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Model systems to the rescue

The relationship between aging and innate immunity

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In humans, there is an interdependent relationship between aging and immune system function, with each process affecting the outcome of the other. Aging can trigger immune system dysfunction, and alterations in the immune response can in turn affect human lifespan. Genetic experiments in model organisms such as *C. elegans* and Drosophila have led to the identification of numerous genes and signaling pathways that can modulate organismal lifespan and immune system function. Importantly, many of these signaling pathways exhibit conserved function in multiple species, including mammals, suggesting that the research in these simpler models could one day pave the way for the modulation of aging and immunity in humans. Here, we review the recent progress in our understanding of aging, innate immunity and the interaction between these two processes using these simple model systems. Additionally, we discuss what this may tell us about aging and the innate immune system in humans.

Aging and Immunity

In humans, the relationship between aging and the immune response is a complicated one. Aging is characterized by an overall decline in immune function termed *immunosenescence*, which affects both the innate and adaptive immune systems.[1,2] With age, cells of the innate immune system exhibit decreased function; for example, macrophages and neutrophils exhibit a weaker phagocytic response and a weaker oxidative burst. Dendritic cells from older individuals have a decreased ability to stimulate T cells. Cells of the adaptive immune system are also affected by age. T cells in older individuals display decreased T cell memory. There is also a decrease in the naïve T cell population in the thymus and decreased B cell production present in the elderly. Although older individuals have a weaker immune response, conversely, they also display a chronic inflammatory state termed *inflamm-aging*,[3] characterized by an increase in inflammatory cytokines present in the serum and an increase in the total NK cell count.[1,2] Immunosenescence leads to a decreased vaccine response and an increased risk of infection; inflamm-aging could contribute to a host of inflammatory diseases such as atherosclerosis. Thus, aging can have dramatic effects on the immune response, and conversely, the immune system can affect the risk of many age-associated diseases and can therefore affect lifespan. Thus, understanding the relationship between aging and immune system function is of critical importance to human health, particularly as the average human lifespan lengthens, increasing the impact of age-related diseases.

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Model Systems to the Rescue: The Genetics of Lifespan Regulation

The quest to understand aging and age-related diseases has been a long and ongoing one, with much of the research focusing on environmental damage and the organism's response to that damage. However, recent genetic experiments in model organisms such as *C. elegans*, Drosophila and mice have led to something of a revolution in aging research, as conserved signaling pathways that affect lifespan have been identified. Interestingly, the pathways that modulate lifespan also regulate the response to various environmental stresses, ranging from oxidative damage to UV exposure to thermal stress. Several of these signaling pathways also regulate the immune response. The genetic tools available in these model systems allow the dissection of the interaction between aging, immunity and stress response.

Much of this revolution in aging research has originated with studies using the nematode *C. elegans*. *C. elegans* was originally developed by Sydney Brenner as a model system to study development and nervous system function,[4] but it has emerged as a leading model system in many fields, including lifespan research.[5] This lifespan research has been aided by the excellent genetic and genomic tools available to study *C. elegans,* as well as the relatively short nematode lifespan (2–3 weeks). The modern explosion of aging research in *C. elegans* started when mutations were identified in genes in the insulin/IGF-1 (insulin-like growth factor 1)-like signaling pathway that affect nematode lifespan. Mutation of either *daf-2* (the nematode homolog of the insulin/IGF-1 receptor) or *age-1* [a phosphatidylinositol 3-kinase (PI3K) that acts in the *daf-2* pathway] was shown to lead to drastically increased nematode lifespan,[6-8] a concomitant increase in nematode health and resistance to many environmental stresses, including oxidative stress, ultraviolet light, heavy metals and thermal stress.[9-17] Mutation of either *daf-2* or *age-1* also increases *C. elegans* DNA repair capacity.[13] Finally, mutation of either *daf-2*

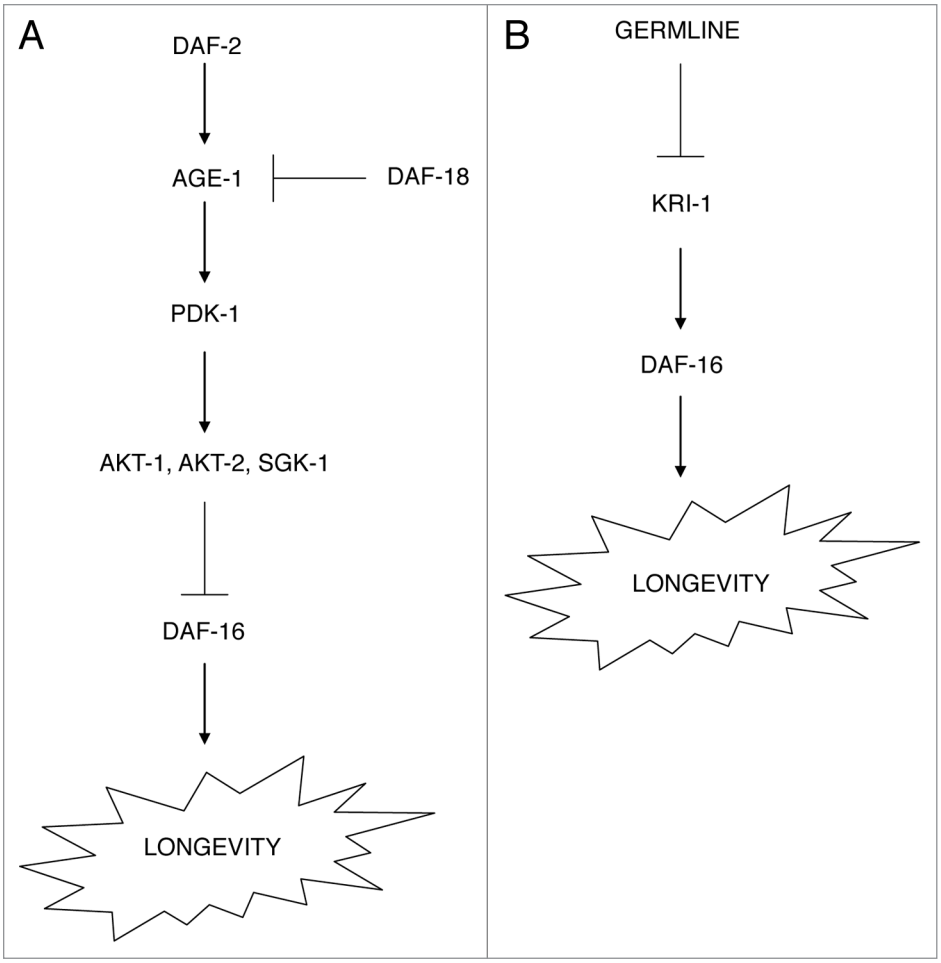

Figure 1. Signaling pathways that regulate longevity in *C. elegans*. The *daf-2* insulin/IGF-1-like signaling pathway (A) and germline proliferation (B) both regulate the DAF-16/FOXO family transcription factor, which in turn regulates nematode longevity.

or *age-1* affects the nematode immune response; *daf-2* and *age-1* mutant nematodes survive longer than wild-type nematodes when exposed to the human bacterial pathogens *P. aeruginosa, S. aureus* and *E. faecalis*.[18]

Since the original demonstration that the insulin/IGF-1 signaling pathway modulates nematode lifespan, numerous other genes, pathways and treatments have been discovered that affect *C. elegans* lifespan, including the action of the nematode germline, mitochondrial activity, caloric restriction and many others (reviewed recently in Kenyon 2010 [5]). Ablation of the nematode germline, either physically using a laser to ablate the germline precursor cells or genetically using sterilizing mutations, leads to increased nematode lifespan[19,20] and resistance to oxidative and thermal stress.[19] The germline has also been found to regulate *C. elegans* immunity, as nematodes lacking a germline are resistant to the pathogens *P. aeruginosa, S. marsescens and S. aureus.*[21-23] The relationship between lifespan regulation and innate immunity is not a universal one in *C. elegans*, however, because some mutations that increase nematode longevity (such as *clk-1,* a regulator of ubiquinone symthesis and *eat-2,* which is often used as a model for caloric restriction) do not increase nematode pathogen resistance.[22] So far, only the insulin/IGF-1 pathway and the germline pathways have been shown to regulate both *C. elegans* lifespan and immunity, so we will focus on these two pathways and discuss how their roles in both processes are interrelated.

Numerous components of the insulin/IGF-1 pathway have been identified in *C. elegans*.[5,24-27] *daf-2* is the sole insulin/IGF-1-like receptor in *C.* elegans[28] although numerous insulin-like peptides are encoded in the genome.[29,30] *daf-2* acts by the activation of the PI3 Kinase, *age-1*,[31] which in turn acts through the downstream kinases *pdk-1, akt-1, akt-2* and *sgk-1* (**Fig. 1A**).[32-34] The actions of these kinases culminate in the phosphorylation and inactivation of a FOXO-family transcription factor, DAF-16, which is sequestered from the nucleus when inactive.[35-38] However, when *daf-2* signaling is reduced, *daf-16* can undergo nuclear translocation,[37] where in conjunction with other transcription factors, it initiates the transcription of numerous genes that affect stress and pathogen resistance.[39,40] Thus, *daf-16* is a critical readout of insulin signaling that regulates lifespan, stress resistance and pathogen resistance. The insulin/IGF-1 pathway is also modulated by the *C. elegans* PTEN homolog *daf-18*.[41]

The *C. elegans* germline can also regulate nematode lifespan. Removal of the germline leads to increased lifespan.[19,20] This effect is due to germline proliferation and not simply loss of progeny production, as removal of the entire gonad (germline and somatic tissues) does not lengthen lifespan.[20] The germline acts through the adaptor protein *kri-1* to ultimately control DAF-16 activation.[42] Like the effect of *daf-2* on lifespan, the effect of the germline also requires the *daf-16* transcription factor (**Fig. 1B**).[19,20]

The *C. elegans* Innate Immune Response

C. elegans lacks an adaptive immune response and also lacks obvious migratory immune cells (the possible exception to this may be the coelomocytes, which are endocytic migratory cells in the nematode, although no immunological role for these cells has been demonstrated[43]). Instead, the main route of host defense in *C. elegans* appears to be the production of a plethora of antimicrobial proteins that are expressed in tissues exposed to pathogens.[44-50] *C. elegans* can be infected by numerous Gram negative and positive bacteria and some fungi.[51] While there are exceptions, the majority of these pathogens infect the nematode

intestine, proliferate there and ultimately kill the nematodes.[51] The production of different subsets of these antimicrobial proteins is induced by different pathogens, with pathogen-specific signatures suggesting that there are multiple pathogen recognition receptors in the nematode.[44-50]

Several genes and pathways have been identified that affect production of these antimicrobials in response to pathogen and therefore, ultimately affect the survival of the infected nematode. These include but are not limited to a p38 MAP Kinase signaling pathway,[45,46,52] a TGFβ signaling pathway,[47,53] and the *daf-2* insulin/IGF-1 signaling pathway[18] (see Ewbank 2006 and Irazoqui and Ausubel 2010,[54,55] for more extensive reviews of these and other *C. elegans* immune signaling pathways). The p38 MAPK pathway (**Fig. 2A**) acts autonomously in the epidermis and intestine to regulate antimicrobial gene expression.[56,57] In contrast, the *dbl-1*/TGFβ and *daf-2*/insulin pathways act in the epidermis and intestine, respectively, but are regulated by non-autonomous signals from neurons (**Fig. 2B and C**).[53,58] For example, the *daf-2* receptor is expressed and functions in the intestine; it is regulated by secretion of the *ins-7* insulin-like ligand from neurons.[58] The p38 MAPK and *daf-2* pathways act in parallel to control overlapping but different sets of genes to fight infection.[44,50] In general, *daf-2* regulates general stress response genes, while the effect of p38 MAPK is more specific to infection.[50] In addition to controlling the nematode innate immune response, these pathways are themselves potential targets for pathogens to inhibit the immune response, as *P. aeruginosa* can activate *daf-2* signaling, thereby inhibiting production of some antimicrobials.[59]

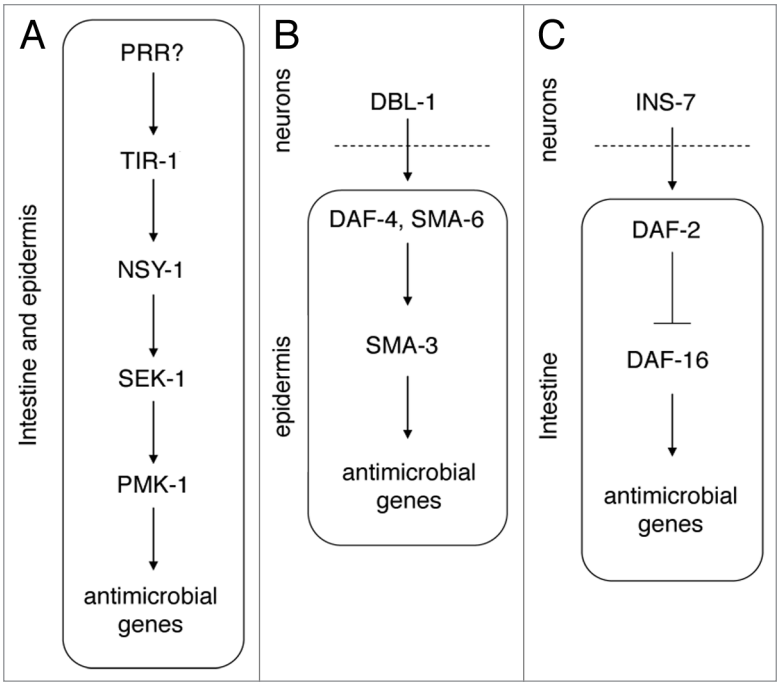

Figure 2. Innate immunity regulation in *C. elegans*. Three key signaling pathways regulate innate immunity in *C. elegans*. The p38 MAPK cascade functions in the intestine and epidermis to regulate antimicrobial gene expression, and includes TIR-1, a protein with a conserved TIR domain, the MAPKKK NSY-1, the MAPKK SEK-1 and the MAPK PMK-1 (A). How this pathway senses pathogens is still unclear, but the specificity of the nematode innate immune response suggests that several pathogen recognition receptors (PRRs) exist in the genome. The TGFβ family member DBL-1 is synthesized in neurons and controls expression of antimicrobial genes in the epidermis (B). DBL-1 signals via the TGFβ receptor (composed of DAF-4 and SMA-6) and a downstream SMAD (SMA-3). Neuronal expression of the insulin-like peptide INS-7 also regulates antimicrobial gene expression non-autonomously, acting on the DAF-2 receptor and downstream signaling components, including DAF-16 in the intestine (C).

daf-2 mutant nematodes live long and are resistant to numerous environmental stresses. All these effects are dependent on the *daf-16* transcription factor, as evidenced by experiments using *daf-2; daf-16* double mutant nematodes. This relationship extends to the regulation of pathogen resistance as well (**Fig. 3A**), with the *daf-16* mutation suppressing the enhanced pathogen resistance of the *daf-2* mutant.[18] However, mutations in certain other kinases that act downstream of *daf-2* are able to separate the effects of insulin signaling on lifespan and innate immunity. While nematodes harboring mutations in either *pdk-1* or *sgk-1* are long-lived, they are not resistant to pathogen.[22] In contrast, mutation of either *akt-1* or *akt-2* has a minimal effect on *C. elegans* lifespan but does induce strong pathogen resistance.[22] This ability to separate the effects of insulin signaling on lifespan and innate immunity suggests that other stresses besides pathogen infection are critical to overall lifespan regulation, at least in this context.

Germline proliferation also controls both lifespan and innate immunity in *C. elegans*, as sterile nematodes are resistant to both Gram-negative and Gram-positive bacteria.[21-23] The long lifespan of sterile nematodes is completely dependent on *daf-16*.[19,20] However, the effect of *daf-16* in relation to germline proliferation and innate immunity varies depending on the growth conditions of the pathogen. Under certain conditions, the pathogen resistance of sterile nematodes is completely dependent on *daf-16* (**Fig. 3B**)[22,23] while under other conditions, it is completely independent.[21] Again, there is no absolute correlation between long life and pathogen resistance. The germline also acts in part or in whole in parallel to the p38 MAPK pathway to control pathogen resistance (**Fig. 3B**).[21] While there is a general correlation between lifespan and pathogen resistance in *C. elegans* and related species,[60,61] the complicated genetic interactions suggest that there is no simple causal interaction between the two.

Evolutionary Conservation

At first glance, it might be tempting to dismiss the genetic experiments in *C. elegans* as having no real bearing on human aging. However, experiments in other organisms, including Drosophila, mice and even humans, suggest the pathways that affect *C. elegans* lifespan also affect lifespan and stress resistance in other organisms, including mammals and thus they theoretically offer the potential as therapeutic targets to delay aging and aging-related diseases.

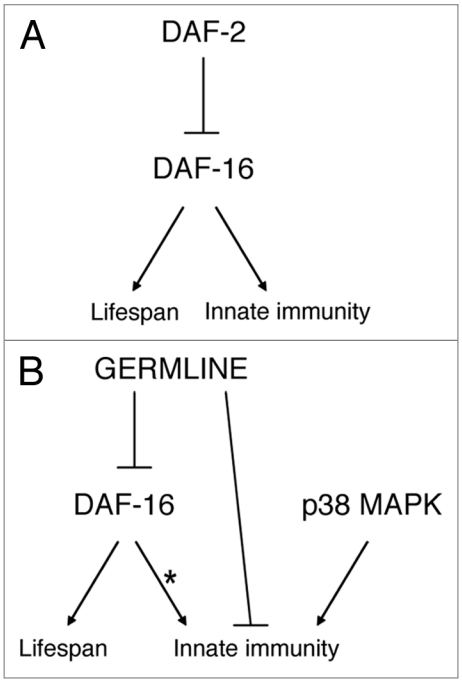

Figure 3. Model for the regulation of lifespan and innate immunity. The *daf-2* (A) and germline (B) pathways both regulate lifespan and innate immunity in *C. elegans*. The effect of *daf-2* on both lifespan and innate immunity depends on the DAF-16 transcription factor (A). Similarly, the effect of the germline on lifespan also requires *daf-16*. However, depending on the growth conditions of the pathogen and the nematodes, the effect of the germline on innate immunity may or may not depend on DAF-16 (B). The germline also acts in parallel to a p38 MAPK pathway to regulate innate immunity. This figure was originally published in Alper et al. "The *Caenorhabditis elegans* germ line regulates distinct signaling pathways to control lifespan and innate immunity." J Biol Chem 2010; 285:1822–8.

As in *C. elegans,* the insulin/IGF-1 pathway controls lifespan, stress resistance and pathogen resistance in Drosophila. Mutation of the insulin receptor in flies increases lifespan.[62] Flies carrying mutations in the insulin-like ligand chico are also long-lived, resistant to some but not all stresses (such as starvation but not thermal stress)[63] and are resistant to the bacterial pathogens *P. aeruginosa* and *E. faecalis*.[64] There is also evidence for the role of the insulin and IGF-1 signaling pathways in lifespan regulation in mammals. While completely knocking out the insulin-signaling pathway has profoundly detrimental consequences, partially turning down the pathway using heterozygous animals or knockouts in particular tissues has allowed investigators to test the function of these pathways in lifespan regulation in mice. These studies have demonstrated that several components in these pathways, including the insulin receptor, the Igf1 receptor and IRS1 (Insulin receptor substrate 1), all affect lifespan;[65-68] mutation of some of these signaling components also increases resistance to oxidative stress in mice.[66,69] There is even evidence suggesting a role of insulin signaling in lifespan regulation in humans; studies of long-lived individuals have led to the identification of DNA polymorphisms in insulin/IGF-1 signaling components that are associated with longevity.[70-75] Thus, there is strong evidence in multiple species that the role of the insulin/IGF-1 pathway in lifespan regulation and stress resistance is evolutionarily conserved.

There is also some evidence suggesting that the germline can regulate lifespan in organisms besides *C. elegans*, although this evidence is so far not as compelling as the evidence for the insulin/IGF-1 pathway. The germline, at least under some conditions, can modulate lifespan in Drosophila.[76] Similarly, the germline may affect lifespan and stress resistance in mammals. Transplantation of young ovarian tissue into older mice leads to an increase in mouse lifespan,[77] and postponement of menopause in mice can delay some age-related health complications.[78] Obviously there is limited evidence that the germline can affect lifespan in humans. The extension of lifespan in nematodes without a germline is dependent on the somatic gonad,[20] indicating that the somatic gonad plays a protective role in lifespan regulation in *C. elegans.* Interestingly, while removal of ovaries in postmenopausal women has a positive survival effect on those at risk of ovarian and breast cancer, this surgery has an overall net negative effect on long-term health, with increased risk of most other diseases, such as cardiovascular disease and other cancers.[79-81] This raises the possibility that somatic gonadal tissue in humans may also serve a protective function.[5]

The Central Role of FOXO-Family Transcription Factors

The DAF-16/FOXO family of transcription factors plays a key role in regulating lifespan, stress resistance and immunity in many systems. As outlined above, *daf-16* plays a critical role in all these processes in *C. elegans,* acting downstream of multiple signaling pathways. The role of *daf-16* includes the regulation of antimicrobial gene expression during infection.[39,40] Likewise, the Drosophila FOXO transcription factor regulates antimicrobial gene expression.[82] In mammals, different forkhead family transcription factors control different aspects of immune cell development and function, including the development of regulatory T cells, the regulation of T cell tolerance and thymic development.[83,84] FOXO also plays a role in the aging of the immune system in mammals. Dis-regulation of the NFκB transcription factor is thought to play a critical role in *inflamm-aging,*[85,86] and FoxO3a in turn has been shown to inhibit NFκB activity.[87-89] Thus, the DAF-16/FOXO transcription factor is at the center of key lifespan and stress response pathways in multiple organisms, and the study of its regulation in the simpler model systems should provide useful insights into the role of forkhead family transcription factors in mammals.

The Relationship Between Stress, Immunity and Aging

What causes aging has been a profound question that has sparked much debate and numerous theories. One current widely-accepted theory is that oxidative damage caused by free radicals causes aging.[90,91] The studies using long-lived mutants in model organisms have largely backed up these ideas, as most long-lived mutants are resistant to one or multiple stresses. However, there

are some exceptions, and mutant combinations or treatments have been identified that uncouple lifespan regulation from resistance to one or multiple stresses, arguing against a simple model in which any single stress is the key factor in aging. This is illustrated by experiments dealing with the role of oxidative damage in aging. For example, the *mev-4* mutation renders *C. elegans* long-lived and resistant to oxidative damage; however the *mev-4; daf-16* double mutant is no longer long-lived but is still resistant to oxidative damage.[92] If oxidative damage were the sole factor in lifespan regulation, then the double mutant would be expected to live long. An even more striking effect is observed with the germline. Nematodes lacking a germline that also lack *daf-16* activity no longer live long but are still resistant to oxidative stress.[93] What is interesting about sterilized *daf-16* mutant nematodes is that they are also still resistant to thermal stress and, at least under some assay conditions, pathogen infection.[21,94] So sterilized *daf-16* mutant nematodes are resistant to oxidative stress, thermal stress and pathogen infection; yet these animals still do not live long! There may be an additional critical stress that affects longevity in these animals, but no single stress is an obvious candidate. Further muddying the role of oxidative stress in lifespan regulation are experiments in *C. elegans* and mice in which the SOD genes are either mutated or overexpressed.[95-97] While there are some caveats to these experiments, the correlation between lifespan and SOD activity in nematodes and mice is contradicted if not completely disproven in these experiments. It is formally possible that oxidative stress could be a key determinant of aging in wild-type animals or in some mutants but not in others. These possibilities will keep investigators busy researching and debating for quite some time. What is clear is that stress, immunity and aging are inter-related; altering one process often perturbs the others, and some of the same signaling pathways and components are shared. A simple global theory that explains how and why is lacking. Undoubtedly, experimentation in model organisms will continue to reap rewards in addressing these issues.

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