a comparable course of bacterial proliferation and alteration in the intestinal epithelia, albeit one with different kinetics. Most compelling have been studies of electron microscopy of aging adults propagated on *E. coli* OP50, which have revealed intestinal accumulation punctuated by variable regions of bacterial packing and adjacent intestinal distension and tissue morphology consistent with local catastrophic pathogenic events (33, 57). These observations strongly suggest bacterial infection and proliferation within the intestine of

aging adults, particularly toward the end of life, and further point to a role for bacteria in terminal events of at least some aging animals.

How do the animals die? The terminal events in life-span assays, or even pathogenesis assays with more virulent bacteria, are not well understood. Increased density of bacteria may also lead to a high local concentration of secreted bacterial toxins. Tissue damage may also result from by-products of the host immune response to bacteria. The preceding accumulation of bacteria and localized areas of bacterial packing suggests the possibility of direct invasion and translocation by bacteria, with imaging providing evidence of intracellular invasion of *C. elegans* intestinal cells by pathogenic bacteria (38, 39).

Variability in the magnitude and patterns of bacterial packing may contribute to the stochastic nature of deaths of isogenic populations of *C. elegans* observed in life-span assays (33). Such variation might result from, or be the cause of, variability in host antimicrobial and stress responses. The variability in stress responses and bacterial accumulation in populations of *C. elegans* suggests that differential susceptibility to bacterial infection may contribute substantially to the observed variation in life span among isogenic populations of *C. elegans* (69, 74, 94).

## **BACTERIAL INDUCTION OF IMMUNE AND STRESS SIGNALING DURING AGING OF *CAENORHABDITIS ELEGANS***

### **Host Defense of *Caenorhabditis elegans***

Multiple facets of *C. elegans* anatomy and physiology contribute to host defense against pathogenic microbes. *C. elegans* relies on its cuticle to serve as a mechanical barrier to infection and a pharyngeal grinder to disrupt ingested bacteria. Stress-activated innate immunity regulates local responses to bacterial infection in the intestine and hypodermis (41, 53, 62, 67), whereas the *C. elegans* nervous system facilitates the recognition and behavioral avoidance of pathogenic bacteria (66, 70, 79, 96).

A forward genetic approach to identify genes required for resistance of *C. elegans* to pathogenic *P. aeruginosa* led to the identification and characterization of a conserved PMK-1 p38 mitogen-activated protein kinase (MAPK) pathway that is required for *C. elegans* survival during intestinal infection with *P. aeruginosa* and other bacterial pathogens (41). The p38 MAPK pathway is a central mediator of innate immunity in mammals (20), and

thus identification of its role in *C. elegans* pathogen resistance suggests an ancient role for this pathway in evolutionarily diverse hosts. Gene expression analysis suggests that the PMK-1 pathway regulates C-type lectins and other putative antimicrobial genes (87). The PMK-1 pathway has also been shown to function in the hypodermis, where PMK-1 regulates the expression of antifungal peptides in response to infection by the fungus *Drechmeria coniospora* (67). Interestingly, a different MAPK signaling pathway, converging on the ERK[\*\*AU: Necessary to define this acronym?\*\*-like MAPK MPK-1, regulates a protective tissue swelling response to the natural pathogen *Microbacterium nematophilum*, which adheres to the cuticle of the perianal region of *C. elegans* (62).

The PMK-1 MAPK functions downstream of NSY-1 (72), the *C. elegans* ortholog of the ASK1 MAPKKK that is involved in diverse stress-activated and immune signaling processes in mammals (54), as well as the Toll-interleukin-1 receptor (TIR-1) domain protein (18, 46), an ortholog of mammalian SARM, which functions in neuronal degeneration with less clear roles in the regulation of immune responses of mammals (12, 42, 64). Toll-like receptors, which have a pivotal role in innate immunity of mammals and *Drosophila*, do not appear to function in intestinal or hypodermal innate immunity of *C. elegans* (68). The mechanisms involved in activating the PMK-1 pathway in response to infection remain enigmatic. The activation of the PMK-1 pathway in response to pore-forming toxins in the intestine (36) and laser-mediated wounding in the epidermis (67) raises the possibility that host-derived molecules released upon intestinal cell damage may be involved. Damage-associated host molecules have been implicated in the activation of mammalian innate immune and inflammatory signaling pathways (84). The possibility that tissue damage may activate PMK-1 also raises the possibility that the function of the PMK-1 pathway in conferring resistance to infection may be related to stress adaptation and tissue-repair activities, and not solely to the mounting of an antimicrobial response.

The characterization of *P. aeruginosa* exotoxin A and *E. coli* Shiga-like toxin, both of which function by ADP-ribosylation of *C. elegans* elongation factor 2 (EF2) revealed that the PMK-1 pathway can be activated by the inhibition of host translation (16, 21, 56). These observations, motivated in part by mechanisms of plant surveillance and defense against pathogen-derived effectors (50), have led to the hypothesis that the inhibition of host translation of *C. elegans* may be detected as a pathogen-triggered event, leading to the

activation of innate immunity. These studies illustrate how pathogen-derived effectors may not only induce host innate immunity but also trigger stress-activated adaptive responses.

### **Pathogen Resistance and Longevity**

Ever since the initial characterization of mutants with enhanced susceptibility to pathogenic bacteria, the wild-type survival of such mutants on nonpathogenic *E. coli* OP50 has served as an important control to ensure that mutants were defective specifically in resistance to pathogenic bacteria and not short-lived from a generally diminished fitness. Mutants in the PMK-1 pathway have been shown to exhibit comparable longevity to wild-type *C. elegans* in life-span assays on *E. coli* OP50 (41, 87). But the evidence that infection, and in turn, activated immunity may contribute to longevity, demonstrates that the interpretation is not straight forward. That is, perhaps mutants deficient in innate immunity might be anticipated to have a shortened life span, as nonpathogenic *E. coli* makes an increasing contribution to mortality in aging animals. Correspondingly, mutants with enhanced resistance to pathogenic bacteria may exhibit life-span extension on *E. coli*.

The *daf-2* mutant carries a reduction-of-function mutation in an insulin-like receptor that confers a dramatic increase in life span that is dependent on the function of the Forkhead family transcription factor DAF-16 (40, 43, 48, 63). Notably, the *daf-2* mutant also exhibits a markedly enhanced resistance to pathogenic bacteria that is mediated by DAF-16 (26). The genomic expression profiling analysis of DAF-16 has identified several classes of transcriptional targets, including genes involved in detoxification and putative antimicrobial factors (55, 61). These data suggest that DAF-16 may promote longevity in part through the increased expression of host defense factors. Interestingly, although the *pmk-1* mutation has little or no effect on the life span of wild-type *C. elegans*, mutations in the PMK-1 pathway have a marked effect on the longevity of the *daf-2* mutant (87), which is also consistent with the idea that the life-span extension of the *daf-2* mutant may in part be due to enhanced pathogen resistance. The PMK-1 pathway is also required for the activation of the transcription factor SKN-1 (37), and *skn-1* mutations also suppress the extended life span of *daf-2* mutants independent of DAF-16 (88). Roles for the PMK-1 p38 MAPK pathway and SKN-1 in mediating resistance to oxidative stress suggest that attributing the life span--altering effects of mutations in the PMK-1 pathway solely to changes in innate immunity may represent an oversimplification.



The enhanced pathogen resistance of *daf-2* mutants has also been utilized to identify additional DAF-16 cofactors that function in longevity, such as SMK-1 (92). The characterization of the *daf-2* mutant survival on pathogenic and relatively nonpathogenic bacteria is consistent with overlapping roles in pathogen resistance and extended longevity for DAF-16. The heat-shock factor HSF-1 has also been shown to mediate the life-span extension of *daf-2* mutants (24), and *hsf-1* mutants show markedly shortened longevity and compromised resistance to various stressors, including infection by pathogenic bacteria (81).

Mutants defective in germ-line proliferation have been shown to have extended longevity through mechanisms that depend on DAF-16 functioning principally in the intestine (35, 47, 49). Consistent with the effects of increased DAF-16 on pathogen resistance in *daf-2* mutants, germ line--deficient mutants also exhibit marked resistance to pathogenic bacteria. However, sterile mutants that are not defective in germ-line proliferation, although reported to not exhibit life-span extension on nonpathogenic bacteria, have been shown to exhibit resistance to pathogenic bacteria (59). The killing of *C. elegans* by some bacteria pathogens, such as *Staphylococcus aureus* and *Enterococcus faecalis* (25), is greatly facilitated by the matricidal hatching of progeny during pathogenesis assays, and sterile mutants exhibit dramatic resistance to killing by these gram-positive pathogens. But independent of the effects on matricidal bagging, sterile mutants (with intact germ-line proliferation) have been reported to have enhanced resistance to killing by pathogenic bacteria, surprisingly also through DAF-16-dependent mechanisms (59). The molecular mechanisms underlying the effects of reproduction on pathogen resistance, particularly the contribution that is independent of germ-line proliferation, remain to be elucidated.

### **Immunosenescence and Aging**

The potentially dynamic nature of the immune response to microbial pathogens during the aging process may influence how host defense contributes to longevity. A decline in immune function, known as immunosenescence, has been principally associated with the thymic involution and the adaptive immune response in vertebrates (76), although recent studies have explored how aging affects the human innate immune response (77). Aging in *Drosophila* has been associated with a decline in pathogen-inducible antimicrobial peptide expression but also with a slight increase in the constitutive expression of antimicrobial



peptides (95). The age-dependent dynamic behavior of innate immune signaling pathways has remained poorly understood in all species.

The characterization of host defense and innate immune signaling in *C. elegans* has focused primarily on larval stage animals and young adults, raising the question of how the aging process might influence immune signaling. The systematic study of age-dependent pathogen susceptibility revealed a decline in pathogen resistance (45, 94), establishing that innate immunosenescence occurs in *C. elegans*. Genetic analysis of host defense pathways during aging revealed that PMK-1 expression decreases dramatically in aging adults (94). The decline in PMK-1 activity may influence antibacterial activity, contributing to increased intraluminal bacterial proliferation as well as increased cellular responses to intestinal damage during infection. The decline in PMK-1 activity in aging animals provides an explanation for the aforementioned lack of diminished longevity of mutants lacking PMK-1 activity that might be otherwise anticipated to protect against infection.

Immunosenescence in *C. elegans* may contribute to mortality in aging animals through a spiraling process in which a decline in PMK-1 activity promotes increased bacterial proliferation and toxicity, which further contributes to tissue aging and damage that may, in turn, further precipitate a decline in PMK-1-mediated defenses. Whether other pathways mediating immunity and host resistance to bacterial infection can compensate for the decline in PMK-1 in pathogen resistance in aging animals remains to be determined.

### **Stress and Tolerance in Aging and Longevity**

The microbial induction of immunity, even as it declines with advancing age, and other stress signaling pathways in aging animals may modulate aging and longevity. The enhanced pathogen resistance and longevity of *daf-2* mutants suggest that the activation of pathways promoting pathogen and stress resistance may promote longevity, but the activation of host immune responses has been increasingly recognized as a potentially double-edged sword. The host response may involve the release of immune mediators that might be toxic not only to the pathogen but also to host cells. The term “tolerance” has been used to describe host mechanisms to help protect against the potentially detrimental consequences of its own immune response (5, 58). The implication is that bacterial pathogenesis in aging animals may function not only to damage host epithelia directly but

also to trigger host responses that, although countering pathogenic bacteria, may contribute to tissue damage and aging.

The production of reactive oxygen species by NADPH-dependent dual oxidase functions as a component of innate immunity in the intestinal mucosa of *Drosophila* and immune tissues of mammals (6). The *C. elegans* dual oxidase homolog BLI-3 has been proposed to play a similar role in host mucosal defense (14), also inducing the activation of SKN-1 to protect the host against the release of reactive oxygen species (34). BLI-3 is known to be expressed in the hypodermis, where BLI-3 is required for collagen cross-links that are essential for cuticle integrity (85). Further work may determine whether BLI-3 is expressed in the intestine, where a role in defense against intestinal bacterial infection might be anticipated, or exerts effects through its activity in the hypodermis. Reactive oxygen species may have distinct effects on host-microbe interactions through their functions in immune effector activities and signaling and induce the activation of stress pathways mediating tolerance.

We have recently shown that the maintenance of endoplasmic reticulum (ER) protein-folding homeostasis has an unanticipated role in tolerance of the *C. elegans* innate immune response (71). The unfolded protein response (UPR) is a conserved signaling mechanism that functions in response to the accumulation of misfolded proteins in the ER (91). The UPR was principally identified through studies utilizing toxins such as tunicamycin, which blocks ER protein glycosylation, but genetic studies have implicated critical physiological roles for UPR signaling in organismal development and physiology (91). Infection of *C. elegans* with *P. aeruginosa* or intoxication of *C. elegans* with bacteria that express pore-forming toxins results in the marked induction of protein-folding stress in the ER (7, 71). Induction is dependent on PMK-1 MAPK, suggesting that ER stress does not arise from the direct toxic effects of the bacteria but through the bacterial induction of the PMK-1-dependent host response to infection and intoxication. Genetic analysis further established a requirement for intact IRE-1-XBP-1-dependent UPR signaling for *C. elegans* to tolerate its own innate immune response to infection with *P. aeruginosa* during larval development (71). These data establish a key physiological role for the UPR in balancing the host response to microbial pathogenesis. Because the UPR signaling has been shown to be required for longevity of *daf-2* mutants (32), establishing a key role for UPR-dependent

stress pathways in the maintenance of longevity, these data provide a clear mechanism by which microbial pathogens may induce cellular stress responses that may modulate the longevity of the host.

Transcriptional profiling of *C. elegans* mutants with altered resistance to killing by bacterial pathogens has identified a nematode-specific class of genes, known as the *abu* gene family, which is differentially regulated in a number of different mutants, including *ced-1* (31) and *octr-1* (82). The *abu* genes were originally identified as genes that were upregulated in mutants deficient in *xbp-1* and that caused lethality when inactivated in an *xbp-1* mutant (89). However, the physiological role for the *abu* genes, which have been proposed to mediate the protective effects downstream of *ced-1* and *octr-1* mutants by acting in the ER (31, 82), remains unclear. Further study of the roles of the *abu* genes may provide insights and validation of the role of the *abu* genes in innate immunity and their proposed regulatory pathways.

The induction of host immune and stress responses by bacterial infection provides an opportunity to dissect how microbes may modulate host pathways involved in the regulation of life span. Much of the preceding discussion has focused on microbial induction of host immune and stress responses, but as alluded to in the prior section, such interactions need not involve infection, as demonstrated by the recent observation that bacterially derived nitric oxide may also modulate host stress physiology and longevity (30).

## **MICROBIAL MODULATION OF THE NEURONAL PATHWAYS THAT REGULATE LONGEVITY**

Much of this review focuses on interactions between host intestinal cells and bacteria. However, interactions between the *C. elegans* nervous system and the microbial environment may also influence host aging and longevity. A role for the nervous system in the regulation of aging and longevity has been established through site-of-action studies for the DAF-2 insulin-like receptor (2, 93), the requirement for SKN-1 in the ASI neuron pair in mediating life-span extension caused by dietary restriction (8), and the analysis of the effects of genetic alteration or laser ablation of specific sensory neurons on *C. elegans* life span (1, 3).

Multiple *C. elegans* behaviors are modulated by the presence of bacterial food, including reproductive egg laying (86), feeding behavior (4), the dauer developmental decision (23), aerotaxis behavior (13, 15, 29), nutritional state--dependent locomotion (75), and pathogen avoidance behavior (66, 70, 96). Many of the corresponding signaling mechanisms, such as the DAF-7/TGF $\beta$  pathway and the neurotransmitter serotonin, are also implicated in the regulation of organismal longevity (60, 78), illustrating how bacterial modulation of *C. elegans* neuronal and endocrine signaling pathways may also affect life span.

The *C. elegans* nervous system can mediate discrimination and choice between bacteria that differ in nutritional quality and in pathogenicity (66, 96). How such considerations might be shown to influence longevity is illustrated in work that focused on the difference in longevity between *E. coli* OP50 and HT115 strains. Genetic studies suggested the NMUR-1 neuromedin receptor mediates differential neuronal responses induced by differences in the lipopolysaccharide (LPS) structure of the respective *E. coli* strains (52). Further studies identifying the sensory mechanisms involved in the discrimination of LPS structure may allow potential contributions from metabolic differences arising from the diets on the respective *E. coli* strains to be defined. The intersection of sensory responses to microbes and the neuronal signaling pathways that regulate aging and longevity represents a fertile area for future investigation, particularly given the emerging appreciation of the diverse effects that commensal bacteria can have on host physiology.

#### **DISCLOSURE STATEMENT**

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

#### **ACKNOWLEDGMENTS**

Work in the D.H.K. lab is supported by grants from National Institute of General Medical Sciences and the Ellison Medical Foundation.

#### **LITERATURE CITED**

1. Alcedo J, Kenyon C. 2004. Regulation of *C. elegans* longevity by specific gustatory and olfactory neurons. *Neuron* 41(1):45--55

2. Apfeld J, Kenyon C. 1998. Cell nonautonomy of *C. elegans daf-2* function in the regulation of diapause and life span. *Cell* 95(2):199--210
3. Apfeld J, Kenyon C. 1999. Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. *Nature* 402(6763):804--9
4. Avery L. 1993. The genetics of feeding in *Caenorhabditis elegans*. *Genetics* 133(4):897--917
5. Ayres JS, Schneider DS. 2012. Tolerance of infections. *Annu. Rev. Immunol.* 30(1):271--94
6. Bae YS, Choi MK, Lee W-J. 2010. Dual oxidase in mucosal immunity and host-microbe homeostasis. *Trends Immunol.* 31(7):278--87
7. Bischof LJ, Kao C-Y, Los FCO, Gonzalez MR, Shen Z, et al. 2008. Activation of the unfolded protein response is required for defenses against bacterial pore-forming toxin in vivo. *PLoS Pathog.* 4(10):e1000176
8. Bishop NA, Guarente L. 2007. Two neurons mediate diet-restriction-induced longevity in *C. elegans*. *Nature* 447(7144):545--49
9. Brenner S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* 77(1):71--94
10. Brooks KK, Liang B, Watts JL. 2009. The influence of bacterial diet on fat storage in *C. elegans*. *PLoS ONE* 4(10):e7545
11. Cabreiro F, Au C, Leung K-Y, Vergara-Irigaray N, Cochemé HM, et al. 2013. Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism. *Cell* 153(1):228--39
12. Carty M, Goodbody R, Schröder M, Stack J, Moynagh PN, Bowie AG. 2006. The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. *Nat. Immunol.* 7(10):1074--81
13. Chang AJ, Chronis N, Karow DS, Marletta MA, Bargmann CI. 2006. A distributed chemosensory circuit for oxygen preference in *C. elegans*. *PLoS Biol.* 4(9):e274
14. Chávez V, Mohri-Shiomi A, Garsin DA. 2009. Ce-Duox1/BLI-3 Generates reactive oxygen species as a protective innate immune mechanism in *Caenorhabditis elegans*. *Infect. Immun.* 77(11):4983--89
15. Cheung BHH, Arellano-Carbajal F, Rybicki I, de Bono M. 2004. Soluble guanylate cyclases act in neurons exposed to the body fluid to promote *C. elegans* aggregation behavior. *Curr. Biol.* 14(12):1105--11

16. Chou T-C, Chiu H-C, Kuo C-J, Wu C-M, Syu W-J, et al. 2013. Enterohaemorrhagic *Escherichia coli* O157:H7 Shiga-like toxin 1 is required for full pathogenicity and activation of the p38 mitogen-activated protein kinase pathway in *Caenorhabditis elegans*. *Cell. Microbiol.* 15(1):82--97
17. Couillault C, Ewbank JJ. 2002. Diverse bacteria are pathogens of *Caenorhabditis elegans*. *Infect. Immun.* 70(8):4705--7
18. Couillault C, Pujol N, Reboul J, Sabatier L, Guichou J-F, et al. 2004. TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. *Nat. Immunol.* 5(5):488--94
19. Croll NA, Smith JM, Zuckerman BM. 1977. The aging process of the nematode *Caenorhabditis elegans* in bacterial and axenic culture. *Exp. Aging Res.* 3(3):175--89
20. Dong C, Davis RJ, Flavell RA. 2002. MAP kinases in the immune response. *Annu. Rev. Immunol.* 20:55--72
21. Dunbar TL, Yan Z, Balla KM, Smelkinson MG, Troemel ER. 2012. *C. elegans* detects pathogen-induced translational inhibition to activate immune signaling. *Cell Host Microbe* 11(4):375--86
22. Félix M-A, Duveau F. 2012. Population dynamics and habitat sharing of natural populations of *Caenorhabditis elegans* and *C. briggsae*. *BMC Biol.* 10:59
23. Fielenbach N, Antebi A. 2008. *C. elegans* dauer formation and the molecular basis of plasticity. *Genes Dev.* 22(16):2149--65
24. Garigan D, Hsu A-L, Fraser AG, Kamath RS, Ahringer J, Kenyon C. 2002. Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. *Genetics* 161(3):1101--12
25. Garsin DA, Sifri CD, Mylonakis E, Qin X, Singh KV, et al. 2001. A simple model host for identifying gram-positive virulence factors. *Proc. Natl. Acad. Sci. USA* 98(19):10892--97
26. Garsin DA, Villanueva JM, Begun J, Kim DH, Sifri CD, et al. 2003. Long-lived *C. elegans* *daf-2* mutants are resistant to bacterial pathogens. *Science* 300(5627):1921
27. Gems D, Riddle DL. 2000. Genetic, behavioral and environmental determinants of male longevity in *Caenorhabditis elegans*. *Genetics* 154(4):1597--610

28. Gomez F, Monsalve GC, Tse V, Saiki R, Weng E, et al. 2012. Delayed accumulation of intestinal coliform bacteria enhances life span and stress resistance in *Caenorhabditis elegans* fed respiratory deficient *E. coli*. *BMC Microbiol.* 12:300
29. Gray JM, Karow DS, Lu H, Chang AJ, Chang JS, et al. 2004. Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue. *Nature* 430(6997):317--22
30. Gusarov I, Gautier L, Smolentseva O, Shamovsky I, Eremina S, et al. 2013. Bacterial nitric oxide extends the lifespan of *C. elegans*. *Cell* 152(4):818--30
31. Haskins KA, Russell JF, Gaddis N, Dressman HK, Aballay A. 2008. Unfolded protein response genes regulated by CED-1 are required for *Caenorhabditis elegans* innate immunity. *Dev. Cell* 15(1):87--97
32. Henis-Korenblit S, Zhang P, Hansen M, McCormick M, Lee S-J, et al. 2010. Insulin/IGF-1 signaling mutants reprogram ER stress response regulators to promote longevity. *Proc. Natl. Acad. Sci. USA* 107(21):9730--35
33. Herndon LA, Schmeissner PJ, Dudaronek JM, Brown PA, Listner KM, et al. 2002. Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. *Nature* 419(6909):808--14
35. Hsin H, Kenyon C. 1999. Signals from the reproductive system regulate the lifespan of *C. elegans*. *Nature* 399(6734):362--66
36. Huffman DL, Abrami L, Sasik R, Corbeil J, van der Goot FG, Aroian RV. 2004. Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins. *Proc. Natl. Acad. Sci. USA* 101(30):10995--1000
37. Inoue H, Hisamoto N, An JH, Oliveira RP, Nishida E, et al. 2005. The *C. elegans* p38 MAPK pathway regulates nuclear localization of the transcription factor SKN-1 in oxidative stress response. *Genes Dev.* 19(19):2278--83
38. Irazoqui JE, Troemel ER, Feinbaum RL, Luhachack LG, Cezairliyan BO, Ausubel FM. 2010. Distinct pathogenesis and host responses during infection of *C. elegans* by *P. aeruginosa* and *S. aureus*. *PLoS Pathog.* 6:e1000982
39. Jia K, Thomas C, Akbar M, Sun Q, Adams-Huet B, et al. 2009. Autophagy genes protect against *Salmonella typhimurium* infection and mediate insulin signaling-regulated pathogen resistance. *Proc. Natl. Acad. Sci. USA* 106(34):14564--69



40. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. 1993. A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366(6454):461--64
41. Kim DH, Feinbaum R, Alloing G, Emerson FE, Garsin DA, et al. 2002. A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* 297(5581):623--26
42. Kim Y, Zhou P, Qian L, Chuang J-Z, Lee J, et al. 2007. MyD88-5 links mitochondria, microtubules, and JNK3 in neurons and regulates neuronal survival. *J. Exp. Med.* 204(9):2063--74
43. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. 1997. *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277(5328):942--46
44. Larsen PL, Clarke CF. 2002. Extension of life-span in *Caenorhabditis elegans* by a diet lacking coenzyme Q. *Science* 295(5552):120--23
45. Laws TR, Harding SV, Smith MP, Atkins TP, Titball RW. 2004. Age influences resistance of *Caenorhabditis elegans* to killing by pathogenic bacteria. *FEMS Microbiol. Lett.* 234(2):281--87
46. Liberati NT, Fitzgerald KA, Kim DH, Feinbaum R, Golenbock DT, Ausubel FM. 2004. Requirement for a conserved Toll/interleukin-1 resistance domain protein in the *Caenorhabditis elegans* immune response. *Proc. Natl. Acad. Sci. USA* 101(17):6593--98
47. Libina N, Berman JR, Kenyon C. 2003. Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. *Cell* 115(4):489--502
48. Lin K, Dorman JB, Rodan A, Kenyon C. 1997. *daf-16*: An HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science* 278(5341):1319--22
49. Lin K, Hsin H, Libina N, Kenyon C. 2001. Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat. Genet.* 28(2):139--45
50. Mackey D, Holt BF III, Wiig A, Dangl JL. 2002. RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in *Arabidopsis*. *Cell* 108(6):743--54
51. Macneil LT, Watson E, Arda HE, Zhu LJ, Walhout AJM. 2013. Diet-induced developmental acceleration independent of TOR and insulin in *C. elegans*. *Cell* 153(1):240--52

52. Maier W, Adilov B, Regenass M, Alcedo J. 2010. A neuromedin U receptor acts with the sensory system to modulate food type-dependent effects on *C. elegans* lifespan. *PLoS Biol.* 8(5):e1000376
53. Mallo GV, Kurz CL, Couillault C, Pujol N, Granjeaud S, et al. 2002. Inducible antibacterial defense system in *C. elegans*. *Curr. Biol.* 12(14):1209--14
54. Matsuzawa A, Ichijo H. 2008. Redox control of cell fate by MAP kinase: physiological roles of ASK1-MAP kinase pathway in stress signaling. *Biochim. Biophys. Acta* 1780(11):1325--36
55. McElwee J, Bubb K, Thomas JH. 2003. Transcriptional outputs of the *Caenorhabditis elegans* forkhead protein DAF-16. *Aging Cell* 2(2):111--21
56. McEwan DL, Kirienko NV, Ausubel FM. 2012. Host translational inhibition by *Pseudomonas aeruginosa* exotoxin A triggers an immune response in *Caenorhabditis elegans*. *Cell Host Microbe* 11(4):364--74
57. McGee MD, Weber D, Day N, Vitelli C, Crippen D, et al. 2011. Loss of intestinal nuclei and intestinal integrity in aging *C. elegans*. *Aging Cell* 10(4):699--710
58. Medzhitov R, Schneider DS, Soares MP. 2012. Disease tolerance as a defense strategy. *Science* 335(6071):936--41
59. Miyata S, Begun J, Troemel ER, Ausubel FM. 2008. DAF-16-dependent suppression of immunity during reproduction in *Caenorhabditis elegans*. *Genetics* 178(2):903--18
60. Murakami H, Murakami S. 2007. Serotonin receptors antagonistically modulate *Caenorhabditis elegans* longevity. *Aging Cell* 6(4):483--88
61. Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, et al. 2003. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* 424(6946):277--83
62. Nicholas HR, Hodgkin J. 2004. The ERK MAP kinase cascade mediates tail swelling and a protective response to rectal infection in *C. elegans*. *Curr. Biol.* 14(14):1256--61
63. Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, et al. 1997. The fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389(6654):994--99
64. Osterloh JM, Yang J, Rooney TM, Fox AN, Adalbert R, et al. 2012. dSarm/Sarm1 is required for activation of an injury-induced axon death pathway. *Science* 337(6093):481--84

65. Piper MDW, Bartke A. 2008. Diet and aging. *Cell Metab.* 8(2):99--104
66. Pradel E, Zhang Y, Pujol N, Matsuyama T, Bargmann CI, Ewbank JJ. 2007. Detection and avoidance of a natural product from the pathogenic bacterium *Serratia marcescens* by *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* 104(7):2295--300
67. Pujol N, Cypowyj S, Ziegler K, Millet A, Astrain A, et al. 2008. Distinct innate immune responses to infection and wounding in the *C. elegans* epidermis. *Curr. Biol.* 18(7):481--89
68. Pujol N, Link EM, Liu LX, Kurz CL, Alloing G, et al. 2001. A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. *Curr. Biol.* 11(11):809--21
69. Rea SL, Wu D, Cypser JR, Vaupel JW, Johnson TE. 2005. A stress-sensitive reporter predicts longevity in isogenic populations of *Caenorhabditis elegans*. *Nat. Genet.* 37(8):894--98
70. Reddy KC, Andersen EC, Kruglyak L, Kim DH. 2009. A polymorphism in *npr-1* is a behavioral determinant of pathogen susceptibility in *C. elegans*. *Science* 323(5912):382--84
71. Richardson CE, Kooistra T, Kim DH. 2010. An essential role for XBP-1 in host protection against immune activation in *C. elegans*. *Nature* 463(7284):1092--95
72. Sagasti A, Hisamoto N, Hyodo J, Tanaka-Hino M, Matsumoto K, Bargmann CI. 2001. The CaMKII UNC-43 activates the MAPKKK NSY-1 to execute a lateral signaling decision required for asymmetric olfactory neuron fates. *Cell* 105(2):221--32
73. Saiki R, Lunceford AL, Bixler T, Dang P, Lee W, et al. 2008. Altered bacterial metabolism, not coenzyme Q content, is responsible for the lifespan extension in *Caenorhabditis elegans* fed an *Escherichia coli* diet lacking coenzyme Q. *Aging Cell* 7(3):291--304
74. Sánchez-Blanco A, Kim SK. 2011. Variable pathogenicity determines individual lifespan in *Caenorhabditis elegans*. *PLoS Genet.* 7(4):e1002047
75. Sawin ER, Ranganathan R, Horvitz HR. 2000. *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron* 26(3):619--31
76. Shanley DP, Aw D, Manley NR, Palmer DB. 2009. An evolutionary perspective on the mechanisms of immunosenescence. *Trends Immunol.* 30(7):374--81
77. Shaw AC, Joshi S, Greenwood H, Panda A, Lord JM. 2010. Aging of the innate immune system. *Curr. Opin. Immunol.* 22(4):507--13

78. Shaw WM, Luo S, Landis J, Ashraf J, Murphy CT. 2007. The *C. elegans* TGF- $\beta$  dauer pathway regulates longevity via insulin signaling. *Curr. Biol.* 17(19):1635--45
79. Shivers RP, Kooistra T, Chu SW, Pagano DJ, Kim DH. 2009. Tissue-specific activities of an immune signaling module regulate physiological responses to pathogenic and nutritional bacteria in *C. elegans*. *Cell Host Microbe* 6(4):321--30
80. Shtonda BB, Avery L. 2006. Dietary choice behavior in *Caenorhabditis elegans*. *J. Exp. Biol.* 209(Pt. 1):89--102
81. Singh V, Aballay A. 2006. Heat-shock transcription factor (HSF)-1 pathway required for *Caenorhabditis elegans* immunity. *Proc. Natl. Acad. Sci. USA* 103(35):13092--97
82. Sun J, Singh V, Kajino-Sakamoto R, Aballay A. 2011. Neuronal GPCR controls innate immunity by regulating noncanonical unfolded protein response genes. *Science* 332(6030):729--32
83. Tan MW, Mahajan-Miklos S, Ausubel FM. 1999. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc. Natl. Acad. Sci. USA* 96(2):715--20
84. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. 2012. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Immunol. Rev.* 249(1):158--75
85. Thein MC, Winter AD, Stepek G, McCormack G, Stapleton G, et al. 2009. Combined extracellular matrix cross-linking activity of the peroxidase MLT-7 and the dual oxidase BLI-3 is critical for post-embryonic viability in *Caenorhabditis elegans*. *J. Biol. Chem.* 284(26):17549--63
86. Trent C, Tsuing N, Horvitz HR. 1983. Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. *Genetics* 104(4):619--47
87. Troemel ER, Chu SW, Reinke V, Lee SS, Ausubel FM, Kim DH. 2006. p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. *PLoS Genet.* 2(11):e183
88. Tullet JMA, Hertweck M, An JH, Baker J, Hwang JY, et al. 2008. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. *Cell* 132(6):1025--38

89. Urano F, Calfon M, Yoneda T, Yun C, Kiraly M, et al. 2002. A survival pathway for *Caenorhabditis elegans* with a blocked unfolded protein response. *J. Cell Biol.* 158(4):639--46
- 89a. van der Hoeven R, McCallum KC, Cruz MR, Garsin DA. 2011. Ce-Duox1/BLI-3 generated reactive oxygen species trigger protective SKN-1 Activity via p38 MAPK signaling during infection in *C. elegans*. *PLoS Pathog.* 7(12):e1002453
90. Virk B, Correia G, Dixon DP, Feyst I, Jia J, et al. 2012. Excessive folate synthesis limits lifespan in the *C. elegans*:*E. coli* aging model. *BMC Biol.* 10:67
91. Walter P, Ron D. 2011. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 334(6059):1081--86
92. Wolff S, Ma H, Burch D, Maciel GA, Hunter T, Dillin A. 2006. SMK-1, an essential regulator of DAF-16-mediated longevity. *Cell* 124(5):1039--53
93. Wolkow CA, Kimura KD, Lee MS, Ruvkun G. 2000. Regulation of *C. elegans* life-span by insulinlike signaling in the nervous system. *Science* 290(5489):147--50
94. Youngman MJ, Rogers ZN, Kim DH. 2011. A decline in p38 MAPK signaling underlies immunosenescence in *Caenorhabditis elegans*. *PLoS Genet.* 7(5):e1002082
95. Zerofsky M, Harel E, Silverman N, Tatar M. 2005. Aging of the innate immune response in *Drosophila melanogaster*. *Aging Cell* 4(2):103--8
96. Zhang Y, Lu H, Bargmann CI. 2005. Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature* 438(7065):179--84







