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Host-microbe interactions and the behavior of *Caenorhabditis elegans*

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ABSTRACT

Microbes are ubiquitous in the natural environment of *Caenorhabditis elegans*. Bacteria serve as a food source for *C. elegans* but may also cause infection in the nematode host. The sensory nervous system of *C. elegans* detects diverse microbial molecules, ranging from metabolites produced by broad classes of bacteria to molecules synthesized by specific strains of bacteria. Innate recognition through chemosensation of bacterial metabolites or mechanosensation of bacteria can induce immediate behavioral responses. The ingestion of nutritive or pathogenic bacteria can modulate internal states that underlie long-lasting behavioral changes. Ingestion of nutritive bacteria leads to learned attraction and exploitation of the bacterial food source. Infection, which is accompanied by activation of innate immunity, stress responses, and host damage, leads to the development of aversive behavior. The integration of a multitude of microbial sensory cues in the environment is shaped by experience and context. Genetic, chemical, and neuronal studies of *C. elegans* behavior in the presence of bacteria have defined neural circuits and neuromodulatory systems that shape innate and learned behavioral responses to microbial cues. These studies have revealed the profound influence that host-microbe interactions have in governing the behavior of this simple animal host.

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Microbiology

Caenorhabditis elegans lives in a microbe-rich environment that defines the ecology and has shaped the evolution of the organism (Schulenburg & Félix, 2017). The basic dichotomy for *C. elegans* in its interactions with this microbial community is that bacteria are an essential source of nutrition but may also be pathogenic and cause infection and death. Ecological survey and sequence analysis of bacteria isolated from the natural environment from which *C. elegans* are isolated has revealed a community of *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (Samuel, Rowedder, Braendle, Félix, & Ruvkun, 2016), with notable enrichment of alpha-*Proteobacteria* genera such as *Ochrobactrum* and *Pseudomonas* in close association with *C. elegans*, likely colonizing the intestine (Dirksen *et al.*, 2016). These associated bacteria may benefit the *C. elegans* host as a food source and in other ways. At the same time, the characterization of *C. elegans* strains in the wild has uncovered a broad range of pathogenic microorganisms, including bacteria, fungi, and viruses, which can cause sickness and death (Schulenburg & Félix, 2017).

The experimental study of *C. elegans* has typically involved laboratory cultivation on monoaxenic lawns of *Escherichia coli* OP50 seeded on agar plates supplemented with cholesterol (Brenner, 1974). Genetic and metabolomic characterization of alternative bacterial food sources for *C. elegans*, such as *Comamonas, Bacillus subtilis* and mutants of *E. coli*, has defined conserved requirements for micronutrients (Qi, Kniazeva, & Han, 2017; Watson *et al.*, 2014), novel mechanisms of host co-option of bacterial siderophores for iron acquisition (Qi & Han, 2018), and metabolic determinants of bacteria that can influence complex phenotypes such as lifespan (Han *et al.*, 2017; Qi & Han, 2018; Saiki *et al.*, 2008; Virk *et al.*, 2012). Semi-defined axenic media has been developed for growth and cultivation of *C. elegans,* but live bacteria support optimal growth and development (Lenaerts, Walker, Van Hoorebeke, Gems, & Vanfleteren, 2008).

Diverse bacteria are pathogenic to *C. elegans*. The human opportunistic pathogen *Pseudomonas aeruginosa*, which resides in soil and water, was shown to infect an evolutionarily diverse range of hosts, including *C. elegans* (Rahme *et al.*, 1995; Tan, Mahajan-Miklos, & Ausubel, 1999). *Bacillus thurigiensis* produces a crystal pore-forming toxin that is highly toxic to *C. elegans* upon ingestion (Marroquin, Elyassnia, Griffitts, Feitelson, & Aroian, 2000) and has attracted interest as a potential biocontrol method for nematodes that are pathogenic to animals and plants. The coryneform bacterium *Microbacterium nematophilum* causes a

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distinct mode of infection and host response through adherence to the rectal and post-anal cuticle, likely reflecting a natural infection of *C. elegans* (Hodgkin, Kuwabara, & Corneliussen, 2000). A broad range of bacterial species, including environmental isolates and human pathogens, exhibit increased virulence towards *C. elegans*, compared with the survival of *C. elegans* on *E. coli* OP50 (Couillault & Ewbank, 2002). Experimental conditions and host status play an important role when considering the pathogenicity of a bacterial strain. As an illustration of this point, even *E. coli* OP50 can be considered as pathogenic in the presence of richer growth media or when colonizing aging or feeding-defective animals (Garigan *et al.*, 2002; Garsin *et al.*, 2001; Herndon *et al.*, 2002; Kumar *et al.*, 2019). Pathogenicity can also be altered by co-ingestion of non-pathogenic bacteria (Montalvo-Katz, Huang, Appel, Berg, & Shapira, 2013; Samuel *et al.*, 2016). For example, factors secreted by *Enterococcus faecium* are protective against *Salmonella* pathogenesis (Rangan *et al.*, 2016). The range of bacteria that can be pathogenic to *C. elegans* in the laboratory setting has been expanded by molecular engineering, for example by having *E. coli* strains carry plasmids expressing specific toxins of *B. thurigiensis* (Wei *et al.*, 2003) or *P. aeruginosa* (McEwan, Kirienko, & Ausubel, 2012), or even RNAi clones targeting essential *C. elegans* genes (Kamath *et al.*, 2003).

Innate recognition

The innate recognition of bacteria by *C. elegans* is evident from observations of behavioral phenotypes of *C. elegans* in the presence of bacteria and its metabolites (Figure 1). Behaviors such as feeding (Avery & Horvitz, 1990), defecation (Thomas, 1990), egg-laying (Trent, Tsuing, & Horvitz, 1983), and locomotion (Sawin, Ranganathan, & Horvitz, 2000) are affected by the presence of bacteria. Insights into the molecular cues sensed by *C. elegans* have come from the characterization of chemotaxis behaviors of *C. elegans,* which demonstrate that *C. elegans* propagated in the laboratory are attracted to a broad range of volatile organic molecules that are produced by bacterial metabolism and may serve as food cues (Bargmann, Hartwieg, & Horvitz, 1993; Bargmann & Horvitz, 1991; Ward, 1973). The genome of *C. elegans* encodes an expanded family of over 1000 chemoreceptor genes (Bargmann, 1998). Genetic analysis of chemotactic responses to diacetyl, which is produced by many bacteria, identified ODR-10 as a chemoreceptor for diacetyl (Sengupta, Chou, & Bargmann, 1996). The chemical characterization of natural bacterial isolates that may serve as food for *C. elegans* in the wild has further revealed a number of volatile organic compounds that attract *C. elegans* (Worthy *et al.*, 2018a). Bacterial food cues modulate dauer entry and exit (Golden & Riddle, 1984), and fatty acids derived from bacteria cause dauer larvae, which do not ingest bacteria, to exit dauer diapause (Kaul *et al.*, 2014). In addition to chemosensation, *C. elegans* also detect the presence of bacteria through mechanosensation. Four classes of ciliated dopaminergic neurons are required for an innate slowing response that *C. elegans* display upon encountering a bacterial food source (Sawin *et al.*, 2000). These neurons express mechanically-sensitive ion channels, display neuronal responses to mechanical forces, and also drive slowing in response to microbeads that are similar in size to bacteria (Kang, Gao, Schafer, Xie, & Xu, 2010; Sawin *et al.*, 2000).

Gradients of molecular oxygen and carbon dioxide are generated by bacterial metabolism, and *C. elegans* exhibits robust detection and behavioral responses to these gases (Bretscher, Busch, & de Bono, 2008, Bretscher *et al.*, 2011; Chang, Chronis, Karow, Marletta, & Bargmann, 2006;

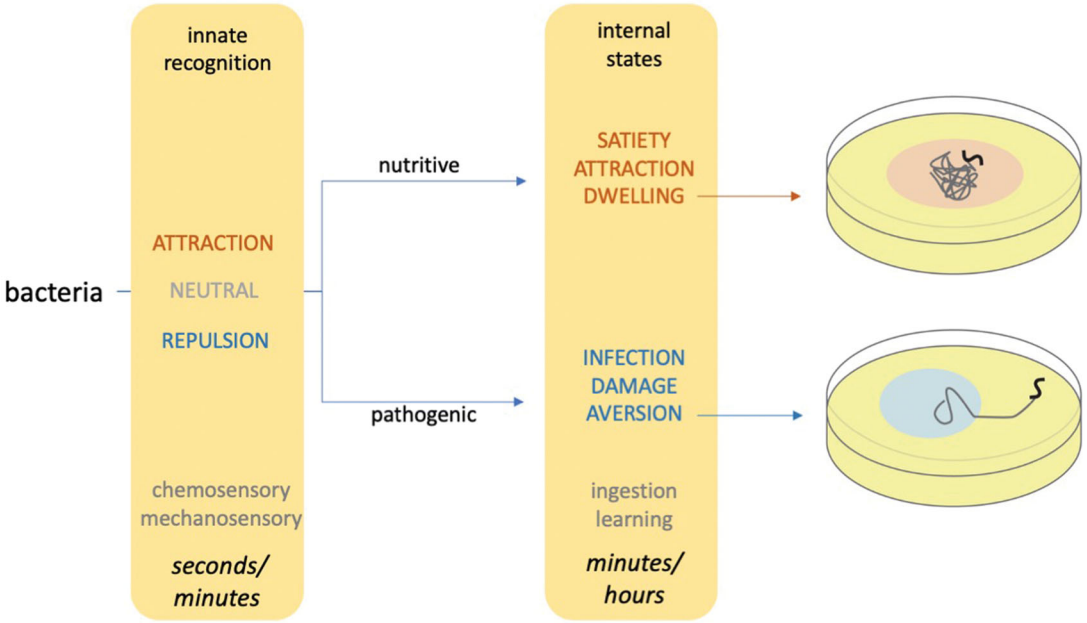

Figure 1. *C. elegans* behavioral responses to bacteria unfold over multiple time scales. *C. elegans* consume diverse bacteria that differ in their nutritive qualities and their pathogenicity. Innate recognition of bacteria allows animals to generate rapid behavioral responses to bacterial odors and textures (left). After bacteria are ingested, animals undergo internal state changes that underlie long-lasting behavioral changes (middle). These long-lasting changes include alterations in their foraging strategies (right) and learned changes in bacterial preference.

Cheung, Cohen, Rogers, Albayram, & de Bono, 2005; Gray *et al.*, 2004; Hallem & Sternberg, 2008), which can drive the behavior of *C. elegans* in a microbial environment. *C. elegans* detects molecular oxygen with cytosolic guanylyl cyclase receptors expressed in the AQR, PQR, URX, and BAG neurons (Cheung *et al.*, 2005; Gray *et al.*, 2004; Zimmer *et al.*, 2009) and exhibits a peaked preference for oxygen levels around ∼8% in the absence of bacterial food (Gray *et al.*, 2004). Multiple sensory neurons including BAG and AFD are responsive to carbon dioxide levels (Bretscher *et al.*, 2008, 2011; Hallem & Sternberg, 2008), which may also serve as a cue that modulates behavioral responses to bacterial density. Ambient laboratory oxygen levels (∼21%) would be expected to drive *C. elegans* into a bacterial lawn, where oxygen levels can be substantially lower (Gray *et al.*, 2004; Reddy, Hunter, Bhatla, Newman, & Kim, 2011). However, the wild-type laboratory strain, N2, exhibits aerotaxis behavior that is altered in the presence of bacteria, such that the peaked preference for ∼8% O_2 levels is lost, with an increased attraction to higher levels of molecular oxygen. The altered aerotaxis behavior of N2 *C. elegans* in the presence of bacteria is caused by allele differences in the *npr-1* gene, encoding a neuropeptide receptor (Chang *et al.*, 2006; Cheung *et al.*, 2005; de Bono & Bargmann, 1998; Gray *et al.*, 2004), and the *glb-5* gene, which encodes a neural globin (McGrath *et al.*, 2009; Persson *et al.*, 2009). The N2 strain carries a laboratory-acquired neomorphic 215 V allele of *npr-1*, whereas natural isolates of *C. elegans* carry an ancestral 215 F allele with reduced NPR-1 activity (de Bono & Bargmann, 1998; McGrath *et al.*, 2009). Bacteria induce NPR-1-dependent differences in not only aerotaxis behavior, but a number of different behavioral phenotypes including differences in CO_2 avoidance (Bretscher *et al.*, 2008; Hallem & Sternberg, 2008), aggregation in feeding behavior, and roaming versus dwelling locomotion on bacterial lawns (Cheung *et al.*, 2005; Gray *et al.*, 2004). Altered behavioral responses to gradients of molecular oxygen also underlie the NPR-1-dependent avoidance of *P. aeruginosa* lawns (Reddy, Andersen, Kruglyak, & Kim, 2009). Of note, behaviors dependent on NPR-1 in the N2 strain can be attenuated in the presence of mucoid Gram-negative bacterial strains, which have an altered surface due to the overproduction of an exopolysaccharide coat (Reddy *et al.*, 2011). The pervasive influence that bacteria can have on the behavior of *C. elegans* is underscored by the modulation of behavior by NPR-1-dependent signaling circuitry (Macosko *et al.*, 2009).

In addition to the innate responsiveness to molecules and mechanical cues that are produced by many bacteria, *C. elegans* also detects molecules that enable discrimination among specific bacterial species. Food choice assays and microdroplet-based assays have enabled the sensitive monitoring of head-turning behaviors to bacterial odors, which demonstrated that *C. elegans* can innately distinguish between odors emanating from *E. coli* OP50 and *P. aeruginosa* PA14 (Ha *et al.*, 2010). *C. elegans* also exhibit chemotactic responses to autoinducer molecules produced by *P. aeruginosa* and *Vibrio cholerae* that mediate quorum sensing (Beale, Li, Tan, & Rumbaugh, 2006; Werner, Perez, Ghosh, Semmelhack, & Bassler, 2014). Innate recognition of pathogenic *Serratia marcescens* has been shown to include the detection of volatile cues (Glater, Rockman, & Bargmann, 2014; Worthy, Rojas, Taylor, & Glater, 2018b), as well as Serrawettin W2, a surfactant-like lipodepsipentapeptide, which acts as a chemical repellent of *C. elegans* (Pradel *et al.*, 2007).

In vivo calcium imaging methods that directly measure activation of individual sensory neurons in response to bacterial cues has corroborated observations from behavioral assays and enabled the dissection of neuronal circuitry mediating innate recognition and preference. The activation of AWB and AWC chemosensory neurons, which mediate repulsive and attractive responses to volatile chemicals, was demonstrated and defined components of a circuit that mediates innate preference for *P. aeruginosa* PA14-conditioned media over *E. coli* OP50-conditioned media (Ha *et al.*, 2010). Activation of the ASH neurons, in response to surfactant-like dodecanoic acid that is secreted by *Streptomyces*, was observed to be dependent on the SRB-6 olfactory receptor, defining a molecular mechanism for innate recognition of a repellant that induces immediate avoidance behavior (Tran *et al.*, 2017). Comprehensive analyses of sensory responses in *C. elegans* have revealed at least 10 classes of sensory neurons that respond to nutritive *E. coli* supernatants (Zaslaver *et al.*, 2015). The ASJ neurons were shown to be activated by both *E. coli* supernatants (Zaslaver *et al.*, 2015), as well as nitric oxide (Hao *et al.*, 2018) and phenazine-1-carboxamide (Meisel, Panda, Mahanti, Schroeder, & Kim, 2014), which are produced by *P. aeruginosa* PA14.

Neuronal activation is accompanied by the rapid induction of gene transcription (Yap & Greenberg, 2018), and ASJ activation is accompanied by the induction of *daf-7*/TGF-beta transcription in the ASJ neurons within six minutes of exposure to *P. aeruginosa* and specifically its secondary metabolites, phenazine-1-carboxamide and the siderophore pyochelin (Meisel *et al.*, 2014). DAF-7 is necessary for *C. elegans* avoidance of *P. aeruginosa,* and increased DAF-7 activity in the ASJ neurons induced by innate recognition of *P. aeruginosa* metabolites alters aerotaxis behavior to promote avoidance behavior (Meisel *et al.*, 2014). Genetic analysis has defined the involvement of distinct cyclic-GMP-dependent signaling pathways in the ASJ neurons that couple the recognition of *P. aeruginosa* metabolites to the selective transcription of *daf-7* (Park, Meisel, & Kim, 2020). The modulation of avoidance behavior induced by infection (discussed further below) by DAF-7 in response to innate recognition in the ASJ neurons of *P. aeruginosa* metabolites contrasts with the immediate attractive or repulsive responses that are induced by activation of the ASH neurons (Tran *et al.*, 2017).

The characterization of *C. elegans* sensory responses to microbe-derived molecules in its environment suggest these host animals recognize molecules produced by broad classes of bacteria as well as molecules that are highly specific to bacterial strains. Many molecules are attractive to *C. elegans,* as might be anticipated for cues of bacterial food, but the

diverse repertoire of molecules that can elicit sensory responses also suggests the ability to recognize and respond to specific strains of bacteria, depending on the physiological context.

Bacterial food and internal state

The ingestion of nutritive bacteria can influence the subsequent behavior of *C. elegans* (Figure 1). Associative learning paradigms that involve pairing either the presence or absence of nutritive, non-pathogenic *E. coli* bacteria with environmental conditions such as temperature result in a learned preference for the conditions associated with the fed, not starved, state (Hedgecock & Russell, 1975; Mori & Ohshima, 1995; Torayama, Ishihara, & Katsura, 2007). In addition, *C. elegans* that are fed non-pathogenic bacteria with a wide range of nutritive qualities – 'good food' versus 'bad food' that differ in their ease of ingestion and metabolic factors – can also exhibit a learned change in preference that is calibrated for better versus poorer food (Shtonda & Avery, 2006). These learned changes in behavior can last from minutes to days, depending on the conditioning protocol, thus reflecting a stable change in preference.

The foraging behaviors of *C. elegans* are also strongly influenced by past and present bacterial feeding conditions. While feeding on *E. coli* OP50, *C. elegans* alternate between active 'roaming' states and inactive 'dwelling' states in which they either explore or exploit their food source, respectively (Fujiwara, Sengupta, & McIntire, 2002). The proportion of time that *C. elegans* spends in roaming versus dwelling states is controlled by satiety levels, chemosensory inputs, and internal sensing of bacterial food ingestion (Ben Arous, Laffont, & Chatenay, 2009; Fujiwara *et al.*, 2002; Shtonda & Avery, 2006). Animals that have been deprived of bacterial food exhibit an 'enhanced slowing response' upon encountering a bacterial lawn, suggesting an important role for satiety state in these modes of food exploration (Sawin *et al.*, 2000). The acute ingestion of bacterial food also plays a central role in food exploration: animals that are exposed to a lawn of bacteria rendered largely inedible as a result of pharmacological treatment, spend almost all of their time in the roaming state (Ben Arous *et al.*, 2009).

Serotonin signaling has been shown to have a key role in mediating the effects of the ingestion of nutritive bacteria on behavior. Serotonin biosynthesis and the serotonergic NSM neurons were shown to be required for the enhanced slowing response (Sawin *et al.*, 2000) and for maintenance of the dwelling state (Flavell *et al.*, 2013). NSM neurons extend a sensory dendrite to the surface of the pharyngeal lumen and are acutely activated upon bacterial food ingestion (Rhoades *et al.*, 2019). Feeding-dependent NSM activation requires the acid-sensing ion channels DEL-3 and DEL-7 that localize to the NSM sensory dendrite and appear to mediate detection of a heat-stable bacterial component, connecting microbial recognition in the *C. elegans* alimentary canal to the modification of feeding behaviors.

While serotonin appears to promote states of slow locomotion, the neuropeptide pigment dispersing factor (PDF) is required for sustained roaming states (Flavell *et al.*, 2013) and for mate search behaviors (Barrios, Ghosh, Fang, Emmons, & Barr, 2012), in which male *C. elegans* leave a bacterial food source to search for a mating partner (Lipton, Kleemann, Ghosh, Lints, & Emmons, 2004). PDF signaling appears to be antagonized by serotonin release (Flavell *et al.*, 2013) and, in males, it promotes expression of *daf-7* in ASJ neurons (Hilbert & Kim, 2018), which promotes mate searching behavior (Hilbert & Kim, 2017). *daf-7* expression in ASJ is also positively regulated by satiety, thus allowing it to serve as a signal that integrates multiple internal cues to promote exploration. Increased motion through bacterial lawns during roaming states also activates dopamine signaling via the dopaminergic PDE neurons that appear to integrate the presence of food with the animal's own motion (Cermak *et al.*, 2020). Activation of dopaminergic neurons during roaming elevates egg-laying rates, allowing animals disperse their eggs across bacterial food sources.

Metabolic changes arising from bacterial food ingestion also influence foraging. Rictor/TORC2 signaling in intestinal cells promotes *daf-7* expression in ASI neurons and elevates roaming behavior in a PDF-dependent manner (O'Donnell *et al.*, 2018). The ETS-5 transcription factor functions in ASG and BAG sensory neurons to limit intestinal fat storage and promote PDF-dependent roaming (Juozaityte *et al.*, 2017). Interestingly, the effects of *ets-5* on roaming can be reversed by altering intestinal fat storage. In addition to ASG and BAG, the URX and ASI sensory neurons also impact fat storage (Palamiuc *et al.*, 2017; Witham *et al.*, 2016). URX has also been shown to detect the mobilization of peripheral fat stores, suggesting bi-directional communication (Witham *et al.*, 2016). Genetic analysis of the sterol response element binding protein pathway for fat metabolism has also suggested a critical role for fat metabolism in the regulation of food-induced quiescence behaviors in *C. elegans* (Hyun *et al.*, 2016). Reduced food intake can also alter the production of other metabolites that act on the nervous system. For example, 2 h of fasting reduces levels of kynurenic acid, which alters NMDA signaling and downstream serotonin signaling to impact feeding (Lemieux *et al.*, 2015). Our current understanding of how specific species of bacteria might alter metabolic state to impact behavior remains more limited. However, a recent study showed that *Providencia* bacteria in the *C. elegans* gut produce the neurotransmitter tyramine, which is converted to octopamine by the *C. elegans* host to alter aversive sensory responses (O'Donnell *et al.*, 2020), which were previously shown to be regulated by feeding state (Chao, Komatsu, Fukuto, Dionne, & Hart, 2004). A large number of neuroactive metabolites are produced by non-pathogenic bacteria, suggesting that other bacterial species-specific signals produced in the gut may similarly influence behavior.

The chemosensory and mechanosensory detection of bacteria can also influence *C. elegans* foraging states. When animals are removed from an *E. coli* food source, they exhibit a 'local search' state where they display a high frequency of high-angle turns for ~15 min, before switching to a 'global search' state where they dramatically reduce turning (Gray,

Hill, & Bargmann, 2005; Hills, Brockie, & Maricq, 2004; Wakabayashi, Kitagawa, & Shingai, 2004). The frequency of turning during the local search state depends on the density of the *E. coli* food lawn from which animals were removed (López-Cruz *et al.*, 2019). In this case, chemosensory and mechanosensory neurons are required for bacterial food detection, as depletion of glutamate from both populations of sensory neurons abolishes local search (López-Cruz *et al.*, 2019). Together, these studies of internal states reveal that the *C. elegans* nervous system surveys the past and present levels of non-pathogenic bacteria through multiple sensory modalities in order to change behavior over long time scales.

Infection and internal state

Infection following the ingestion of *P. aeruginosa* PA14, which is not only a nutritive food source for *C. elegans,* but also highly pathogenic (Tan *et al.*, 1999), causes an aversive learned response that is distinct from the effect of feeding on nutritive non-pathogenic bacteria. The initial innate preference of *C. elegans* for *P. aeruginosa* PA14 over *E. coli* OP50 was found to be reversed after feeding on *P. aeruginosa* PA14, with a subsequent preference for *E. coli* OP50 and aversion to *P. aeruginosa* PA14 (Ha *et al.*, 2010; Zhang, Lu, & Bargmann, 2005).

P. aeruginosa can kill *C. elegans* through multiple modes of toxicity that depend on bacterial strain and experimental conditions, including rapid toxicity from the secretion of diffusible toxins over the course of minutes (Darby, Cosma, Thomas, & Manoil, 1999; Kirienko *et al.*, 2013; Mahajan-Miklos, Tan, Rahme, & Ausubel, 1999), or the development of an intestinal infection associated with intralumenal, extracellular bacterial proliferation and distention of the intestinal lumen with effacement of epithelial cells (Irazoqui *et al.*, 2010; Tan *et al.*, 1999) over the course of several hours (Tan *et al.*, 1999). Infection of *C. elegans* by *P. aeruginosa* induces the activation of host innate immunity (Kim & Ewbank, 2018). The host response integrates innate immunity with cellular stress response pathways, such as the endoplasmic reticulum Unfolded Protein Response (Richardson, Kooistra, & Kim, 2010) and mitochondrial stress pathways (Pellegrino *et al.*, 2014), as well as responses to exogenous, toxin-mediated effects on mRNA translation (Dunbar, Yan, Balla, Smelkinson, & Troemel, 2012; McEwan *et al.*, 2012). The widespread induction of stress-activated signaling pathways, immune and stress-responsive genes, and morphological changes to the host reflect the disruption of normal physiology and homeostasis caused by infection with pathogenic bacteria. The broad activation of cellular stress responses with infection has itself been proposed to be a mechanism by which immune defense is activated (Liu, Samuel, Breen, & Ruvkun, 2014; Pukkila-Worley, 2016; Reddy, Dunbar, Nargund, Haynes, & Troemel, 2016). Whereas specific microbial cues for the activation of innate immunity in *C. elegans* remain elusive, evidence points to a key role for host damage resulting from infection in activating innate immunity. The PMK-1 p38 mitogen-activated protein kinase pathway is activated by both bacterial infection (Fletcher, Tillman, Butty, Levine, & Kim, 2019; Kim *et al.*, 2002; Troemel *et al.*, 2006) and pore-forming toxin activity (Huffman *et al.*, 2004). In the epidermal response to fungal infection, host damage may be signaled by the endogenously produced metabolite 4-hydroxyphenyllactic acid, which acts through GPCR signaling to activate PMK-1 and antifungal immunity (Zugasti *et al.*, 2014).

The evolutionarily ancient role for host damage in the activation of innate immunity parallels its apparent role in the development of aversive behavior. Consistent with the kinetics of infection and modified choice behaviors, *C. elegans* exhibits avoidance of a lawn of pathogenic bacteria following bacterial infection (Melo & Ruvkun, 2012; Pradel *et al.*, 2007; Pujol *et al.*, 2001; Reddy *et al.*, 2009; Schulenburg & Müller, 2004). Whereas morphological changes such as intestinal distention accompany the development of pathogen infection (Tan *et al.*, 1999), such changes are only correlative, and host damage in the absence of such changes has been shown to be sufficient for *C. elegans* lawn avoidance behavior. *C. elegans* avoids bacteria such as *Bacillus thurigiensis* (Schulenburg & Müller, 2004), which produces a pore-forming toxin that rapidly kills *C. elegans* over a time scale of minutes without associated intestinal proliferation of bacteria (Marroquin *et al.*, 2000). The development of a lawn avoidance response is also observed in the presence of *Microbacterium nematophilum*, which causes a distinct mode of infection resulting in the induction of a rectal swelling response and sickness (McMullan, Anderson, & Nurrish, 2012; Yook & Hodgkin, 2007). Moreover, *E. coli* strain HT115, which is used for feeding RNAi-based experiments and is a nutritious food source of *C. elegans* can be engineered to be toxic to *C. elegans* through the expression of dsRNA targeting genes that are essential for viability of *C. elegans* (Kamath *et al.*, 2003). Aversion to the *E. coli* HT115 lawn develops over the time course that RNAi exerts toxic effects on the host, and even the addition of abiotic toxins to the lawn can also induce *C. elegans* to leave a bacterial lawn (Melo & Ruvkun, 2012). Notably, the subsequent lawn aversive behavior is not specific for *E. coli* HT115 only but also observed in response to other *E. coli* and even other bacterial species.

In addition to this generalized aversive response, exposure to pathogenic *P. aeruginosa* PA14 also causes a learned change in bacterial preference, where the preference for *P. aeruginosa* PA14 over *E. coli* OP50 is reversed. This change can be elicited by 4 h of *P. aeruginosa* PA14 exposure in adulthood or 12 h of exposure during the L1 larval stage (Jin, Pokala, & Bargmann, 2016; Zhang *et al.*, 2005). In addition, 24 h of PA14 exposure beginning at the L4 larval stage can also reduce PA14 preference in progeny for up to four generations later (Moore, Kaletsky, & Murphy, 2019). The molecular and circuit mechanisms by which exposure to *P. aeruginosa* elicits a change in *C. elegans* preference have been carefully examined. Infection with *P. aeruginosa* induces the increased transcription of *tph-1* from the ADF neurons, and *tph-1* mutants are defective for learned aversive choice behavior following *P. aeruginosa* PA14 infection

(Zhang *et al.*, 2005). *tph-1* mutants exhibit increased susceptibility to killing by *P. aeruginosa* compared to wild-type, a difference that is abrogated when animals are constrained such that they cannot avoid the *P. aeruginosa* lawn (Shivers, Kooistra, Chu, Pagano, & Kim, 2009).

Circuit-level studies have localized the site of learning within sensorimotor circuits that underlies the learned change in bacterial preference. AWB and AWC olfactory neurons detect *P. aeruginosa* PA14 and *E. coli* OP50 odors, but their sensory responses to these cues are not altered after *P. aeruginosa* PA14 exposure (Ha *et al.*, 2010). In addition, a subset of neurons in the downstream circuitry, such as AIY, AIZ, and AIB neurons, are required for navigation towards food sources in naïve animals, but not required for learned changes. In contrast, RIA and SMD neurons are not required for naïve food choice, but are required for the learned change in food preference after *P. aeruginosa* PA14 exposure (Jin *et al.*, 2016; Zhang *et al.*, 2005). These results suggest that RIA and SMD neurons are likely modulated during *P. aeruginosa* PA14 exposure. This modulation appears to require serotonergic signaling from ADF (Ha *et al.*, 2010) and *ins-6* and *ins-7* insulin-like peptides whose expression also changes after learning (Chen *et al.*, 2013). Thus, *P. aeruginosa* PA14 elicits changes in neuroendocrine signaling that impact specific nodes in the sensorimotor circuit to alter food preference. Interestingly, neuroendocrine signals from the gut are also critical for food aversion, as the dynamic expression of an intestinal insulin, *ins-11*, modulates aversive responses to *P. aeruginosa* (Lee & Mylonakis, 2017). In addition, changes in neuroendocrine signaling that modulate learning may be accompanied by changes in neuroendocrine signaling that alter innate behaviors, as is observed in the modulation of *P. aeruginosa* PA14 avoidance behavior by dynamic *daf-7* expression (Meisel *et al.*, 2014).

The observations that *C. elegans* exhibits a preference for one bacterial species over another, and that this preference can be changed based on experience, suggest that *C. elegans* can discriminate among bacterial species, which may be enabled in part by the diversity of microbial ligands that it can recognize. At the same time, the observations that *C. elegans* will leave a lawn of bacteria following infection and damage, and that the subsequent aversive response may not be specific for the bacteria causing the damage, suggest that a behavioral state characterized by a more general aversion to bacteria may also develop. This distinction underscores differences in assays for aversive behavior following infection. Lawn-leaving behavior may be influenced by changes in internal states that confer both specific and non-specific responses to bacteria. It is also possible that non-specific lawn aversion determinants, such as differential oxygen or carbon dioxide levels, may act differentially on the attraction or repulsion of *C. elegans* to particular bacteria to also influence the choice of *C. elegans* between two different bacterial strains.

Summary

The behavioral responses of *C. elegans* animals to microbial cues in their environment rely on the innate recognition of a vast and diverse set of bacterial sensory cues. Behavioral responses to these molecular and mechanosensory cues are influenced by experience and context, endowing *C. elegans* with a great deal of flexibility in how it responds and adapts to its microbial environment. Changes in satiety, tissue damage resulting from pathogenic infection, and recognition of specific bacterial metabolites can alter how the neural circuits in this animal process subsequent microbial cues. Nutritive bacteria can elicit a range of adaptive behavioral changes including learned attraction and stable switches to exploitative foraging behaviors. Pathogenic bacteria can elicit generalized responses, like bacterial aversion, as well as highly specific changes, like learned avoidance of harmful food sources. Thus, evolutionary ancient mechanisms to sense bacteria, as well as host damage arising from pathogenic bacteria, triggers not only cell-autonomous innate immune responses, but also organism-wide behavioral responses that are controlled by a nervous system that can flexibly respond to microbial sensory cues. These studies of host-microbe interactions in *C. elegans* may ultimately inform our understanding of how microbes impact nervous system function in more complex animals (Li & Liberles, 2015; Yang & Chiu, 2017).

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