The relationship between the mechanisms that control an organism’s lifespan and its ability to respond to environmental challenges are poorly understood. In Caenorhabditis elegans, an insulin-like signaling pathway modulates lifespan and the innate immune response to bacterial pathogens via a common mechanism involving transcriptional regulation by the DAF-16/FOXO transcription factor. The C. elegans germ line also modulates lifespan in a daf-16-dependent manner. Here, we show that the germ line controls the innate immune response of C. elegans somatic cells to two different Gram-negative bacteria. In contrast to the insulin-like signaling pathway, the germ line acts via distinct signaling pathways to control lifespan and innate immunity. Under standard nematode culture conditions, the germ line regulates innate immunity in parallel to a known p38 MAPK signaling pathway, via a daf-16-independent pathway. Our findings indicate that a complex regulatory network integrates inputs from insulin-like signaling, p38 MAPK signaling, and germ line stem cells to control innate immunity in C. elegans. We also confirm that innate immunity and lifespan in C. elegans are distinct processes, as nonoverlapping regulatory networks control survival in the presence of pathogenic and nonpathogenic bacteria. Finally, we demonstrate that the p38 MAPK pathway in C. elegans is activated to a similar extent by both pathogenic and nonpathogenic bacteria, suggesting that both can induce the nematode innate immune response.

In Caenorhabditis elegans, inhibition of either daf-2 insulin/IGF-1-like signaling or germ line proliferation increases nematode lifespan (1–4), increases resistance to environmental stress (1, 5–11), and increases nematode survival in the presence of pathogen (12–14). Insulin-like signaling also affects lifespan and stress resistance in Drosophila and rodents (15–19), and possibly in humans (20–23), indicating that this pathway may also be evolutionarily conserved. The germ line may also affect lifespan and stress resistance in mammals, as transplantation of young ovarian tissue into older mice can lengthen mouse lifespan (25), and postponement of menopause in mice delays some age-related health complications (26). Thus, the role of the germ line in the regulation of lifespan may also be evolutionarily conserved.

Here, we show that the C. elegans germ line controls nematode resistance to two different Gram-negative bacterial pathogens, Pseudomonas aeruginosa and Serratia marcescens. However, unlike the case with daf-2, the effects of the germ line on lifespan and pathogen resistance are mediated by separate signaling pathways. While the FOXO family transcription factor DAF-16 is necessary for germ line-induced changes in lifespan (1, 2), daf-16 does not affect germ line-induced changes in innate immunity under standard nematode culture conditions. Our findings also indicate that the germ line acts in parallel to a p38 MAPK2 pathway to induce changes in innate immunity, suggesting that there are additional regulatory components of C. elegans host defense. Surprisingly, the extent of p38 MAPK phosphorylation was not different in nematodes exposed to either nonpathogenic or pathogenic bacteria, suggesting that either both bacteria induce the innate immune response to a similar extent or that the p38 pathway in C. elegans may act permisively rather than actively in controlling the nematode innate immune response.

**EXPERIMENTAL PROCEDURES**

C. elegans mutant strains obtained for this work are: JK1107 glp-1(q224) III (27); CF1903 glp-1(e2141) III and CI1880 daf-16(mu86) I; glp-1(e2141) III (28); SS104 glp-4(bn2) I (29); CI1038 daf-16(mu86) I (30); CF1700 daf-16(mu86) I; mes-1(bn7) X that had lost the muEx248 array (31); SI149 mes-1(bn7) X (32); KU25 pmk-1(km25) IV (33); JTI366 vhp-1(sa366) II (34); AU147 daf-16(mgDf47) I; glp-1(e2141) III (14); daf-16(mgDf47) I; CB1370 daf-2(e1370) III; and GR1309 daf-16(mgDf47) I; daf-2(e1370) III (35); and VC390 nsy-1(ok593) II from the C. elegans knock-out consortium. We constructed two additional double mutant strains for this work: nsy-
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FIGURE 1. Mutations that prevent germ line proliferation in C. elegans increase resistance to pathogenic bacteria. Depicted are representative survival plots for the indicated C. elegans wild-type or mutant strains, which were exposed to either P. aeruginosa (A–D) or S. marcescens (E–G). Survival studies were initiated with nematodes in the late L4 larval stage. The tested alleles and further statistical data (medians, n, and p values) are listed in supplemental Table 1. The glp-1(e2124) allele was used in all experiments except for B and F, where the glp-1(q224) allele was used. mes-1 F refers to fertile nematodes; mes-1 S refers to sterile nematodes. Some wild-type (w.t.) curves in individual panels are from the same experiment as in other panels. All survival studies were carried out at least three times; the data is representative of these multiple experiments.

1(ok593); glp-1(e2141) III and glp-1(e2141) III; pmk-1(km25) IV. Both double mutant strains were generated by first rendering either pmk-1 or nsy-1 homozygous (using PCR to follow the deletion in either pmk-1 or nsy-1) and then rendering glp-1 homozygous by monitoring sterility of the strains at 25 °C. Temperature-sensitive sterile strains (glp-1 and glp-4) were maintained at 15 °C but were shifted to 25 °C as young L1s prior to initiation of survival assays. Sterile and fertile mes-1(bn7) animals were identified by their appearance on the dissecting microscope; sterility was confirmed by the absence of progeny production during the course of the survival assay. Survival assays were initiated with nematodes in the late L4 stage. Survival assays were carried out largely as described (36). For P. aeruginosa survival assays, nematode growth medium (NGM) plates (37) were seeded with bacteria and incubated overnight at room temperature, with the exception of the experiments in Fig. 2, G and H, in which either modified NGM plates (38) or standard NGM plates, respectively, were seeded with bacteria and incubated for 24 h at 37 °C prior to adding nematodes. All survival studies were carried out at least three times, except for the epistasis experiments with daf-16, which were replicated more than six times with multiple strains. Nematode survival was analyzed using Graphpad Prism 4; the Mantel-Cox log-rank test was used to determine the statistical significance of differences in survival. The Escherichia coli lifespan studies were carried out in the presence of 5 μg/ml of the sterilizing agent 5-fluoro-2’ deoxyuridine (39); 5-fluoro-2’ deoxyuridine does not alter the lifespan of these mutants (1). Bacterial strains used in the survival assays were E. coli OP50 (37), P. aeruginosa PA14 (40–42), and S. marcescens IGX2 (43, 44).

Antimicrobial gene expression was monitored by quantitative PCR as described previously (45). In these experiments, eggs from either wild-type (N2), glp-1, or vhp-1 mutant animals were collected and synchronized by bleaching (37), allowed to hatch at 15 °C, and then shifted 12 h later as young L1s to 25 °C. Nematodes were collected for RNA preparation as young adults. For the P. aeruginosa exposures, nematodes were shifted from E. coli to P. aeruginosa 12 h prior to nematode collection.

To monitor phosphorylation of the p38 MAPK PMK-1, eggs from wild-type, glp-1 mutant, or glp-4 mutant animals were collected and synchronized by bleaching (37), allowed to hatch, and were then grown and exposed as described above for the quantitative PCR experiments. Mixed stage nematodes were collected for the wild-type versus nsy-1 mutant nematode comparison. For the arsenic exposure, mixed stage nematodes were exposed to 5 mM sodium arsenite for 30 min as described in Ref. 33. Nematodes were collected by centrifugation, and pellets were then rapidly frozen in liquid nitrogen. Protein was isolated, and Western blot analysis was performed as described (33, 46). A BCA protein assay (Thermo Scientific, Rockford, IL) was then performed to quantify the protein in the lysate. Each sample was analyzed by Western blotting using three different antisera: anti-phospho-p38 (Thr180/Tyr182) antibody (Cell Signaling Technology), rabbit polyclonal antisera specific to PMK-1 protein (gift of Kuni Matsumoto), and anti-β-tubulin monoclonal (E7, Developmental Studies Hybridoma Bank, Iowa City, IA). Western blots were developed using ECL Plus (GE Healthcare), and the STORM 860 Molecular Imager (Amersham Biosciences) was used to capture images. ImageQuant software (GE Healthcare) was used to quantify bands on the Western blots. PMK-1 and phospho-PMK-1 quantities were normalized using the anti-β-tubulin blots to control for protein concentration in the samples.

RESULTS

To examine the role of the C. elegans germ line in innate immunity, we evaluated survival of nematodes harboring muta-
tions in any of several genes required for germ line proliferation. These mutations are known to increase nematode lifespan in the presence of *E. coli* (1) (the standard experimental food source, considered nonpathogenic to *C. elegans*). As observed previously (47), mutation of *glp-4*, a gene required for germ line proliferation (29), enhanced nematode survival in the presence of the pathogen *Pseudomonas aeruginosa* (Fig. 1A; alleles used and statistical information for all survival plots are presented in supplemental Table 1). To verify that this effect is due to loss of the germ line and not due to some other potential function of *glp-4*, we demonstrated that two different mutations in *glp-1*, which is also required for germ line proliferation (27, 48), both extended nematode survival in the presence of *P. aeruginosa* (Fig. 1, B and C). The *mes-1* gene controls initial development of the nematode germ line; some *mes-1* mutant animals are completely fertile, whereas others are completely sterile (32, 49, 50). *mes-1* mutant nematodes that are fertile survive as long as wild-type when exposed to *P. aeruginosa*, whereas *mes-1* mutant nematodes that are sterile are much more resistant (Fig. 1D). Together, these findings indicate that germ line proliferation normally increases *C. elegans* susceptibility to *P. aeruginosa* as nematodes lacking a germ line are more resistant.

The effect of the germ line on pathogen resistance was not unique to *P. aeruginosa*, as removal of the germ line by mutation of either *glp-4* or either of two *glp-1* alleles also increased survival when *C. elegans* was exposed to a second Gram-negative bacterial pathogen, *S. marcescens* (Fig. 1, E–G). Thus, nematodes lacking a germ line are resistant to at least two different Gram-negative bacterial pathogens. Because we observed that mutant nematodes that lack a germ line exhibit enhanced host defense, we can conclude that the germ line actively inhibits host defense in wild-type nematodes.

The longer lifespan of germ line-deficient nematodes in the presence of the nonpathogenic bacterium *E. coli* depends on the activity of the FOXO family transcription factor DAF-16 (Fig. 2, A and B) (1). In contrast, we find that the enhanced resistance to *P. aeruginosa* caused by mutation of either *glp-1* or *mes-1* is completely independent of the activity of DAF-16. *daf-16(mu86);mes-1* sterile double mutant nematodes live as long as *mes-1* sterile single mutant nematodes in the presence of *P. aeruginosa* (Fig. 2C). Likewise, *daf-16(mu86);glp-1* double mutant nematodes live as long as (or even slightly longer than) *glp-1* single mutant nematodes in the presence of pathogen (Fig. 2D). To confirm that the *daf-16* mutation could still exert an effect in these strains, we verified that the presence of the *daf-16(mu86)* mutation did diminish the lifespan of either *mes-1* or *glp-1* mutant nematodes grown in the presence of *E. coli* (Fig. 2, A and B). Thus, *daf-16* is required for lifespan...
extension in the presence of sterilizing mutations but not for the sterility-induced increase in pathogen resistance. This is in contrast to the role of daf-16 in the daf-2 insulin-like signaling pathway, where daf-16 is required for the long lifespan and pathogen resistance of daf-2 (Fig. 2E) (13).

It has been reported that under some conditions, daf-16 could suppress the increased pathogen resistance of sterile nematodes (12, 14). We tested the daf-16 allele previously used (daf-16(mgDf47)) and found that daf-16 still failed to affect the pathogen resistance of glp-1 mutant animals (Fig. 2F). One key difference between our survival assays and those previously reported (12, 14) is that our assays were carried out under standard nematode culture conditions using NGM (37). Even et al. and Miyata et al. (12, 14) both use a slightly modified growth medium supplemented with additional peptone (38) and grow bacterial lawns at 37 °C for 24 h prior to adding nematodes. In the studies presented in this report, bacteria are grown at room temperature overnight prior to adding nematodes. When we carried out survival assays under conditions that were similar to these previous reports (12, 14), we found that both daf-16(mub86) and daf-16(mgDf47) mutations did at least partially suppress the increased pathogen resistance of glp-1 (Fig. 2G). Thus, the growth conditions of the bacteria or the nematode host had profound consequences on C. elegans pathogen resistance. To differentiate between these two possibilities, we grew the bacteria at high temperature (37 °C) on standard culture medium and then monitored nematode survival, conditions that could alter the pathogen but not the nematode. Under these conditions, we found that a daf-16(mgDf47) mutation did partially suppress the increased pathogen resistance of glp-1 (Fig. 2H), indicating that at least some of the differences in the effects of daf-16 under different conditions is due to changes in the pathogen and not the host.

As in mammals, a p38 MAPK pathway affects C. elegans host defense to numerous pathogens including P. aeruginosa (47). To investigate whether the p38 MAPK pathway might mediate the role of the germ line in host defense, we tested whether the pathogen resistance of glp-1 sterile mutant nematodes required the activity of genes in the p38 MAPK pathway. As observed previously (47), we found that loss of pmk-1 (p38 MAPK) or nsy-1 (MAPKKK) activity in otherwise wild-type nematodes shortened survival in the presence of P. aeruginosa (Fig. 3, A and C). Mutation of either the MAPK pmk-1 or the MAPKKK nsy-1 also shortened survival of glp-1 mutant nematodes when exposed to P. aeruginosa (Fig. 3, A and C). However, the glp-1;pmk-1 or nsy-1;glp-1 double-mutant nematodes did not survive as short as the MAPK pathway single-mutant animals (Fig. 3, A and C). This indicates that the effect of the germ line on pathogen resistance may be due, at least in part, to a pathway other than the pmk-1 p38 MAPK pathway. These findings are consistent with previous studies showing that mutations in nsy-1 or the MAPKKK sek-1 decrease the pathogen resistance of glp-4 (47). These results indicate that the role of the p38 MAPK pathway in regulating C. elegans host defense is additive to that of the germ line and are consistent with models where the p38 MAPK pathway acts either downstream or in parallel to the germ line to regulate pathogen resistance.

In contrast to our findings with the pathogen P. aeruginosa, the addition of either a pmk-1 or a nsy-1 mutation to glp-1 mutant animals had little or no effect on nematode lifespan in the presence of nonpathogenic E. coli (Fig. 3, B and D). We note that pmk-1;glp-1 double mutant nematodes survived for a slightly shorter period of time than glp-1 mutant nematodes in the experiment depicted in Fig. 3D; however, in several other experiments, the lifespan of glp-1 and pmk-1;glp-1 mutant animals was indistinguishable (data not shown). Thus, in the absence of the nematode germ line, the daf-16 and p38 MAPK pathways act independently to control survival in the presence of nonpathogenic E. coli (lifespan) and pathogenic P. aeruginosa (innate immunity), respectively.

These epistasis experiments indicate that the p38 MAPK pathway is acting either downstream or in parallel to the germ line in the control of innate immunity. To distinguish between these two possibilities, we used quantitative PCR to monitor the expression of four putative antimicrobial genes (lys-8, clec-85, dod-22, and K08D8.5) whose expression is regulated by the p38 MAPK signaling pathway. Transcription of these genes in the presence of either the nonpathogenic bacterium E. coli or the pathogenic bacterium P. aeruginosa requires the nsy-1 MAPKKK (45). As observed previously (45), expression of all four of these genes is induced by P. aeruginosa in wild-type nematodes (Fig. 4A). Similarly, expression of all four genes is induced by P. aeruginosa in nematodes harboring a mutation in glp-1 (Fig. 4A). However, the glp-1 mutation did not significantly alter expression of these four p38-dependent genes when the nematodes were exposed to either bacterium, suggesting that glp-1 does not alter p38 activity (Fig. 4A) (t test, p > 0.05 for all comparisons). This is consistent with the germ line and MAPK pathways acting in parallel rather than in serial fashion. To verify that expression of these genes could be induced when
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p38 MAPK activity is increased, we also monitored expression of these genes in nematodes harboring a mutation in \( \text{glp-1} \), a negative regulator of the p38 pathway (51). The expression of \( \text{lys-8} \) and \( \text{dod-22} \) increased significantly when the MAPK pathway inhibitor \( \text{vhp-1} \) was mutated (Fig. 4B) (t test, \( p = 0.040 \) and \( p = 0.021 \), respectively).

To confirm that the germ line was not directly affecting the p38 MAPK pathway, we monitored phosphorylation of the MAPK PMK-1 using an antiserum specific to the phosphorylated form of the protein. We compared wild-type nematodes to those containing a mutation in either \( \text{glp-1} \) or \( \text{glp-4} \). The amount of total PMK-1 protein (phosphorylated and unphosphorylated) was not significantly different in either of the mutant lines tested (Fig. 5A), whether the nematodes were exposed to \( \text{E. coli} \) or \( \text{P. aeruginosa} \). Nematodes containing a mutation in either \( \text{glp-1} \) or \( \text{glp-4} \) did not have substantially more phospho-PMK-1 than did wild-type nematodes, whether the nematodes were exposed to either \( \text{E. coli} \) or \( \text{P. aeruginosa} \) (Fig. 5B). This is consistent with the observation that a \( \text{glp-1} \) mutation did not alter the expression of the MAPK-dependent antimicrobial genes (Fig. 4A) and suggests that the \( \text{C. elegans} \) germ line has little or no effect on p38 MAPK activity. As a control, we quantified phosphorylation of PMK-1 in a \( \text{nys-1} \) MAPKKK mutant background. As expected, nematodes harboring a mutation in \( \text{nys-1} \) produced substantially less phosphorylated PMK-1 than wild-type animals (Fig. 5C). We also verified that we could detect an increase in PMK-1 phosphorylation by exposing nematodes to arsenic, which is known to increase PMK-1 phosphorylation (Fig. 5C) (33). Interestingly, the amount of phosphorylated PMK-1 did not increase when nematodes were exposed to \( \text{P. aeruginosa} \), as compared with nematodes exposed to \( \text{E. coli} \) (Fig. 5B), even though \( \text{P. aeruginosa} \) induces the expression of many p38-dependent antimicrobial genes (Fig. 4) (45, 52).

**DISCUSSION**

The germ line in \( \text{C. elegans} \), like the insulin signaling pathway, can control both lifespan in the presence of nonpathogenic bacteria and host defense in the presence of pathogenic bacteria. However, unlike the insulin signaling pathway, where \( \text{daf-16} \) is critical to both the lifespan and host defense pathways (Fig. 6A), the germ line requires \( \text{daf-16} \) to exert its effect on lifespan but acts independently of \( \text{daf-16} \) in host defense (Fig. 6B). This observation demonstrates that innate immunity and lifespan are genetically separable and are therefore distinct processes in \( \text{C. elegans} \) even though both occur in the presence of bacteria and even though the timescale of infection and aging is much more similar in nematodes than in mammals.

Our results are consistent with the prior observation that the germ line affects the response to oxidative damage and thermal stress independently of \( \text{daf-16} \) (31, 53). This raises an interesting paradox. The long lifespan of \( \text{daf-2} \) mutant animals is thought to be due to the increased stress resistance of those animals. Sterile nematodes are likewise long lived and stress resistant. However, while sterile, \( \text{daf-16} \) double mutant nematodes are not long lived; these nematodes are still resistant to multiple stresses (thermal, oxidative, and pathogen stress), which raises the question, why are sterile nematodes long lived? It is possible that another stress is critical in lifespan regulation by the

![FIGURE 4. The glp-1 mutation does not alter the expression of genes that are regulated by the p38 MAPK pathway. A, wild-type (N2) or glp-1(e2141) mutant animals were exposed to \( \text{E. coli} \) or \( \text{P. aeruginosa} \), RNA was collected, and antimicrobial gene expression was assayed by quantitative PCR using \( \text{mlc-1} \) to normalize RNA concentration. Expression was measured relative to N2 grown in the presence of \( \text{E. coli} \). Depicted are the results (±S.E.) of three independent experiments. B, as a control, wild-type and \( \text{vhp-1} \) mutant animals were treated similarly, and antimicrobial gene expression was monitored. Expression levels that were significantly different from control (\( p < 0.05 \), t test) are indicated with an asterisk.](image-url)

![FIGURE 5. Mutations that prevent production of the \( \text{C. elegans} \) germ line do not increase phosphorylation of PMK-1. Production of PMK-1 (A) and phospho-PMK-1 (B and C) in the indicated wild-type (w.t.) or mutant strains and in the presence of the indicated bacteria was quantitated using antibodies specific to the protein and the phosphorylated form of the protein and normalized using antibodies specific for \( \beta \)-tubulin as a reference as described under “Experimental Procedures.” Depicted are the results (±S.E.) of three independent experiments. Alleles used were: \( \text{glp-1} \) (e2141), \( \text{glp-4} \) (bn2), and \( \text{nys-1} \) (ok593). As indicates wild-type nematodes that were treated with arsenic as described under “Experimental Procedures.” Phospho-PMK-1 levels that were significantly different from wild-type (\( p < 0.05 \), t test) are indicated with an asterisk.](image-url)
germ line. Regardless, the link between lifespan and stress resistance may be more complicated than surmised.

The complexity of the relationship between lifespan and stress resistance is also supported by the observation that under some environmental conditions, the pathogen resistance of sterile nematodes requires *daf-16* (Figs. 2G and 6B) (12, 14). These modified conditions could either alter host defense in the nematode or virulence of the pathogen. We found that pathogen resistance of sterile nematodes also depended in part on *daf-16* when the pathogen was grown at higher temperature on standard NGM (Fig. 2H), conditions that could alter bacterial virulence but not nematode host defense. Alterations in growth conditions (depicted with an asterisk), *daf-16* does mediate the effect of the germ line on host defense (12, 14). The p38 MAPK pathway also plays a role in lifespan regulation in wild-type (52) but not germ line-deficient nematodes; for simplicity, this is not shown in these models.

**Figure 6. A model for the regulation of *C. elegans* lifespan and innate immunity.** A, a schematic demonstrating that *daf-2* regulates both nematode lifespan and the innate immune response in a *daf-16*-dependent manner (based largely on the work in Refs. 3, 4, 13, 52). In contrast, as depicted in B, while the germ line acts through *daf-16* to control lifespan (1, 2) under standard nematode growth conditions, the germ line acts independently of *daf-16* and in parallel to the p38 MAPK pathway (this work). Under modified nematode growth conditions (depicted with an asterisk), *daf-16* does mediate the effect of the germ line on host defense (12, 14). The p38 MAPK pathway also plays a role in lifespan regulation in wild-type (52) but not germ line-deficient nematodes; for simplicity, this is not shown in these models.

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