



Take a Walk to the Wild Side of *Caenorhabditis elegans*-Pathogen Interactions

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SUMMARY Microbiomes form intimate functional associations with their hosts. Much has been learned from correlating changes in microbiome composition to host organismal functions. However, in-depth functional studies require the manipulation of microbiome composition coupled with the precise interrogation of organismal physiology—features available in few host study systems. *Caenorhabditis elegans* has proven to be an excellent genetic model organism to study innate immunity and, more recently, microbiome interactions. The study of *C. elegans*-pathogen interactions has provided in depth understanding of innate immune pathways, many of which are conserved in other animals. However, many bacteria were chosen for these studies because of their convenience in the lab setting or their implication in human health rather than their native interactions with *C. elegans*. In their natural environment, *C. elegans* feed on a variety of bacteria found in rotting organic matter, such as rotting fruits, flowers, and stems. Recent work has begun to characterize the native microbiome and has identified a common set of bacteria found in the microbiome of *C. elegans*. While some of these bacteria are beneficial to *C. elegans* health, others are detrimental, leading to a complex, multifaceted understanding of bacterium-nematode interactions. Current research on nematode-bacterium interactions is focused on these native microbiome components, both their interactions with each other and with *C. elegans*. We will summarize our knowledge of bacterial pathogen-host interactions in *C. elegans*, as well as recent work on the native microbiome, and explore the incorporation of these bacterium-nematode interactions into studies of innate immunity and pathogenesis.

KEYWORDS *Caenorhabditis elegans*, host-pathogen interactions, innate immunity, microbiome

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INTRODUCTION

Nematodes are the most abundant multicellular organism on Earth and consist of over 25,000 species, both free-living and parasitic (1). *Caenorhabditis elegans* is a free-living nematode that can be found in the natural environment worldwide (2–6). It feeds on a variety of bacteria found in rotting organic matter and is most commonly isolated from rotting fruits, flowers, and stems (reviewed in reference 7). In the natural environment, *C. elegans* is in constant contact with many other organisms, including other small invertebrates, bacteria, and fungi. *C. elegans* travels between locations via vectors, such as isopods and snails (4, 8). Nematodes are also prey to a variety of insects and fungi, in addition to playing host to a number of pathogenic and symbiotic bacteria (3; reviewed in reference 7).

C. elegans is an excellent genetic model organism for studying many biological processes, including development, cell biology, innate immunity, and neurobiology. Some of the features that make *C. elegans* a tractable model system include its many progeny (up to 300), short generation time (3.5 days), small size (1 mm), and transparency, allowing for visualization of development, bacterial colonization, and protein localization and expression. In addition, there are a variety of tools for determining *C. elegans* health, including fecundity, life span, and stress response assays. *C. elegans* is also amenable to genetic manipulation, allowing for an in-depth understanding of the genetics underlying host-microbe interactions. In the laboratory, *C. elegans* can be grown on plates seeded with *Escherichia coli* OP50. This artificial lab setting differs significantly from the natural environment and lacks ecologically important biotic components. In addition, many recent studies have determined that bacteria, both in the surrounding environment and in a microbiome of many organisms, influence behavior, aging, and overall health (9–11). These observations led to studies investigating the effects of diverse microorganisms, including bacteria, fungi, and viruses, on *C. elegans*.

This review will begin with an overview of types of *C. elegans*-bacterium interactions and then focus more specifically on innate immunity in *C. elegans* in response to bacteria. The remaining sections will review more recent studies characterizing bacteria found in the natural environment of *C. elegans* and the role of pathogens in *C. elegans*' environment. Although *C. elegans* encounters numerous pathogenic microbes in its natural environment, including bacteria, fungi, and viruses, extensive identification and characterization of fungi and viruses in *C. elegans*' environment has yet to be performed. Therefore, we focus here on *C. elegans* interactions with bacterial pathogens.

C. ELEGANS-BACTERIUM INTERACTIONS

Bacteria interacting with *C. elegans* can be classified into three different categories: (i) as prey, not influencing the host and having a detrimental relationship with the host; (ii) as mutualists, where both host and bacteria benefit from the association; and (iii) as pathogens, where the bacteria have a detrimental effect on the host. These interactions are dynamic, and a single species can fit into more than one category. This compound relationship causes various responses to bacteria, including a neuronal response, a nutrition response, and a pathogen response (12) (Fig. 1). The neuronal response, or more specifically the olfactory response, is the detection of bacteria as either food or pathogen and leads to further neuronal and endocrine signaling that can affect behavior and longevity (12, 13). For example, *C. elegans* was shown to preferentially choose food that promotes growth, and this behavior is dependent on the amphid AIY neurons (14). Another study found that tyramine produced by the commensal bacteria *Providencia* can alter *C. elegans* neurosensory behavior that is dependent on the octopamine receptor in ASH neurons (15). There is also evidence that neuroendocrine signaling is involved in recognition of pathogens. For example, *C. elegans* can learn to avoid pathogenic bacteria, such as *Pseudomonas aeruginosa* and *Serratia marcescens*, that are dependent on a serotonin signaling pathway (16) and a neurotransmitter NPR-1, in the case of *P. aeruginosa* (17). Furthermore, signaling between

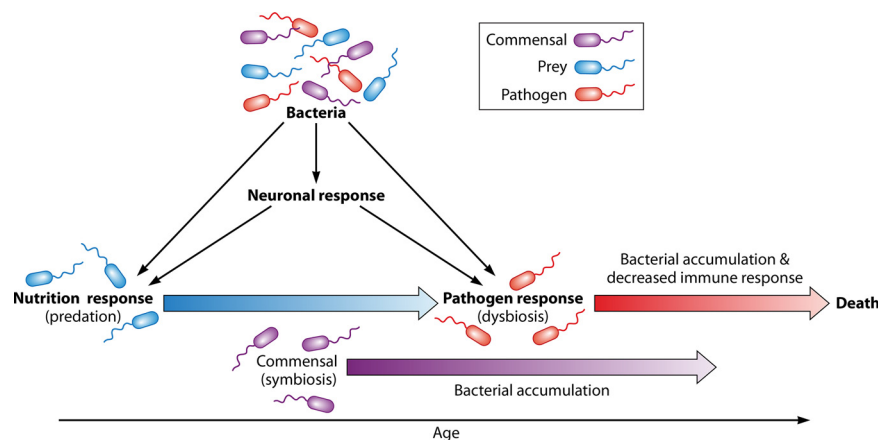


FIG 1 Dynamic interactions between *C. elegans* and bacteria. The *C. elegans* response to bacteria involves interactions among three response types: neuronal, nutritional, and pathogenic. The overlapping shifts from predator-prey to pathogen-host as *C. elegans* age are also shown.

neurons and the intestine plays a role in the immune response to some pathogenic bacteria (18, 19).

The nutrition response to bacteria is based on both nutrient availability and nutrient quality (12, 20). More specifically, *C. elegans* requires metabolically active bacteria for optimal fitness (21). *C. elegans* will arrest development at multiple life cycle points based on the presence of bacteria (20). In addition, some bacteria, including *Bacillus anthracis* and *Bacillus megaterium*, are physically harder to ingest, and *C. elegans* can display lawn avoidance behavior in response to indigestible food (13, 22). *C. elegans* also respond to different bacterially derived nutrients. For example, *C. elegans* requires several essential amino acids from its food for survival, and sensing of these amino acids occurs via the DAF-2/16 insulin-like pathway and the TOR pathway (23). More specifically, providing ethanol plus amino acids to starved L1 worms triggered release from developmental arrest (23). Vitamin B₁₂ is produced by some bacteria and is required for many metabolic reactions. Bacterial food sources lacking vitamin B₁₂ affect gene expression in *C. elegans*, resulting in slowed development and a decrease in fecundity (24, 25).

The pathogen response depends on the activation of several innate immune, defense, and stress pathways that are discussed in detail in the next section. A gradual shift from predator-prey to pathogen-host often occurs as *C. elegans* ages and correlates with accumulation of bacteria within the intestine (26). This change involves three phases: (i) predation, primarily during development, which involves mastication, or breakdown, of bacteria in the pharyngeal grinder, followed by uptake of nutrients from bacterial cell material in the intestine; (ii) symbiosis, primarily in young adults, in which live bacteria that are able to survive pharyngeal grinding inhabit the intestine and provide nutrients to the nematode through metabolism (21, 23–25); and (iii) dysbiosis, primarily in older adults, where bacteria accumulate in the intestine and cause damage to tissues (27) (Fig. 1). These phases are dynamic and can occur simultaneously, with progression through these phases being largely dependent on the food source. In addition, these stages are dependent on several factors, including the efficiency of the pharynx, the ability of bacteria to proliferate in the intestine, and the ability of the host to reduce bacterial accumulation through digestion and/or defense responses.

The neuronal, nutrition, and pathogen responses in *C. elegans* are not mutually exclusive, and the interplay of these responses leads to a multifaceted relationship between *C. elegans* and bacteria. Furthermore, *C. elegans* encounters a multitude of bacteria in the natural environment, with the dynamic and simultaneous responses becoming even more complex. Thus, to understand the complexity of *C. elegans*-bacterium interactions in nature, the most tractable approach is to first simplify these interactions to individual bacterial species and responses. For example, one particularly

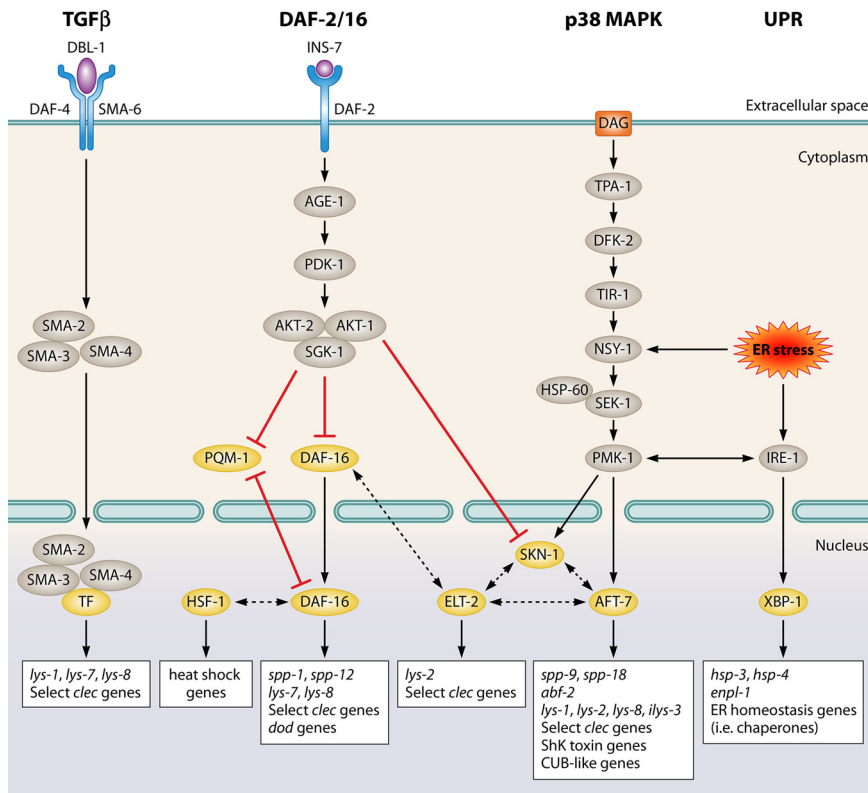


FIG 2 Overview of innate immune and defense pathways: the TGF- β pathway, DAF-2/16 insulin-like pathway, p38 MAPK pathway, and unfolded protein response (UPR) pathway. Purple indicates ligands, blue indicates receptors, gray indicates downstream signaling components, and gold indicates transcription factors. Boxes below pathways identify selected major downstream effectors (65, 80, 82, 83, 86, 88, 93, 96, 99, 104, 109). DAG, diacylglycerol; TF, transcription factor.

interesting and widely studied relationship is *C. elegans*' response to pathogens. Determination of the behavioral and genetic changes *C. elegans* undergoes upon exposure to virulent bacteria will provide insight into the underlying mechanisms of the pathogen response.

C. ELEGANS PATHOGEN RESPONSE

The study of *C. elegans* genes and pathways involved in pathogen response is medically relevant, since many of these genes and pathways are conserved. Since approximately 40% of genes found in *C. elegans* have orthologs in humans, many processes in *C. elegans* are conserved in mammals (28). For example, conservation of innate immune proteins between nematodes, insects, and mammals has revealed important immune factors in *C. elegans*, indicating similarities between innate immunity in *C. elegans* and other metazoa (reviewed in references 29, 30, 30, 31, and 32). However, *C. elegans* does not have dedicated innate immune cells as are found in vertebrates. Therefore, a primary component of its immune response occurs through physical barriers where pathogenesis begins, such as the cuticle and intestine (28).

In general, the innate immune response involves three steps, each of which is carried out by different classes of proteins (28). The first step is the recognition of the pathogen. This step can be species specific, by proteins that recognize particular toxins or bacterial proteins, or more generally respond to pathogen-induced damage. The second step involves signaling pathways which activate downstream proteins and eventually transcription factors (Fig. 2). The final step involves the expression of effector genes that are regulated downstream of signaling pathways, including antimicrobial peptides (AMPs) (28). Because the scope of this review covers bacterial pathogens and bacteria found in the microbiome of *C. elegans*

(primarily the intestine), our emphasis is on the response to bacterial pathogens in the intestine. However, shared and specific innate immune responses to fungi and viruses occur, as has been studied and reviewed extensively (33–35).

Recognition Proteins

Pathogen recognition can involve direct recognition of structural components or secreted proteins of the pathogen, termed microbe-associated molecular patterns (MAMPs), or indirectly via perturbations induced by infection, termed damage-associated molecular patterns (DAMPs). Interestingly, many conserved receptors involved in MAMP recognition, including peptidoglycan recognition proteins, Gram-negative binding proteins, or nucleotide-binding oligomerization domain (NOD)-like receptors, are not found in *C. elegans* (31). Toll-like pathway receptors play a role in immunity in insects and higher-order metazoa (36, 37). The sole Toll-like receptor (TLR) in *C. elegans*, TOL-1, is vital for proper development and function of sensory neurons (38). Although the p38 mitogen-activated protein kinase (MAPK) pathway downstream of the TLR is important for response to many pathogens in *C. elegans*, TOL-1 does not appear to play a role in recognition of or response to *Staphylococcus aureus* or *Pseudomonas aeruginosa* (30, 39, 40). The lack of traditional MAMP recognition mechanisms suggests that pathways involved in innate immunity in *C. elegans* may not be responding directly to the pathogen but instead to cell damage or other stressors that are a consequence of infection.

Exposure to several pathogens, including *B. thuringiensis*, *S. aureus*, and *P. aeruginosa*, leads to intestinal distension (39, 41). Intestinal damage by *B. thuringiensis* is at least partially dependent on pore-forming cytotoxins (42). Singh and Aballay found that bloating of the intestinal lumen in *C. elegans* due to bacterial colonization leads to avoidance of pathogens and activates innate immune signaling (43). The mechanisms for how damage induces downstream defense and immune signaling is largely unclear, but Singh and Aballay found that neuroendocrine signaling through NPR-1 and DAF-7 was required for pathogen avoidance behavior (43).

In addition, reactive oxygen species (ROS) are generated during infection due to mitochondrial damage and upon *Enterococcus faecalis* infection through the NADPH oxidase/dual oxidase BLI-3 (44, 45). ROS then acts as a signal for downstream defense responses, including activation of the DAF-16 and p38 MAPK pathways (reviewed in reference 46).

In other animals, C-type lectin domain (CTLD) containing proteins recognize and bind bacterial cell walls via pathogen recognition receptors (47). In *C. elegans*, two C-type lectin domain proteins, CLEC-39 and CLEC-49, can directly bind *S. marcescens*, and mutations that inactivate these genes cause increased susceptibility to *S. marcescens* infection (48). In another case, mutations in *clec* gene *C54G4.4* results in increased avoidance behavior in response to *B. thuringiensis*, suggesting that it plays a negative role in *B. thuringiensis* lawn avoidance (49). However, CTLD containing proteins appear to play a variety of roles in innate immune response in *C. elegans*, and whether these roles are implicated in pathogen recognition or as downstream effectors is still largely unknown. For example, many *clec* genes are also differentially expressed in response to several pathogens and are regulated by innate immune pathways (50). Therefore, it is unclear whether these proteins play a role in recognizing pathogens or function as downstream antimicrobial peptides.

The lack of conserved receptors suggests that there may be noncanonical mechanisms involved in pathogen recognition. Recent evidence has shown that the nervous system may be involved in upstream signaling that leads to pathogen responses in the intestine. For example, INS-7, an insulin-like ligand that binds to and activates the insulin-like receptor DAF-2 (discussed below), is expressed mainly in neuronal cells and at low levels in the intestine (51). *ins-7* expression is increased upon exposure to *P. aeruginosa* via excretion from dense core vesicles, leading to activation of the DAF-2 pathway and suppression of the DAF-16 transcription factor (18, 52). Mutations in *ins-7* cause

resistance to *P. aeruginosa*, and transgenic expression of INS-7 in neuronal cells alone is able to suppress *ins-7* mutant resistance (18, 52).

In addition, several studies have suggested that neuronal G-protein coupled receptors (GPCRs), such as FSHR-1, NPR-1, NPR-9, and OCTR-1, are required for pathogen resistance and downstream innate immune signaling (reviewed in references 53, 54, and 55). The mechanism for how these neuronal receptors activate the immune response and resistance to pathogens remains unclear, but it is possible that neuronal signaling serves as an intermediate between pathogen recognition and innate immune pathway activation in the intestine.

Signaling Pathways

p38 MAPK pathway. MAPK pathways play significant roles in a variety of cellular responses such as development, differentiation, stress, and apoptosis (56). The p38 MAPK pathway in mammals is activated by cytokines and other stressors in immune cells (57). Analysis of *C. elegans* mutants that enhanced susceptibility to *P. aeruginosa* PA14 led to the identification of conserved components of the p38 MAPK signaling pathway, including SEK-1, NSY-1, and PMK-1 (30). NSY-1, a MAPK kinase kinase (MAPKKK), phosphorylates and activates SEK-1, a MAPKK, which signals via the MAPK PMK-1 (30) (Fig. 2). This pathway acts cell autonomously in the intestine in response to bacterial pathogens and in the epidermis in response to fungal pathogens and wounding (58, 59). In mammalian studies, a variety of transcription factors were identified as direct targets of PMK-1, many of which are conserved in *C. elegans* (60). A forward genetic screen identified AFT-7, ortholog of human AFT2, as being an important p38 MAPK transcription factor regulating transcription of innate immune genes (61). AFT-7 functions as a transcriptional repressor until it is phosphorylated by PMK-1, when it then becomes a transcriptional activator of innate immune genes (61, 62) (Fig. 2).

In *Drosophila*, TLRs and Toll-Interleukin-1 Receptor (TIR) domain adaptor proteins function upstream of p38 MAPK cascades (36). TIR domain adapter proteins specifically bridge the gap between TLR and MAPK signaling, initiating p38 MAPK pathways. In *C. elegans*, the TLR pathway does not appear to play a role in intestinal pathogenesis (40, 63), but the sole TIR domain protein in *C. elegans*, TIR-1, does activate MAPK signaling upstream of PMK-1 (64). The mechanism of TIR-1 activation remains unclear but may be related to diacylglycerol (DAG). TPA-1, a protein kinase C in *C. elegans*, is activated by DAG and phosphorylates DKF-2, a protein kinase D (63, 65) (Fig. 2). Mutations in *dkf-2* result in increased susceptibility to *P. aeruginosa* and *E. faecalis* (65). DKF-2 is required for immune response via p38 MAPK signaling, since overexpression of DKF-2 causes increase in phosphorylated PMK-1, but DKF-2 acts independently of PMK-1 as well (65). The mechanism of activation between DKF-2 and MAPK signaling remains unknown, but DKF-2 may directly phosphorylate TIR-1 (28, 63).

HSP-60, a mitochondrial chaperone and heat shock protein, mediates resistance to *P. aeruginosa* and activates p38 MAPK signaling (66). Further evidence found that HSP-60 binds to and stabilizes SEK-1 in the cytosol, thereby modulating immunity to bacterial pathogens (66).

Pore-forming toxins (PFTs), produced by many human bacterial pathogens, have also been shown to activate MAPK signaling in several organisms, including *C. elegans*, insects, and mammalian cells (reviewed in references 67 and 68). In *C. elegans*, mutations in *pmk-1* and *sek-1* caused increased susceptibility to PFTs produced by *B. thuringiensis* (69). In addition, a proteomic analysis of the response of *C. elegans* to a PFT produced by *S. aureus* revealed the involvement of MAPK pathways, as well as galectins and heat shock proteins (70).

DAF-2/16 insulin-like pathway. The insulin-like signaling pathway was originally identified in *C. elegans* for its role in life span, reproduction, and regulating dauer entry, an alternative life stage that occurs under stressful environmental conditions (71, 72). Mutations in the sole insulin/IGF-1-like receptor *daf-2* in *C. elegans* leads to a near doubling of life span when exposed to many bacteria, including pathogens such as *P. aeruginosa*, *E. faecalis*, *Staphylococcus aureus*, and *Salmonella Typhimurium* (73, 74).

This effect is dependent on the Forkhead transcription factor DAF-16 (73). Other components of this pathway include the phosphoinositide 3-kinase, AGE-1, which is phosphorylated by DAF-2 resulting in the recruitment of kinases PDK-1, AKT-1, AKT-2, and SGK-1. PDK-1 phosphorylates AKT-1, AKT-2, and SGK-1, which then form a complex that phosphorylates DAF-16 (75), resulting in its localization to the cytoplasm, preventing it from entering the nucleus and regulating gene expression (reviewed in reference 76) (Fig. 2). When the pathway is inactivated (e.g., in *daf-2* mutants), DAF-16 localizes to the nucleus, which results in upregulation of genes involved in longevity and stress resistance. PQM-1, another transcription factor downstream of DAF-2, has an antagonistic relationship with DAF-16, where nuclear localization of one transcription factor excludes nuclear localization of the other (77). Under normal conditions PQM-1 is primarily transcriptionally active and targets genes involved in growth, development, and proteostasis, whereas DAF-16 nuclear localization occurs under stress conditions (77, 78).

Although the DAF-2/16 pathway is involved in both longevity and defense responses, linking these responses, it also plays an important role in innate immunity. In fact, many of the genes targeted by DAF-16 have antimicrobial activities, such as lysozymes and detoxification enzymes (79, 80). Heat shock proteins are also downstream components of DAF-2/16 signaling that may play a role in innate immunity. HSF-1, a transcription factor that regulates a subset of heat shock proteins, is required for life span extension of *daf-2* mutants (81) and activation of innate immune genes through DAF-2/16 pathway (82).

In addition, *daf-2* mutant worms display a decrease in bacterial packing, suggesting that regulation of genes by DAF-16 defends against accumulation of bacteria in the intestine (74, 80). Interestingly, one study found that *P. aeruginosa* infection suppresses the activity of DAF-16, which is mediated by several *P. aeruginosa* virulence factors (52). However, this phenomenon appears to be pathogen specific, since exposure to *S. marcescens*, *E. faecalis*, and *S. Typhimurium* induce the expression of several DAF-16 targets that have putative antimicrobial activity (32, 52). In addition, *pqm-1* is required for resistance to *P. aeruginosa*, suggesting PQM-1 targets may confer resistance in that case, rather than DAF-16 targets (83). Finally, recent evidence suggests that aging plays a role in DAF-2/16 regulated immunity, where DAF-16 becomes more important for immune activation and increased longevity later in adulthood (84). Clearly, there is much yet to be discovered regarding the complexities of the DAF-2/16 pathway involvement in pathogen response.

Unfolded protein response pathway. The endoplasmic reticulum (ER) is responsible for protein processing, including folding and posttranslational modifications, and transportation. Perturbations resulting in accumulation of unprocessed proteins activates the unfolded protein response (UPR). The IRE-1-XBP-1 branch of the UPR regulates expression of genes involved in ER homeostasis, leading to defense responses and increased longevity (85). The UPR^{ER} is conserved in animals, as well as some fungi. This pathway involves activation of IRE-1, which leads to the alternative splicing and activation of *xbp-1* mRNA in response to accumulation of unfolded proteins in the ER (Fig. 2). In addition to unfolded proteins, activation of IRE-1-XBP-1 occurs in response to several pathogens likely to protect against ER stress resulting from innate immune responses (86). For example, activation of the UPR^{ER} occurs in response to the pore-forming toxins of *B. thuringiensis*, Cry5, and *ire-1* and *xbp-1* mutations cause hypersensitivity to Cry5 (87). In addition, intestinal infection of *C. elegans* with *P. aeruginosa* induces expression of the heat shock protein HSP-4, a downstream effector of the IRE-1-XBP-1 pathway, and *xbp-1* mutants are susceptible to *P. aeruginosa* and have perturbed ER morphology (86). Both UPR^{ER} signaling and p38 MAPK signaling in response to *P. aeruginosa* are dependent on the oligosaccharyl transferase complex, which mediates protein glycosylation in the ER (88).

In addition to UPR in the ER being involved in innate immunity, another study identified an overlap of upregulated genes in response to mitochondrial stress and infection to *P. aeruginosa* (89). The activation of several of these genes was dependent on

the mitochondrial UPR transcription factor ATFS-1 (89). Therefore, the mitochondrial UPR is also able to protect against pathogens that induce mitochondrial stress by coupling antimicrobial and mitochondrial homeostasis gene expression.

TGF- β pathway. The transforming growth factor β (TGF- β) pathway is involved in development and embryogenesis (90, 91). In mammals, the TGF- β pathway is required for T cell development and differentiation (reviewed in reference 92). In *C. elegans*, mutation of the TGF- β ligand *dbl-1*, causes increased susceptibility to *S. marcescens* and *Salmonella* Typhimurium (32, 74). In addition, several families of antimicrobial peptides, including C-type lectin domain proteins and lysosomes, are regulated by this pathway (90, 93). The canonical DBL-1/TGF- β pathway components include the type I and type II receptors SMA-6 and DAF-4, as well as the Smad signal transducers SMA-2, SMA-3, and SMA-4 (91) (Fig. 2). Expression of several AMPs are dependent on SMA-2 (94). Further details on the involvement of other downstream components are largely unknown; however, mutations in *dbl-1*, *sma-6*, *sma-2*, *sma-3*, and *sma-4* in *C. elegans* cause increase susceptibility to *S. maltophilia* (95).

Pathway cross talk. Each of these pathways are complex in nature, involving a variety of coregulators that have been shown to mediate pathway cross talk. For example, the increase in life span of *daf-2* mutants is dependent on the p38 MAPK pathway, suggesting that it acts in parallel or downstream of DAF-2/16 (96). In addition, many DAF-16 targets contain a GATA motif, termed the DAF-16 associated element (DAE) (80). Two GATA transcription factors, ELT-3, specific to the epidermis, and ELT-2, specific to the intestine, both regulate expression of DAF-16 targets in a tissue-specific manner (97). Conflicting results on whether ELT-2 or ELT-3 can suppress the longevity of *daf-2* mutants suggests that the interplay of these pathways is complex and condition specific (97–99). A meta-analysis of gene expression studies in response to pathogens found that the GATA motif is the most enriched across these studies (100). Mutations in *elt-2* cause increased mortality to *P. aeruginosa*, *S. enterica*, and *E. faecalis* and bacterial distention of the intestine (83, 99), and ELT-2 is required for recovery from *P. aeruginosa* infection (101). ELT-2 activity seems to be specific to pathogen response, since *elt-2* mutants are not susceptible to oxidative stress, heat stress, or cadmium exposure (83). Further, *elt-2* appears to have a strain-specific role in *C. elegans* in response to pathogenic *B. thuringiensis*, since knocking down *elt-2* increased survival in response to one strain of *B. thuringiensis* but decreased survival in response to another (102).

SKN-1, a putative transcription factor involved in stress responses in the intestine, can be phosphorylated by AKT-1, resulting in repression of SKN-1 target gene expression (103) (Fig. 2). Mutations in *skn-1* in a *daf-2* background suppress the longevity phenotype of *daf-2* mutants, suggesting that SKN-1 contributes to increased life span and stress responses (103). To further complicate this response, Block et al. suggest a complex interplay between ELT-2, SKN-1, and ATF-7, where a combination of factors is required for expression of particular immune genes (104) (Fig. 2).

UPR^{ER} activation is also dependent on p38 MAPK signaling (86, 87). However, whereas p38 MAPK signaling functions by reducing accumulation of pathogenic bacteria, it appears that the role of the UPR^{ER} is to protect from ER damage induced by the immune response (86).

Antimicrobial Peptides

Most known antimicrobial peptides have been identified by homology to other AMPs and by expression profiling. Genes that are commonly differentially expressed in response to bacterial pathogens include caenopores, lysozymes, defensin-like AMPs, and C-type lectin domain proteins (reviewed in reference 29). The function of these proteins in *C. elegans* has largely been predicted based on sequence structure, and further functional analyses of these AMPs have not been well studied.

Caenopores, or saposin-like proteins in *C. elegans*, share a structural similarity with saposin-like proteins (SAPLIPS) in protozoa and mammals (105). Although there are 28 saposin-like protein family (SPP) proteins identified in *C. elegans*, only a few have been identified as immune effectors. *spp-9* and *spp-18* are regulated by DKF-2 and are

upregulated by *P. aeruginosa* exposure (65). *spp-1* and *spp-12* are regulated by DAF-16 (106), and mutations in *spp-12* result in increased susceptibility to pathogenic *B. thuringiensis*, whereas mutations in *spp-1* result in decreased susceptibility (107). Further, knockdown of *spp-1* and *spp-5* results in accumulation of bacteria in the intestine (105, 106). Functional analysis of SPP-5 revealed its ability to form pores in and damage bacterial cell walls (105).

Lysozymes are involved in the hydrolysis of peptidoglycan, a major component of bacterial cell walls. Not surprisingly, lysozymes also play a role in digestion (reviewed in reference 29). *C. elegans* lysozyme genes are classified into 10 protist types (*lys-1* to *lys-10*) and 5 invertebrate types (*ilys-1* to *ilys-5*). This is the largest class of genetically diverse lysozymes found in any organism to date (108). All lysozymes studied to date are expressed mainly in the intestine (reviewed in reference 108). Similar to caenopores, many lysozymes are regulated by defense pathways such as TGF- β (*lys-1*, *lys-7*, and *lys-8*), DAF-2/16 (*lys-7* and *lys-8*), and p38 MAPK (*lys-1*, *lys-2*, *lys-8*, and *ilys-3*) (80, 93, 96, 109). Many lysozyme proteins are differentially expressed in response to pathogens (29, 110, 111). In fact, *ilys-1* and *lys-9* are the only lysozymes that are not differentially expressed upon bacterial pathogen exposure (29). In addition, recombinant ILYS-3 exhibits hydrolytic activity and affects cell wall integrity of Gram-positive bacteria, presumably in the pharynx and intestines, where it is expressed (109).

Defensin-like peptides, termed antibacterial factors ABF-1 to ABF-6 in *C. elegans*, were identified based on sequence homology to proteins of *Ascaris suum*, an intestinal parasitic nematode (112). ABF-2 displays *in vitro* antimicrobial activity and knockdown increases pathogen accumulation (106, 112). Regulation of these genes is not well understood, but the M-box motif-class transcription factor HLH-30 appears to be required for *abf-2* expression, as well as several other antimicrobial genes, in response to *S. aureus* (113).

C-type lectin domain (CTLN) proteins were originally characterized for their Ca⁺-dependent carbohydrate binding ability (reviewed in reference 114). However, this superfamily has now grown to include proteins with structural similarities that do not display these functional characteristics (114). In *C. elegans*, CTLN proteins are the most diverse group of effector molecules, containing 283 members, 81% of which contain signal peptides, suggesting they are secreted (50). Although CLEC-39 and CLEC-49 have been shown to directly bind to *S. marcescens* (48), CTLN proteins have not been shown to have antimicrobial activities. Evidence of interaction between several CTLN proteins and LYS-7 could suggest downstream signaling or coregulation of immune partners (115). In addition, CTLN proteins exhibit differential expression in response to a variety of pathogens (29, 39, 93, 96, 111). Mutation or knockdown of many CTLN genes have been analyzed and result in increased and decreased susceptibility to pathogens (39, 48, 49, 116). Although there is a clear role for CTLN proteins in innate immune response, their functional roles in *C. elegans* are not well known (50).

Analyses of expression data from many experiments in response to a variety of pathogens revealed that expression of effectors is taxon specific, but some members of common classes of AMPs, specifically caenopores and lysozymes, are differentially expressed in response to almost all pathogens. Defensin-like peptides, on the other hand, play a less prominent and more species-specific role in response to bacterial pathogens (29).

In summary, innate immune responses to pathogenic bacteria have been widely studied in *C. elegans* and have led to the identification of several major, well-defined pathways involved in defense and immune response and their downstream genes. These studies have revealed that many of the pathways are complex, with cross talk between pathways and the genes they regulate. In addition, although many common innate immune pathways and effectors have been identified, there are differences in responses to different bacteria and even strains of bacteria. For example, only 11% overlap in differentially expressed genes was found in a comparison of the responses to the intestinal pathogens *S. marcescens*, *E. faecalis*, and *Photobacterium luminescens* (117). Other studies found that the *C. elegans* response to pathogenic strains of bacteria of the same species involve both a common response to both strains and different

responses to each of the strains (102, 118). This phenomenon could be due to species- or strain-specific responses to different pathogens or to the ability of bacteria to manipulate different host responses. Therefore, it is essential to study a variety of pathogens, their virulence factors, and responses to these pathogens in order to fully understand the complexity of genetic mechanisms underlying pathogen defense.

BACTERIA FOUND IN THE NATURAL ENVIRONMENT OF *C. ELEGANS*

Previously, many bacteria used to study *C. elegans*-bacterium interactions, including *S. enterica*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*, were chosen because of their convenience in the laboratory setting or their implication in human health rather than their native interactions with *C. elegans* in the environment. However, in order to understand pathogen response and other host-bacterium interactions, it is necessary to focus efforts on species that have been found to interact with *C. elegans* in their native environments. *C. elegans* interact intimately with many bacteria in their environment, and this symbiosis between microbes and host can lead to adaptation and evolution of both species. For example, a study that coevolved *C. elegans* with a natural pathogen, *B. thuringiensis*, found that the worms became more resistant to killing by *B. thuringiensis* when grown together over many generations and this led to an increase in genetic changes in both bacteria and host compared to bacteria and host maintained individually (119). While the previous example refers to antagonistic coevolution, where the organisms evolve as separate entities with opposing goals, coevolution of bacteria and host can also occur in mutualistic relationships, where increased fitness under favorable conditions leads to genetic changes. This later idea supports the hologenome theory, that suggests the genetic information of microbes and their host act as one unit of selection in evolution (120). A key feature of the hologenome theory is transmission of microbes through host generations, and it is important to note that to date, there is no evidence of vertical transmission of microbes in *C. elegans* across generations. However, because of the essential nature of the *C. elegans*-microbiome relationship, the effect of microbes on *C. elegans* fitness, and the ability of these interactions to lead to genetic changes in both host and microbes, it is intriguing to consider the host and microbiome as one unit.

Characterization of the Native Microbiome of *C. elegans*

Several groups recently determined the bacterial repertoire associated with substrates where *C. elegans* were found as well as within the worm microbiome (3, 121, 122). Berg et al. created artificial microcosms to simulate natural environments using soil supplemented with various produce, including plants and fruit, and then subjected wild-type worms to these microcosms for several days before characterizing bacterial composition within both worms and soils (121). Samuel et al. identified the bacterial community associated with substrates where *C. elegans* were collected (122). Dirksen et al. combined the approaches of the previous studies by analyzing the microbial communities in the native habitats of *Caenorhabditis* species, as well as the microbiome of sampled nematodes from those habitats (3). These researchers also performed "lab enrichment" on some worms, in which the microbial communities of worms found in the natural environment were determined after growing them on *E. coli* OP50 for several weeks in order to determine bacterial species that persist in the worm and do not simply pass through the intestine. In all three studies, researchers found highly diverse microbiomes within the substrates and within *C. elegans*, but diversity within the worms was lower than within the substrates (3, 121, 123). In addition, worm microbiomes, regardless of worm origin or experiment, were more similar to each other than to the microbes associated with the substrates (123), suggesting some constraints on microbiome assembly, either by competition of bacteria within the intestine or selection of bacteria by the worm. Furthermore, a recent study found that many of the bacteria found in worm microbiomes are not found in their corresponding substrates, suggesting that these bacteria can persist in the worm microbiome for some time (124).

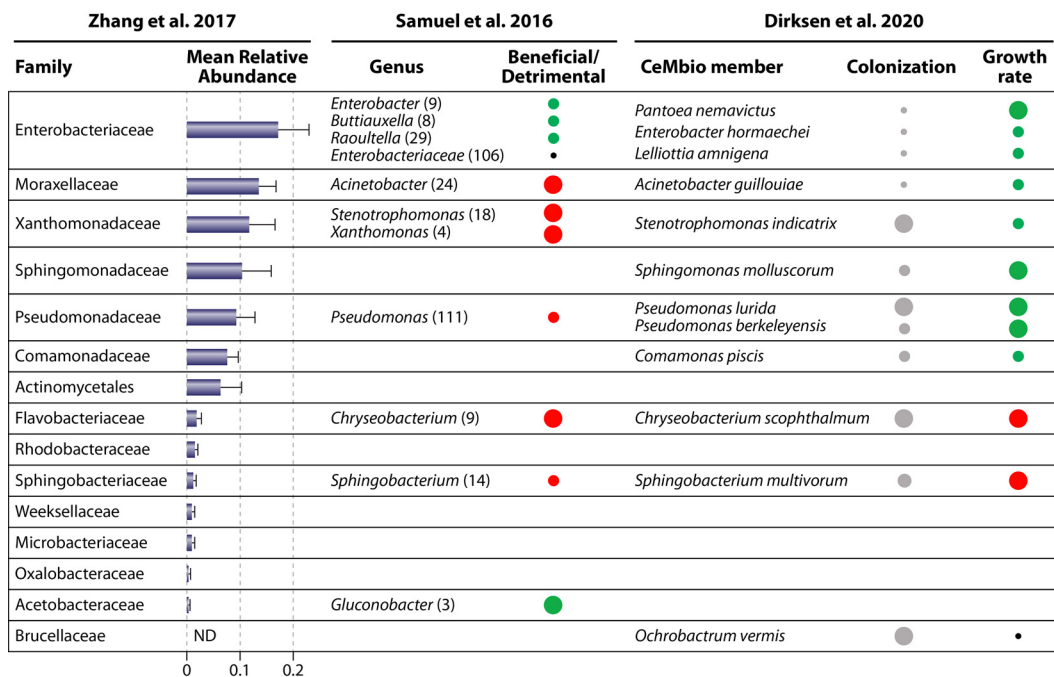


FIG 3 Functional analysis of *C. elegans* microbiome members. Functional analysis of bacteria representative of common families identified across *C. elegans* microbiomes (102) and CeMbio members (104). Mean relative abundance indicates the relative abundance of each bacterial family across 23 *C. elegans* isolates in samples from the natural environment (102). The beneficial/detrimental status was determined for each isolate (the number of isolates for each genus is indicated in parentheses) based on growth rate and induction of stress reporters of *C. elegans* upon exposure to each isolate (101). Colonization was calculated by analysis of the CFU in individual *C. elegans* intestines grown on each bacteria at 1 and 3 days of adulthood. Growth rate indicates time to adulthood of *C. elegans* upon exposure to each bacteria compared to *E. coli* OP50 (104). The dot size represents the value for each functional assay, green indicates the beneficial/higher growth rate, red indicates detrimental/lower growth rate, and black indicates similarity to OP50. ND, no data. See references 123 (Zhang et al. 2017), 122 (Samuel et al. 2016), and 125 (Dirksen et al. 2020).

The similarity of microbial repertoires across experiments resulted in the identification of 14 bacterial families present in all natural worm microbiomes: *Xanthomonadaceae*, *Pseudomonadaceae*, *Moraxellaceae*, *Enterobacteriaceae*, *Oxalobacteraceae*, *Comamonadaceae*, *Sphingomonadaceae*, *Acetobacteraceae*, *Rhodobacteriaceae*, *Sphingobacteriaceae*, *Weeksellaceae*, *Flavobacteriaceae*, *Microbacteriaceae*, and *Actinomycetales* (123) (Fig. 3). Interestingly, many of these families are also found in higher abundance within the worm microbiome than within the substrate, suggesting they can colonize the intestine (3, 123). Recently, a collaborative consortium has established a 12-species experimental microbiome, termed CeMbio, based on common, representative strains found in *C. elegans* microbiomes to serve as a tool for future work on microbiome interactions and assembly (125).

Functional Analysis of Microbiota

Previous studies have determined that the microbiome plays a role in life span, aging, and disease. In humans, gut microbiome dysbiosis is linked to several diseases, including colorectal cancer, diarrheal diseases, liver diseases, and diabetes (reviewed in reference 126). In addition, microbiota of elderly people correlated with health markers such as frailty and nutritional status (127).

In *C. elegans*, microbiota are capable of a variety of metabolic functions, and collectively is able to produce all necessary metabolites needed for *C. elegans*' survival (128). Certain metabolites, such as vitamin B₁₂, are only produced by some bacteria, such as *Ochrobactrum* and *Pseudomonas*, which could affect nematode fitness (125, 128). Several studies have directly analyzed how bacteria in *C. elegans*' natural environment affect their health. Samuel et al. determined the growth rate and induction of stress reporters in response to 565 bacterial isolates found in the natural habitat of *C. elegans*

and used these data to characterize each isolate as either “beneficial,” “detrimental,” or “intermediate” (122) (Fig. 3). Of all 565 isolates, approximately 40% were beneficial, 40% were intermediate, and 20% were detrimental. A range of effects across bacterial genera exists, suggesting differences between strains of closely related bacteria could account for differences in their impact on *C. elegans* (122). Dirksen et al. also characterized the effects of strains from *C. elegans*’ microbiome on aspects of its life history and found that a majority (67%) of the 24 strains tested decreased population size of *C. elegans* (3). Most of these detrimental isolates belonged to *Actinobacteridae*, *Bacilli*, *Flavobacteriia*, and *Sphingobacteriia* (3). Of the 12 strains chosen for the CeMbio experimental microbiome, all isolates are able to colonize the intestine, but only two slow the growth of *C. elegans* (125) (Fig. 3).

Utilizing Microbiome Bacteria To Understand Natural Host-Pathogen Interactions

Based on these functional analyses, it is clear that *C. elegans* encounter bacteria in their microbiome and environment that are pathogenic, but little is known about the molecular mechanisms of these pathogens or their interactions with other species. Samuel et al. found that mixing detrimental strains with beneficial strains found in the environment resulted in protective functions between some species, but not others (122). When mixed in a culture with other core microbiome components, the detrimental effects seen by individual isolates is mitigated, and these mixed cultures are more beneficial for *C. elegans* (3, 125, 129). This suggests an interplay between species within the microbiome and their interactions with *C. elegans* (i.e., an innate immune response). For example, Montalvo-Katz et al. found that two species found in compost samples, *Pseudomonas mendocina* and *Bacillus megaterium*, both conferred resistance to *P. aeruginosa* PA14, and the protective function of *P. mendocina* appears to be an early activation of p38 signaling that primes the immune response to PA14 (130). In addition, several strains of *Enterobacter cloacae* isolated from *C. elegans* protected *C. elegans* upon *E. faecalis* infection but did not protect *C. briggsae* (131). There is also evidence for interkingdom interactions within the microbiome, since several strains of *Pseudomonas* displayed antifungal effects *in vitro*, and one *Pseudomonas* strain decreased the susceptibility of *C. elegans* to fungal infection (3).

These studies have determined that bacteria found in the environment are able to play protective roles in response to clinical pathogens but suggests that they have similar functions in *C. elegans* natural habitat and within the microbiome. Interestingly, innate immune response does occur in *C. elegans* upon exposure to compost and associated bacteria (129), suggesting that *C. elegans* is defending against pathogens in its natural environment. This activated immune response could explain the lack of detrimental effects of a mixed microbiome culture, since *C. elegans* is able to combat the diluted effects of these pathogens. However, few studies have begun to characterize and molecularly analyze these natural pathogens. In one early study, two strains of *Leucobacter* were isolated from a *Caenorhabditis* worm, and each strain displayed distinct, diseased phenotypes in *C. elegans* (132). Interestingly, worms that were resistant to one strain of *Leucobacter* were hypersusceptible to the other strain and vice versa, suggesting a trade-off in susceptibility to these strains (132). Page et al. found that two environmental isolates of *Chryseobacterium* are detrimental to *C. elegans* (133). These researchers determined that these bacteria are able to break down the pharyngeal wall, and comparative genomics revealed differences in proteases between pathogenic and nonpathogenic strains that could be responsible for the virulence of these strains (133).

Stenotrophomonas is another mildly detrimental bacterium found in high abundance within *C. elegans* microbiomes (3, 122). A recent comparison of gene expression in *C. elegans* in response to two pathogenic environmental isolates of *S. maltophilia* and one nonpathogenic clinical isolate of *S. maltophilia* showed that *C. elegans* exhibit both common and strain-specific responses to *S. maltophilia* strains of various pathogenicities (118). One of these *S. maltophilia* strains, JCMS, was isolated in association with soil nematodes and evades the DAF-2/16 pathway (95). Another study looking at the transcriptional response to two pathogenic strains of *B. thuringiensis* also found

that both common and strain-specific responses are displayed by *C. elegans* (102). In that study, the transcription factor ELT-2 was found to have contrasting effects on the two *B. thuringiensis* strains (102). Further analyses to understand the role of these pathogens within the microbiome will uncover a more representative role of these bacteria in their natural environment and their interaction with *C. elegans*.

CONCLUSIONS AND FUTURE DIRECTIONS

C. elegans is a major model organism used to uncover gene and protein functions and relate them to homologous functions in other organisms. However, despite decades of research, 40% of gene functions in *C. elegans* are still unknown (134). One reason for this is that the use of artificial laboratory settings to study *C. elegans* limits responses to environmental stimuli. *C. elegans* and bacteria engage in intimate relationships in their natural environment, so utilizing these interactions to understand gene function in *C. elegans* has already elucidated novel genetic mechanisms (reviewed in reference 134). Before high-throughput characterization of the microbiome, identification of organisms in the natural habitat of *C. elegans* through simple isolation has encouraged use of these species in further studies (118, 130, 132, 133). Further, recent extensive characterization of the microbial communities within *C. elegans*, as well as in the surrounding environment, has expanded our understanding of natural *C. elegans*-bacterium interactions and can be used for further study of microbiome interactions (3, 121, 122). However, because studies of *C. elegans* ecological interactions are only relatively recent, there is still much to be uncovered.

Of particular interest are interactions between *C. elegans* and detrimental bacteria, since this could shed light on novel innate immune responses in the host and bacterial virulence mechanisms. As highlighted in this review, much has been learned through studies with medically relevant pathogens. Studying interactions with naturally encountered pathogens can provide further insight into how *C. elegans* respond to pathogen infection, such as:

- To what extent do the major *C. elegans* innate immune pathways function to combat natural pathogens? Which major pathways play the largest role in natural settings?
- Do distinct effectors function in response to different natural pathogens? Recent work has suggested that *C. elegans* deploys both common and strain-specific responses to different bacterial taxa and strains of the same taxa (102, 118, 135). Does this hold for other natural pathogens?
- Do the responses to novel natural pathogens help to elucidate functions of genes whose function has not yet been described?
- What virulence mechanisms are employed by natural pathogens in their natural hosts? Do taxa related to natural pathogens employ similar or different virulence mechanisms?

Development of the 12-species experimental microbiome, CeMbio, can be used as a universal tool within the community and allows for direct manipulation of the microbiome (125). This advance opens the door to investigate new questions that are intractable in other study systems. Establishment and initial characterization of CeMbio has laid the groundwork for further studies to explore the role of various microbiome members within the microbial community, as well as on the host (125). Manipulation of the CeMbio microbiome through experiments that omit, add, or substitute bacteria within the community will help elucidate the roles of microbial taxa within the microbiome and the host:

- Do microbiome components impact host fitness directly or through interactions with other microbes (i.e., competition, mutualism, pathogenesis, etc. between microbes)? For example, some components might function to limit the growth of other, perhaps more detrimental, microbes (125).
- Do the roles fulfilled by microbiome components follow phylogenetic relationships or are they more related by the functions they perform within the community?

- What role does the host play on shaping these microbial interactions?

Finally, we find detrimental bacteria in relatively high abundance in natural microbiomes (3, 122). Studying these natural pathogens both individually and as components of experimental microbiomes, we can address the following:

- How does the incorporation of microbes of various pathogenicities into the microbiome affect the host? How does this affect the microbiome?
- Are microbes that are detrimental to *C. elegans* also detrimental to other microbes?
- What role do natural pathogens play in the microbiome?

As highlighted here, *C. elegans* has been used for studies involving innate immunity and bacterial virulence for many years, but only recently have we begun to intersect this research with microbiome composition and function. Future research exploring the questions above promises to further our understanding of gene function, host-microbiome interactions, and the evolution of innate immunity in nematodes and other organisms.

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