An organismal understanding of *C. elegans* innate immune responses from pathogen recognition to multigenerational resistance

Tuan D. Tran[1], Robert J. Luallen[1*]
[1] Department of Biology
San Diego State University
5500 Campanile Dr.
San Diego, CA, 92182, USA
[*] Corresponding author: rluallen@sdsu.edu

Abstract

The nematode *Caenorhabditis elegans* has been a model for studying infection since the early 2000s and many major discoveries have been made regarding its innate immune responses. *C. elegans* has been found to utilize some key conserved aspects of immune responses and signaling, but new interesting features of innate immunity have been discovered in the organism that might have broader implications in higher eukaryotes such as mammals. Some of the distinctive features of *C. elegans* innate immunity involve the mechanisms this bacterivore uses to detect infection and mount specific immune responses to different pathogens, despite lacking putative orthologs of many important innate immune components, including cellular immunity, the inflammasome, complement, or melanization. Even when orthologs of known immune factors exist, there appears to be an absence of canonical functions, most notably the lack of pattern recognition by its sole Toll-like receptor. Instead, recent research suggests that *C. elegans* senses infection by specific pathogens through contextual information, including unique products produced by the pathogen or infection-induced disruption of host physiology, similar to the proposed detection of pattern of pathogenesis in mammalian systems. Interestingly, *C. elegans* can also transfer information of past infection to their progeny, providing robust protection for their offspring in face of persisting pathogens, in part through the RNAi pathway as well as potential new mechanisms that remain to be elucidated. Altogether, some of these strategies employed by *C. elegans* share key conceptual features with vertebrate adaptive immunity, as the animal can differentiate specific microbial features, as well as propagate a form of immune memory to their offspring.

Key words: innate immunity, pattern of pathogenesis, intergenerational, transgenerational, pattern recognition, invertebrate, adaptive immunity.

1. Introduction

Advances in our understanding of the vertebrate immune system, particularly the adaptive arm which can specifically detect and respond to any number of epitopes on a pathogen [1], have highlighted the question of how animals without adaptive immunity protect themselves against the myriad of pathogenic microbes in their environments. *Caenorhabditis elegans* and other invertebrate organisms are great models to address this question, allowing us to discover and dissect new mechanisms of innate immunity. Interestingly, *C. elegans* not only lacks an adaptive immune system, but also lacks many aspects found in the vertebrate innate immune system, including cellular immunity, the inflammasome, and complement. Additionally, it lacks essential components found in other invertebrate innate immune responses, including orthologs required for melanization, coagulation, and nitric oxide synthesis, as well as the NF-κB transcription factor that play a central role in innate immune signaling for many organisms [2]. As such, *C. elegans* represents an opportunity for discovering potential novel mechanisms of innate immune responses that allow the animal to cope with the plethora of microbes that it encounters, including food sources and pathogens.

Early studies of *C. elegans* immunity focused on adapting a selection of common clinically relevant pathogens like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella enterica* Typhimurium to unravel mechanisms of host-pathogen interaction. These studies have resulted in major discoveries in our understanding of

innate immunity. For instance, *C. elegans* possesses several immune signaling pathways that are conserved across many species in the animal kingdom, including mammals, such as the p38 MAPK, TGF-β, and HIF-1 pathways [3-6]. Recently, however, there has been wide sampling of wild *Caenorhabditis* nematodes from decaying vegetation by a number of groups in the field. This ecological sampling has opened up new avenues of research that have increased our understanding of host-microbe interactions and innate immunity, due to the discovery of a myriad of new naturally-associated microbes and a variety of host genetic backgrounds [7, 8]. These different host genetic backgrounds have been used for identification of important genetic variation in innate immunity [9-13]. Additionally, the diverse collection of nematode-associated microbes have resulted in the discovery of new mechanisms of innate immune responses, such as the intracellular pathogen response (IPR) to microsporidia and viral infection [14]. In this review, we aim to highlight some recent advances in our understanding of *C. elegans* innate immunity that suggest that the animal detects the presence of pathogens through pathogenic effects imposed on the host rather than the pathogen themselves, and the new paradigm of inter- and transgenerational immune inheritance that allows for this short-lived organism to invest resources in protecting future generations.

2. Detection of pathogens

In many respects, the capacity of *C. elegans* to detect pathogens does not follow many canonical principles that have been discovered in other hosts thus far. For other multicellular eukaryotes, the fight against pathogens starts with the detection of certain molecules that are highly conserved between large categories of microbes, called microbe-associated molecular patterns (MAMPs), including lipopolysaccharides (LPS), peptidoglycans (PGN), flagellin, mannan-rich structures, and microbial-specific nucleic acids [15]. These molecules are detected by hosts using various pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), AIM2, and cGAS [16, 17]. However, the *C. elegans* orthologs of known PRRs in other animals have yet to be shown to play a role in signaling or induction of an immune response, and there is a lack of biochemical evidence showing direct binding to their corresponding MAMP ligands [6, 18-22]. Yet, similar to other host animals, *C. elegans* has been shown to be capable of detecting infection and differentiating between microbes, since they mount non-identical transcriptional responses to different pathogens, albeit with some overlap [14, 23-26]. Therefore, with regards to pathogen detection in *C. elegans*, there are three non-mutually exclusive scenarios. First, it is still a formal possibility that these PRRs play a yet undiscovered role in detecting microbial products in *C. elegans,* as the putative ortholog of RIG-I in *C. elegans,* DRH-1*,* does play a role in immune defense against viruses [9, 27, 28]. Second, the animal may use unknown, novel classes of PRRs to detect MAMPs. Third, *C. elegans* may employ alternative paradigms to detect infection, which include utilizing an array of non-MAMP molecules derived from the pathogens, detecting the pathogenic effects of infection on the animal, or a combination of both. These different contextual signals are likely integrated and converge on key *C. elegans* stress and immune pathways to determine the appropriate immune output (Fig. 1). This last mode of pathogen detection bears resemblance to the “patterns of

pathogenesis" paradigm proposed in mammalian systems, a concept referring to the detection of contextual information of infection, such as pathogen growth and metabolic products, microbial access to the cytosol, or disruption of core cellular processes (e.g., integrity of membrane or cytoskeleton). This mechanism allows for discrimination between pathogens versus nonpathogens to account for the fact that MAMPs are widely distributed in microbes regardless of their pathogenicity [29]. As we will discuss in the following sections, data from various studies during the last decade show that detection of patterns of pathogenesis is at least one major component of *C. elegans* innate immunity.

2.1. Expanded gene families as candidates for detecting infection

C. elegans has several expanded gene families that could potentially function as receptors to detect infection, including C-type lectins (CLECs) [30], G-protein coupled receptors (GPCRs) [31], nuclear hormone receptors (NHRs) [32], F-box proteins (FBXs) [33], and proteins containing ALS2CR12 signature (PALSs) [34]. Genes encoding PRRs usually belong to expanded gene families, as diversification of family members results in a larger array of structures that can be distinguished from each other, while generally conserving the signaling output (response). For example, TLR diversification in mammals allows for the detection of viral, bacterial, and fungal products by different receptors, but the downstream signaling pathway is largely similar between them [35]. In *C. elegans*, members of these gene families have been implicated in immune responses in various infection models.

Among these gene families, the CLEC family represents one of the most probable candidates to play a canonical PRR role in immune detection, having about 280 members and traditionally being associated with binding to sugar moieties. The *C. elegans* CLEC family includes both predicted transmembrane proteins as well as secreted members. Membrane-bound CLECs may act as signaling receptors whereas secreted CLECs can potentially act as effectors that bind microbes or host self-molecules and block their interaction. Many CLECs have been observed to be transcriptionally upregulated upon infection in *C. elegans*, potentially acting as immune effectors (reviewed in [30] and [36]). Some CLECs have been shown to play an immune role. For example, *clec-39* and *clec-49* are required for defense against *Serratia marcescens* infection and the extracellular domains of the proteins were shown to directly bind to live and dead bacteria [19]. More recently, CLEC-4, CLEC-41 and CLEC-42 have been reported to bind a variety of bacterial species and CLEC-41 and CLEC-42 have been shown to have antimicrobial activity at high concentrations [20]. Despite these promising data, there has been little direct evidence for the signaling capacity of predicted transmembrane *clec* genes to regulate immune responses and the molecular identity of their ligands remains largely unknown for *C. elegans*. This problem may be a consequence of the difficulty in studying the role of large gene families, due to inherent redundancy masking the effects of single gene knockouts and knockdowns.

With the emerging theme of *C. elegans* detecting contextual information of infection, other expanded gene families have been suggested to broadly serve as noncanonical receptors for patterns of pathogenesis. The GPCR family is comprised of approximately 1300 genes. Many GPCRs are neuronally-expressed and function in chemoperception of the environment or internal physiology, with some of them

implicated in immunoregulation [31, 37]. Other members of the GPCR family have been found to be expressed in tissues such as intestine and hypodermis where they have more direct functions in host defense. For instance, DCAR-1 is expressed in hypodermal cells and senses cuticle damage to activate an immune response [38], whereas FSHR-1 is expressed in intestine and turns on immune genes through putative detection of oxidative damage from infection [39]. Similarly, several of 284 members of the NHR family have also been shown to activate immune genes in response to different pathogens and different contexts of pathogenesis. Examples include NHR-45 regulating immune and detoxification programs upon mitochondrial dysfunction [40], NHR-86 recognizing secondary metabolites from pathogens and triggering an immune response [41], NHR-49 regulating immune genes in the context of infection-induced oxidative stress [42, 43] and NHR-14 likely coupling iron uptake with an immune response during infection [44].

During intracellular infection, *C. elegans* has expanded gene families that are potentially playing a role in immune detection in the host cell cytoplasm. The sole DRH-1 has been implicated in detecting intracellular cytosolic nucleic acids to activate stress and antiviral response [27]. *C. elegans* has greatly expanded the F-box family of proteins, with a strong selection for variations in the substrate binding domain in wild isolates. It has been suggested that these F-box proteins play a role in defense against intracellular pathogens where their diversity may allow for specific detection and proteolytic destruction of pathogen effectors and/or finetuning of host immune signaling through ubiquitination [11, 45-48]. Separately, the PALS family has also been shown to regulate the IPR response against intracellular pathogens [14]. While it is currently unknown what contextual information of infection the *pals* gene might sense, the fact that the IPR is triggered by a variety of stress stimuli such as proteasome inhibition, heat stress, and viral and microsporidia infection [49, 50], suggests that it is reasonable to suspect that *pals* may act down stream of these stimuli or sense yet unknown infection-induced stressor. An interesting feature of the PALS family is that several paralogs act as antagonistic pairs to switch the IPR on and off [51-53], somewhat reminiscent of the Guard Hypothesis in plant immunity. Finally, it is also worth noting that these receptors may act in a non-cell autonomous manner to elicit an innate immune response [54], therefore uncoupling the “detector” tissue from the “responder” tissue and further expanding the coverage for pathogen detection.

Altogether, these expanded gene families represent a large repertoire of receptors that can detect a diversity of microbes and potentially distinguish the harmful from the benign, likely through contextual information. One can imagine a scenario in which *C. elegans* simultaneously employs GPCRs and NHRs to detect small molecules derived from microbes or the damage from infection, perhaps at different tiers of the more upstream immune response cascade. The combination of signals from these receptors could constitute a pathogenic profile, or pattern of pathogenesis, and help determine the level of response to pathogens (i.e., activation of stress and detoxification programs and/or production of release of antimicrobial immune effectors) and the specific effector profiles (e.g., CLECs, antimicrobial peptides, neuropeptide-like proteins).

2.2. Detection of patterns of pathogenesis

a) Tissue damage

Host tissue damage is one of the most prominent hallmarks of pathogenic infections. Consequently, damage molecules, or damage associated molecular patterns (DAMPs) can be directly detected by hosts as indicators of infection and turn on an immune response. In *C. elegans*, this mechanism of pathogen detection is perhaps best characterized through the studies of hypodermal infection by the fungal pathogen *Drechmeria coniosprora.* This fungus will germinate a conidium to penetrate the cuticle and grow hyphae into the epidermal cells. This invasion leads to destruction of the cuticle, causing an increase in a host endogenous molecule, 4-hydroxyphenyllactic acid (HPLA). This damage molecule is thought to activate the host hypodermal G-protein coupled receptor DCAR-1 and the PMK-1/STA-2 immune signaling pathway resulting in induction of antimicrobial peptides, including neuropeptide-like proteins NLP-29 and NLP-31, and caenacins CNC-2 and CNC-4 [38, 55]. Interestingly, this HPLA signaling can also be induced through cuticular damage without *D. coniospora* infection, suggesting that epidermal immunity uses DAMP detection to trigger a general response, as epidermal damage is often accompanied by microbial invasion in the wild.

The intestine is another tissue that is in constant contact with microbes. In *C. elegans*, a visible characteristic and a sign of intestinal infection is lumenal distention. This "bloating" phenotype in the intestine has recently been shown to induce immune and protective aversive responses, suggesting that the worms may perceive microbial-induced intestinal bloating as a physiological danger signal [56]. While the mechanism by which lumenal distention leads to activation of immune genes is yet to be elucidated, it was shown that distention-induced pathogen avoidance requires transient receptor potential melastatin (TRPM) channels, likely as a sensor, and neuropeptide communications between the intestine and neurons [57, 58].

b) Bacterial secondary products

A common theme emerging in *C. elegans* immunity is the detection of nontoxic secondary products to initiate a response to pathogens that includes either activation of immune genes or induction of behavioral avoidance. Many MAMPs are conserved, structural components at the microbial surface, such as bacterial LPS, peptidoglycan, flagellin, fungal mannan. However, microbes produce an array of other secreted or cytoplasmic molecules that have the potential to be selectively detected as a pattern of pathogenesis. Recently, *C. elegans* was shown to sense the presence of the nontoxic metabolite phenazine-1-carboxamide (PCN) through the nuclear hormone receptor NHR-86 resulting in the activation of an immune response against the pathogenic strain *P. aeruginosa* PA14 in a p38 MAPK-independent manner. No response was observed to *P. aeruginosa* strains PAO1 and PAK that produced significantly less PCN [41, 59]. The stable binding of NHR-86 to PCN is specific, however, as its precursor phenazine-1-carboxylic acid (PCA) does not bind stably to NHR-86 and thus does not trigger the immune response. Separately, *C. elegans* has been shown to activate a protective avoidance response after detecting a bacterial small RNA produced by pathogenic *P. aeruginosa* PA14 [60]. This bacterial small RNA P11 is not unique to PA14 [61], but it is highly upregulated when PA14 is grown under "virulent" conditions and P11 itself does not affect the worms' health or brood size [60].

From these data, a common theme emerges in which nontoxic secondary products are detected by *C. elegans* to initiate different responses, all contributing to the overall resistance of the organism in face of a pathogen. These secondary products apparently make up the pathogenic profile of *P. aeruginosa* toward *C. elegans*. To extrapolate this idea further, it is possible that *C. elegans* possesses mechanisms to detect a range of molecules that are characteristics of frequently encountered pathogens in the environment (e.g., different GPCRs and/or NHRs signaling axes), with inputs from different receptors leading to an additive effect on immune induction. Independently, detecting different secondary products from the same microbe can serve as functionally redundant mechanisms to ensure the robustness of an immune response against a pathogen and complicating its capacity to evolve immune escape.

c) Homeostasis of core cellular processes – surveillance immunity

Surveillance immunity is another theme that has emerged from studies of innate immunity in *C. elegans*, a concept closely related to effector-triggered immunity in plants. In surveillance immunity, hosts detect pathogens by monitoring the homeostasis of key physiological processes, including translation, mitochondrial function, and the proteasome [62-64]. Disruption of these core activities from microbial assault will then activate an immune response. Many of these immune responses overlap with some stress and detoxification programs [64], which have been reviewed in detail elsewhere [65, 66].

Infection by viruses and other intracellular pathogens can result in a dramatic decrease in translation of host proteins. As such, *C. elegans* has been shown to detect host translational repression by pathogens. Infection with *P. aeruginosa* results in bacterial production of the toxin Exotoxin A that suppresses host translation. *C. elegans* uses surveillance to detect loss of translation through an increased expression of the ZIP-2 transcription factor, which in turn activates a set of innate immune genes [62, 67, 68]. Separately, a large-scale RNAi screen found that knocking down key cellular components, including translational machinery components, results in aversion behavior and induction of immune responses in *C. elegans* [64]. More recently, another study suggested an additional mechanism from *P. aeruginosa* may inactivate host translation independent of Exotoxin A. Here, it was found that exposure of *C. elegans* to a *toxA* mutant of *P. aeruginosa* resulted in the accumulation of cleaved rRNA that ultimately resulted in a *zip-2*-mediated immune response. This result further strengthens translation as a key host physiological process that is targeted by pathogens and surveilled by hosts to induce a response [69].

In a similar manner, mitochondrial dysfunction in *C. elegans* was shown to activate immune and detoxification genes. Recently, the nuclear hormone receptor NHR-45, in association with ARIP-4 DNA helicase, was found to induce detoxification and immune genes in response to mitochondrial dysfunction. It was suggested that NHR-45 may bind to a unique lipid (perhaps cardiolipin that normally resides in the inner membrane of mitochondria) that is released from distressed mitochondria and therefore may also serve as a DAMP signal [70]. Separately, a study on *C. elegans* iron metabolism revealed a surprising connection between intestinal iron uptake and innate immune responses. It was found that animals increase the production of the iron-uptake transporter SMF-3 and increase expression of immune genes upon exposure to *P.*

aeruginosa through another nuclear hormone receptor signaling axis, NHR-14, although in this case, it is the suppression of NHR-14 that upregulates iron uptake and immune genes [44]. Considering mitochondria require iron for the electron transport chain, it is possible that during infection, NHR-14 is suppressed by either an inhibitory signal produced by dysfunctional mitochondria in infected cells or the lack of activation signal from normally functional mitochondria. These new results on mitochondrial function, together with those on translation, further solidify surveillance immunity as an important component of *C. elegans* defense. Furthermore, the emerging associations of *nhr* genes with immune response support the model that expanded gene families are used for the detection of pattern of pathogenesis.

3. Stage-dependent immunity in *C. elegans*

A key distinguishing aspect of *C. elegans* immunity is that responses are sometime stronger in larval stages compared to adult stages, before maturation of the reproductive system. Observations from studies that first established *C. elegans* as an infection model for different pathogens found a correlation between the strength of resistance and developmental stages, with larval stages tending to have better survival compared to adult animals. *C. elegans* has four larval (L) stages, designated L1 to L4, before developing into a reproductively active adult. Previous work found that L4 larvae are less susceptible to *P. aeruginosa* PA14 slow killing compared to adult animals [71]. Similarly, L4s exhibited a slower killing rate compared to adult animals after infection with *S. typhimurium* SL1344, suggesting some level of pathogen resistance in L4 stage [72]. Infection with *S. marcescens* also showed a negative correlation between resistance and developmental stages [73], with younger animals showing higher resistance. In infection with *S. aureus*, L4s also showed a slightly higher resistance compared to adults. While stronger resistance in larvae might be the case for many pathogens, it is by no means a universal trait and may be affected by factors from both the host and the pathogen. For example, the L1-L3 larval stages of *C. elegans* are readily killed by *S. aureus* infection [74]. Similarly, L1 animals are unable to survive *B. pseudomallei* and *B. thailandensis* infection, whereas other stages display varied susceptibility, with L2/L3 animals are more resistant compared to L4/adult stages [75]. Beside bacterial pathogens, studies in infections with microsporidia – an obligate intracellular, fungal-like pathogen – also support this notion of enhanced resistance at early developmental stages. For instance, L1 animals of a wild isolate strain of *C. elegans* (CB4856) can clear initial infection by the microsporidia *Nematocida parisii* in the intestine, and this resistance is sharply reduced by L2 stage onwards [12]. In agreement with this finding, in a recent high-throughput screen for genetic loci responsible for susceptibility/resistance against microsporidian infection, *C. elegans* strain JU1400 exhibited resistance toward a related microsporidian species *N. ironsii* infection by clearing the pathogens at L1 stage, but this resistance waned off at L4 stage [10].

These observations are largely the opposite of those seen in mammals, where immunity is stronger in reproductively mature stages [76]. This difference may be related to the differences in lifespan. Host organism must make a cost-benefit calculation to balance an appropriate immune response to protect the host versus investing energy in protection of progeny. For short-lived animals like nematodes,

especially in the wild, investing resources in reproduction and protective mechanisms for the progeny many have several benefits as compared to allocating those limited resources to clear infection in adult animals that have passed their peak of reproduction (day 2 or 3 of adulthood) and produced the majority of progeny (day 5) [77]. Such an investment would likely protect the embryos and hatchlings from the lethal effects of the persisting pathogens that the parents encountered, as well as potentially allowing the progeny to allocate their own resources for better development to reach reproductive maturation, all contributing to the continuation of the strain/species.

4. Intergenerational and transgenerational resistance

For animals lacking adaptive immunity, parental investment to better protect offspring has been suggested to be functionally equivalent to vertebrate adaptive immunity [78]. In *C. elegans*, information from ancestral encounters with pathogens can be passed down for a single generation or multiple generations, termed intergenerational and transgenerational immunity, respectively. The transferred information can manifest as regulation of immune genes and/or protective aversion behaviors in the progeny. Additionally, the transferred information appears to vary depending on the pathogen and the course of infection since not all infections elicit an observable inter- or transgenerational phenotype. For behavioral avoidance of pathogens, a transgenerational effect has been observed for *P. aeruginosa* PA14 infection. Specifically, exposure of *C. elegans* to PA14 at the L4 stage for 24 hours will induce a transgenerational avoidance behavior that lasts for exactly 4 generations. This avoidance is triggered, in part, by the bacterial small RNA molecule P11, which is taken up and processed by the host small-interference RNA (siRNA) arm. Amplification of this siRNA down-regulates the receptor MACO-1 in the ASI neuron resulting in avoidance of *P. aeruginosa* [60]. Although avoidance behaviors are a common protective response to different pathogenic bacteria (*S. marcescens*, *E. faecalis*, *S. aureus*), transgenerational transmission of the phenotype appears to be species-specific since *S. marcescens* avoidance is not passed down to offspring. Small RNA (sRNA)-induced avoidance is also species specific, as treatment with sRNA from *S. marcescens* or RNA extract from *E. faecalis* did not elicit the same response [57, 60, 79]. It remains unclear whether other sRNAs from PA14 can elicit a similar response, or more broadly, whether sRNAs from other pathogenic bacteria can affect the transcriptional immune response of the parents and multigenerational immune inheritance.

While *C. elegans* avoidance behaviors can reduce the chance of encountering pathogens, they are likely insufficient to provide robust protection for the progeny. This is because *C. elegans* is a bacterivore and pathogenic and nutritional microbes are often mixed in wild environments. As such, pathogens have been observed to induce immune inheritance, whereby information of past infections is transmitted to future generations through transcriptional changes of immune genes in progeny. Indeed, infection with several different classes of pathogens also result in either inter- or transgenerational immune priming. In one study, parental exposure of *C. elegans* to the natural bacterial pathogen *Pseudomonas vranovensis* induced a transgenerational protective transcriptional program in the offspring that resulted in the upregulation of cysteine synthase genes *cysl-1* and *cysl-2*, and the regulatory protein *rhy-1.* This induction was independent of the small RNA pathways. Surprisingly, this protection was

advantageous for F1 embryo survival and hatching into L1 progeny, but there was no significant difference in resistance when these F1 were at the adult stage [80]. In a different study examining the immune inheritance effect against microsporidia, exposure of *C. elegans* parents to the microsporidian *N. parisii* or artificial activation of part of their IPR conferred intergenerational resistance against subsequent infection with microsporidia or *P. aeruginosa* in their F1 progeny throughout development [81]. Mechanistically, intergenerational immunity may be mediated by maternal deposition of effectors (transcripts or proteins) while transgenerational immunity may be propagated by epigenetics in a similar fashion of transgenerational stress responses [82-84]. However, known epigenetic regulators of transgenerational responses did not appear to have an effect on transgenerational response to *P. vranovensis*, suggesting other yet unknown mechanisms are in play. It is also worth noting that one study of infection with Orsay virus – a natural viral pathogen of *C. elegans* – reported inheritance of antiviral response through the RNAi pathway [85], but a second study found that neither systemic nor inherited antiviral 22G RNAs were readily detected in offspring of exposed parents [86]. Thus, the inherited antiviral response, as well as small RNA-mediated transgenerational immunity, largely remain an open question in *C. elegans*.

5. Conclusion and perspectives

It has been increasingly recognized that *C. elegans* possesses some unique as well as conserved aspects of their innate immunity compared to other traditionally well-studied organisms. Some of these unique features may be nematode or *C. elegans*-specific, or they may be conserved in other eukaryotes and waiting to be discovered. For instance, the detection of secondary products (i.e., secondary metabolites or microbial small regulatory RNAs) may also be used in other animals such as mammals, considering that the detection of microbial (primary) metabolites can activate and modulate immune responses [87-90], but it may be employed more prominently in *C. elegans* as a result of their lifestyle.

Despite the progress that has been made over the past two decades, our understanding of *C. elegans* innate immunity is still imcomplete. Like an incomplete jigsaw puzzle, the current body of data gives us a glimpse of what could be the overall picture of *C. elegans* innate immunity. We propose that *C. elegans* may not detect any specific canonical structural MAMPs of incoming bacterial species from their food source. Instead, there may be two main lines of defense. First, general surveillance of key cellular processes and/or DAMPs can initiate a stress/detox response (which also include some immune genes and therefore may constitute the “baseline” response), perhaps corresponding to an early stage of infection when pathogen load is still low and its effects on the animals are at the “stress” level. Then, detection of a more specific signature of a particular pathogen can trigger a more specific response. This specific signature is likely the secondary-product profile produced by the pathogen once its population has grown sufficiently large, corresponding to the latter stage of infection. Therefore, as the pathogen load increases, detection of the pathogen-specific, quorum-sensing-induced secondary products would tailor a more appropriate response to control its growth and repair/detoxify damage induced by virulence factors and host immune effectors. Of note, this second line of defense may work simultaneously with the ongoing surveillance immune response started at an early stage of infection. In this

case, the transcription factors activated by the later secondary products may confer the distinctiveness and specificity of the immune responses, despite the fact that the upstream detection of early infection is broad and nonspecific. Thus, these two main lines of defense ensure that the *C. elegans* immune response is both general as well as specific. This concept is reminiscent of the innate and adaptive arms of vertebrate immunity, with respect to the idea that innate immunity detects patterns of pathogens and adaptive immunity detects specific signatures.

However, this model also brings up a question on the specificity and efficiency of such an immune response, i.e. how does a given secondary product turn on the specific set of immune effectors required for a particular pathogen given the large set of available immune genes encoded in the *C. elegans* genome? One possible scenario is that coevolution could have shaped the associations of specific sets of secondary products with specific sets of "efficient" effectors, likely through the use of expanded gene families (CTLs, NHRs, FBXs, GPCRs, and PALSs) to detect those secondary products. Consequently, this would mean a response to a newly emerging pathogen would result in a general surveillance immune response as part of the pattern-of-pathogenesis response but lack specificity, resulting in a less efficient immune response. Under this scenario selective pressure on the host would evolve new receptors capable of specifically detecting the secondary products of this new pathogen, resulting in further expansion of certain gene families. Why has *C. elegans* evolved to detect these secondary bacterial products? It is possible that many bacterial species are producing specific compounds that aid in their survival and growth in vivo or in the environment, perhaps when their populations have reached a limit. Many compounds are often under the control of quorum-sensing and likely to have a negative impact on *C. elegans*, through lysing host cells, hampering cell physiologies that could free up resources, or sequestering key metabolites [91-94]. As such, *C. elegans* might have evolved to "read" these secondary products as an indication of actively replicating and harmful bacteria during an ongoing infection. Future discoveries on new secondary products that can trigger *C. elegans* immunity will further shed light on this topic.

With regards to inter- and transgenerational immunity, it has emerged that this aspect of *C. elegans* innate immunity may be more widespread than previously thought. In higher eukaryotes adaptive immunity is conceptually characterized by immune memory, whereby re-exposure to the same pathogen activates a quicker and stronger response. If this characteristic of adaptive immunity is broadened to the population level, then the use of immune inheritance from ancestral exposure may be considered conceptually similar to immune memory, as short-lived animals like *C. elegans* can protect offspring from re-exposure to a persisting pathogen in the environment resulting in inherited immune and behavioral responses. The main difference is that this form of immune memory is vertically propagated, similar to maternal antibodies in mammals. The mechanisms behind *C. elegans* inter- and transgenerational immunity are only beginning to emerge and further studies will be needed to understand the inner working of this mode of immune response at the molecular level. Many questions remain to be investigated, for example, whether inter- and transgenerational immunity is relatively universal to infections by different pathogens, or how different stressors, abiotic and biotic, affect the outcome of such immune inheritance, as it was recently suggested that different abiotic stressors can reset sRNA-induced inheritance [95]. Altogether, *C.*

elegans immunity contains conserved paradigms shared by all animals, even though the mechanisms differ. Surveillance of host physiology allows for broad recognition of pathogens, similar to innate immunity detection of patterns. Detection secondary pathogen products allows for specific recognition, similar to adaptive immune recognition of signatures. And inter- and transgenerational immunity may confer the "memory" feature of the adaptive arm. Undoubtedly, future studies will give more insights to these intriguing questions and *C. elegans* will continue as a model for host-pathogen interactions and innate immunity and further pioneer a fundamental understanding of animal immunity.

Funding

This work was supported by NIH grant R35 [GM146836] and NSF IOS CAREER grant [2143718] to RJL, and the SDSU University Grant Fellowship and the Rees Steely Research Foundation Fellowship to TDT.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] F.A. Bonilla, H.C. Oettgen, Adaptive immunity, J Allergy Clin Immunol 125(2 Suppl 2) (2010) S33-40.

[2] H. Schulenburg, C.L. Kurz, J.J. Ewbank, Evolution of the innate immune system: the worm perspective, Immunol Rev 198 (2004) 36-58.

[3] D.H. Kim, R. Feinbaum, G. Alloing, F.E. Emerson, D.A. Garsin, H. Inoue, M. Tanaka-Hino, N. Hisamoto, K. Matsumoto, M.W. Tan, F.M. Ausubel, A conserved p38 MAP kinase pathway in Caenorhabditis elegans innate immunity, Science 297(5581) (2002) 623-6.

[4] G.V. Mallo, C.L. Kurz, C. Couillault, N. Pujol, S. Granjeaud, Y. Kohara, J.J. Ewbank, Inducible antibacterial defense system in C. elegans, Curr Biol 12(14) (2002) 1209-14.

[5] L.G. Luhachack, O. Visvikis, A.C. Wollenberg, A. Lacy-Hulbert, L.M. Stuart, J.E. Irazoqui, EGL-9 controls C. elegans host defense specificity through prolyl hydroxylation-dependent and -independent HIF-1 pathways, PLoS Pathog 8(7) (2012) e1002798.

[6] D.H. Kim, J.J. Ewbank, Signaling in the innate immune response, WormBook 2018 (2018) 1-35.

[7] A. Barriere, M.A. Felix, Isolation of C. elegans and related nematodes, WormBook (2006) 1-9.

[8] D.E. Cook, S. Zdraljevic, J.P. Roberts, E.C. Andersen, CeNDR, the Caenorhabditis elegans natural diversity resource, Nucleic Acids Res 45(D1) (2017) D650-D657.

[9] A. Ashe, T. Belicard, J. Le Pen, P. Sarkies, L. Frezal, N.J. Lehrbach, M.A. Felix, E.A. Miska, A deletion polymorphism in the Caenorhabditis elegans RIG-I homolog disables viral RNA dicing and antiviral immunity, Elife 2 (2013) e00994.

[10] M.A.X. Calvin Mok, Yin C. Wan, Winnie Zhao, Shanzeh M. Ahmed, Robert Luallen, Aaron W. Reinke, High-throughput phenotyping of C. elegans wild isolates reveals specific resistance and susceptibility traits to infection by distinct microsporidia species, 2022.

[11] F. Ma, C.Y. Lau, C. Zheng, Large genetic diversity and strong positive selection in F-box and GPCR genes among the wild isolates of Caenorhabditis elegans, Genome Biol Evol 13(5) (2021).
[12] K.M. Balla, E.C. Andersen, L. Kruglyak, E.R. Troemel, A wild C. elegans strain has enhanced epithelial immunity to a natural microsporidian parasite, PLoS Pathog 11(2) (2015) e1004583.
[13] P. Lansdon, M. Carlson, B.D. Ackley, Wild-type Caenorhabditis elegans isolates exhibit distinct gene expression profiles in response to microbial infection, BMC Genomics 23(1) (2022) 229.
[14] K.C. Reddy, T. Dror, J.N. Sowa, J. Panek, K. Chen, E.S. Lim, D. Wang, E.R. Troemel, An Intracellular Pathogen Response Pathway Promotes Proteostasis in C. elegans, Current Biology 27(22) (2017) 3544-3553.
[15] T. Boller, G. Felix, A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors, Annu Rev Plant Biol 60 (2009) 379-406.
[16] D. Li, M. Wu, Pattern recognition receptors in health and diseases, Signal Transduct Target Ther 6(1) (2021) 291.
[17] M. Motwani, S. Pesiridis, K.A. Fitzgerald, DNA sensing by the cGAS-STING pathway in health and disease, Nat Rev Genet 20(11) (2019) 657-674.
[18] E. Pradel, Y. Zhang, N. Pujol, T. Matsuyama, C.I. Bargmann, J.J. Ewbank, Detection and avoidance of a natural product from the pathogenic bacterium Serratia marcescens by Caenorhabditis elegans, Proc Natl Acad Sci U S A 104(7) (2007) 2295-300.
[19] S.M. Miltsch, P.H. Seeberger, B. Lepenies, The C-type lectin-like domain containing proteins Clec-39 and Clec-49 are crucial for Caenorhabditis elegans immunity against Serratia marcescens infection, Dev Comp Immunol 45(1) (2014) 67-73.
[20] B. Pees, W. Yang, A. Kloock, C. Petersen, L. Peters, L. Fan, M. Friedrichsen, S. Butze, A. Zarate-Potes, H. Schulenburg, K. Dierking, Effector and regulator: Diverse functions of C. elegans C-type lectin-like domain proteins, PLoS Pathog 17(4) (2021) e1009454.
[21] N. Pujol, E.M. Link, L.X. Liu, C.L. Kurz, G. Alloing, M.W. Tan, K.P. Ray, R. Solari, C.D. Johnson, J.J. Ewbank, A reverse genetic analysis of components of the Toll signaling pathway in Caenorhabditis elegans, Curr Biol 11(11) (2001) 809-21.
[22] W. Pan, X. Huang, Z. Guo, R. Nagarajan, E. Mylonakis, Identification and Functional Analysis of Cytokine-Like Protein CLEC-47 in Caenorhabditis elegans, mBio 12(5) (2021) e0257921.
[23] J.E. Irazoqui, E.R. Troemel, R.L. Feinbaum, L.G. Luhachack, B.O. Cezairliyan, F.M. Ausubel, Distinct Pathogenesis and Host Responses during Infection of C. elegans by P. aeruginosa and S. aureus, PLoS Pathogens 6(7) (2010) e1000982-Article No.: e1000982.
[24] G.J. Yuen, F.M. Ausubel, Both live and dead Enterococci activate Caenorhabditis elegans host defense via immune and stress pathways, Virulence 9(1) (2018) 683-699.
[25] G.A. Osman, M.K. Fasseas, S.L. Koneru, C.L. Essmann, K. Kyrou, M.A. Srinivasan, G. Zhang, P. Sarkies, M.A. Felix, M. Barkoulas, Natural Infection of C. elegans by an Oomycete Reveals a New Pathogen-Specific Immune Response, Curr Biol 28(4) (2018) 640-648 e5.
[26] D. Wong, D. Bazopoulou, N. Pujol, N. Tavernarakis, J.J. Ewbank, Genome-wide investigation reveals pathogen-specific and shared signatures in the response of Caenorhabditis elegans to infection, Genome Biol 8(9) (2007) R194.

[27] J.N. Sowa, H. Jiang, L. Somasundaram, E. Tecle, G. Xu, D. Wang, E.R. Troemel, The Caenorhabditis elegans RIG-I Homolog DRH-1 Mediates the Intracellular Pathogen Response upon Viral Infection, J Virol 94(2) (2020).
[28] R. Lu, E. Yigit, W.X. Li, S.W. Ding, An RIG-I-Like RNA helicase mediates antiviral RNAi downstream of viral siRNA biogenesis in Caenorhabditis elegans, PLoS Pathog 5(2) (2009) e1000286.
[29] R.E. Vance, R.R. Isberg, D.A. Portnoy, Patterns of pathogenesis: discrimination of pathogenic and nonpathogenic microbes by the innate immune system, Cell Host Microbe 6(1) (2009) 10-21.
[30] H. Schulenburg, M.P. Hoeppner, J. Weiner, 3rd, E. Bornberg-Bauer, Specificity of the innate immune system and diversity of C-type lectin domain (CTLD) proteins in the nematode Caenorhabditis elegans, Immunobiology 213(3-4) (2008) 237-50.
[31] J.H. Thomas, H.M. Robertson, The Caenorhabditis chemoreceptor gene families, BMC Biol 6 (2008) 42.
[32] A. Antebi, Nuclear hormone receptors in C. elegans, WormBook (2006) 1-13.
[33] E.T. Kipreos, M. Pagano, The F-box protein family, Genome Biol 1(5) (2000) REVIEWS3002.
[34] E. Leyva-Diaz, N. Stefanakis, I. Carrera, L. Glenwinkel, G. Wang, M. Driscoll, O. Hobert, Silencing of Repetitive DNA Is Controlled by a Member of an Unusual Caenorhabditis elegans Gene Family, Genetics 207(2) (2017) 529-545.
[35] R. Medzhitov, Toll-like receptors and innate immunity, Nat Rev Immunol 1(2) (2001) 135-45.
[36] B. Pees, W. Yang, A. Zárate-Potes, H. Schulenburg, K. Dierking, High Innate Immune Specificity through Diversified C-Type Lectin-Like Domain Proteins in Invertebrates, Journal of Innate Immunity 8(2) (2016) 129-142.
[37] S.R. Venkatesh, V. Singh, G protein-coupled receptors: The choreographers of innate immunity in Caenorhabditis elegans, PLoS Pathog 17(1) (2021) e1009151.
[38] O. Zugasti, N. Bose, B. Squiban, J. Belougne, C.L. Kurz, F.C. Schroeder, N. Pujol, J.J. Ewbank, Activation of a G protein-coupled receptor by its endogenous ligand triggers the innate immune response of Caenorhabditis elegans, Nat Immunol 15(9) (2014) 833-8.
[39] J.R. Powell, D.H. Kim, F.M. Ausubel, The G protein-coupled receptor FSHR-1 is required for the Caenorhabditis elegans innate immune response, Proc Natl Acad Sci U S A 106(8) (2009) 2782-7.
[40] K. Mao, F. Ji, P. Breen, A. Sewell, M. Han, R. Sadreyev, G. Ruvkun, Mitochondrial Dysfunction in C. elegans Activates Mitochondrial Relocalization and Nuclear Hormone Receptor-Dependent Detoxification Genes, Cell Metab 29(5) (2019) 1182-1191 e4.
[41] N.D. Peterson, S.Y. Tse, Q.J. Huang, K.A. Wani, C.A. Schiffer, R. Pukkila-Worley, Non-canonical pattern recognition of a pathogen-derived metabolite by a nuclear hormone receptor identifies virulent bacteria in C. elegans, Immunity (2023).
[42] K.A. Wani, D. Goswamy, S. Taubert, R. Ratnappan, A. Ghazi, J.E. Irazoqui, NHR-49/PPAR-alpha and HLH-30/TFEB cooperate for C. elegans host defense via a flavin-containing monooxygenase, Elife 10 (2021).
[43] G.Y.S. Goh, J.J. Winter, F. Bhanshali, K.R.S. Doering, R. Lai, K. Lee, E.A. Veal, S. Taubert, NHR-49/HNF4 integrates regulation of fatty acid metabolism with a protective transcriptional response to oxidative stress and fasting, Aging Cell 17(3) (2018) e12743.

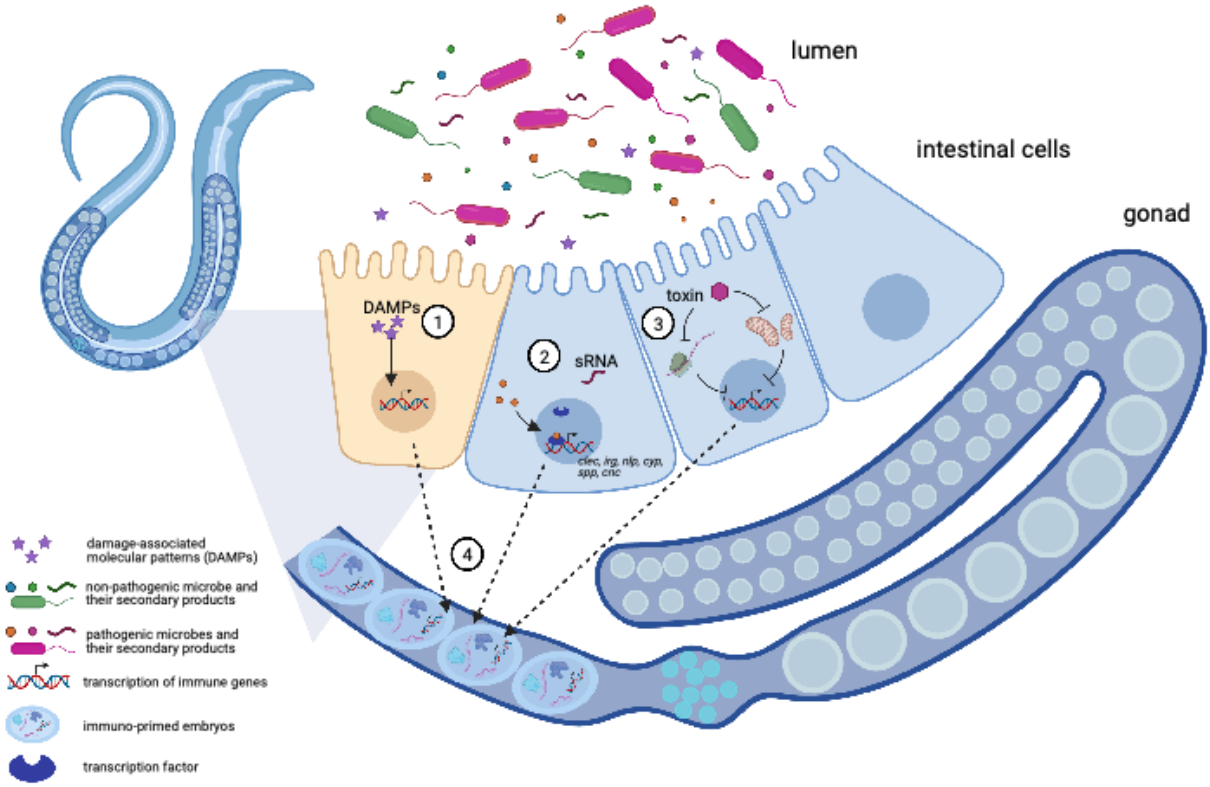
[44] M. Rajan, C.P. Anderson, P.M. Rindler, S.J. Romney, M.C. Ferreira Dos Santos, J. Gertz, E.A. Leibold, NHR-14 loss of function couples intestinal iron uptake with innate immunity in C. elegans through PQM-1 signaling, Elife 8 (2019).
[45] M.A. Bakowski, C.A. Desjardins, M.G. Smelkinson, T.A. Dunbar, I.F. Lopez-Moyado, S.A. Rifkin, C.A. Cuomo, E.R. Troemel, Ubiquitin-Mediated Response to Microsporidia and Virus Infection in C. elegans, PLoS Pathogens 10(6) (2014) e1004200-Article No.: e1004200.
[46] J.H. Thomas, Adaptive evolution in two large families of ubiquitin-ligase adapters in nematodes and plants, Genome Res 16(8) (2006) 1017-30.
[47] J. Panek, S.S. Gang, K.C. Reddy, R.J. Luallen, A. Fulzele, E.J. Bennett, E.R. Troemel, A cullin-RING ubiquitin ligase promotes thermotolerance as part of the intracellular pathogen response in Caenorhabditis elegans, Proc Natl Acad Sci U S A 117(14) (2020) 7950-7960.
[48] A. Wang, W. Chen, S. Tao, Genome-wide characterization, evolution, structure, and expression analysis of the F-box genes in Caenorhabditis, BMC Genomics 22(1) (2021) 889.
[49] V. Lazetic, F. Wu, L.B. Cohen, K.C. Reddy, Y.T. Chang, S.S. Gang, G. Bhabha, E.R. Troemel, The transcription factor ZIP-1 promotes resistance to intracellular infection in Caenorhabditis elegans, Nat Commun 13(1) (2022) 17.
[50] E. Tecle, C.B. Chhan, L. Franklin, R.S. Underwood, W. Hanna-Rose, E.R. Troemel, The purine nucleoside phosphorylase pnp-1 regulates epithelial cell resistance to infection in C. elegans, PLoS Pathog 17(4) (2021) e1009350.
[51] K.C. Reddy, T. Dror, R.S. Underwood, G.A. Osman, C.R. Elder, C.A. Desjardins, C.A. Cuomo, M. Barkoulas, E.R. Troemel, Antagonistic paralogs control a switch between growth and pathogen resistance in C. elegans, PLoS Pathog 15(1) (2019) e1007528.
[52] M.J.B. Vladimir Lažetić, Theresa Bui, Emily R. Troemel, Multiple pals gene modules control a balance between immunity and development in Caenorhabditis elegans, 2023.
[53] S.S. Gang, M. Grover, K.C. Reddy, D. Raman, Y.T. Chang, D.C. Ekiert, M. Barkoulas, E.R. Troemel, A pals-25 gain-of-function allele triggers systemic resistance against natural pathogens of C. elegans, PLoS Genet 18(10) (2022) e1010314.
[54] K.A. Wani, D. Goswamy, J.E. Irazoqui, Nervous system control of intestinal host defense in C. elegans, Curr Opin Neurobiol 62 (2020) 1-9.
[55] C. Couillault, N. Pujol, J. Reboul, L. Sabatier, J.F. Guichou, Y. Kohara, J.J. Ewbank, TLR-independent control of innate immunity in Caenorhabditis elegans by the TIR domain adaptor protein TIR-1, an ortholog of human SARM, Nat Immunol 5(5) (2004) 488-94.
[56] J. Singh, A. Aballay, Microbial Colonization Activates an Immune Fight-and-Flight Response via Neuroendocrine Signaling, Dev Cell 49(1) (2019) 89-99 e4.
[57] A. Filipowicz, J. Lalsiamthara, A. Aballay, TRPM channels mediate learned pathogen avoidance following intestinal distention, Elife 10 (2021).
[58] J. Singh, A. Aballay, Intestinal infection regulates behavior and learning via neuroendocrine signaling, Elife 8 (2019).
[59] N.D. Peterson, H.K. Cheesman, P. Liu, S.M. Anderson, K.J. Foster, R. Chhaya, P. Perrat, J. Thekkiniath, Q. Yang, C.M. Haynes, R. Pukkila-Worley, The nuclear hormone receptor NHR-86 controls anti-pathogen responses in C. elegans, PLoS Genet 15(1) (2019) e1007935.
[60] R. Kaletsky, R.S. Moore, G.D. Vrla, L.R. Parsons, Z. Gitai, C.T. Murphy, C. elegans interprets bacterial non-coding RNAs to learn pathogenic avoidance, Nature 586(7829) (2020) 445-451.

[61] J. Livny, A. Brencic, S. Lory, M.K. Waldor, Identification of 17 Pseudomonas aeruginosa sRNAs and prediction of sRNA-encoding genes in 10 diverse pathogens using the bioinformatic tool sRNAPredict2, Nucleic Acids Res 34(12) (2006) 3484-93.
[62] T.L. Dunbar, Z. Yan, K.M. Balla, M.G. Smelkinson, E.R. Troemel, C. elegans detects pathogen-induced translational inhibition to activate immune signaling, Cell Host Microbe 11(4) (2012) 375-86.
[63] Y. Liu, B.S. Samuel, P.C. Breen, G. Ruvkun, Caenorhabditis elegans pathways that surveil and defend mitochondria, Nature 508(7496) (2014) 406-10.
[64] J.A. Melo, G. Ruvkun, Inactivation of conserved C. elegans genes engages pathogen- and xenobiotic-associated defenses, Cell 149(2) (2012) 452-66.
[65] R. Pukkila-Worley, Surveillance Immunity: An Emerging Paradigm of Innate Defense Activation in Caenorhabditis elegans, PLoS Pathogens 12(9) (2016) e1005795-Article No.: e1005795.
[66] L.B. Cohen, E.R. Troemel, Microbial pathogenesis and host defense in the nematode C. elegans, Curr Opin Microbiol 23 (2015) 94-101.
[67] D.L. McEwan, N.V. Kirienko, F.M. Ausubel, Host translational inhibition by Pseudomonas aeruginosa Exotoxin A Triggers an immune response in Caenorhabditis elegans, Cell Host Microbe 11(4) (2012) 364-74.
[68] K.A. Estes, T.L. Dunbar, J.R. Powell, F.M. Ausubel, E.R. Troemel, bZIP transcription factor zip-2 mediates an early response to Pseudomonas aeruginosa infection in Caenorhabditis elegans, Proc Natl Acad Sci U S A 107(5) (2010) 2153-8.
[69] A. Vasquez-Rifo, E.P. Ricci, V. Ambros, Pseudomonas aeruginosa cleaves the decoding center of Caenorhabditis elegans ribosomes, PLoS Biol 18(12) (2020) e3000969.
[70] K. Mao, P. Breen, G. Ruvkun, The Caenorhabditis elegans ARIP-4 DNA helicase couples mitochondrial surveillance to immune, detoxification, and antiviral pathways, Proc Natl Acad Sci U S A 119(49) (2022) e2215966119.
[71] M.W. Tan, S. Mahajan-Miklos, F.M. Ausubel, Killing of Caenorhabditis elegans by Pseudomonas aeruginosa used to model mammalian bacterial pathogenesis, Proc Natl Acad Sci U S A 96(2) (1999) 715-20.
[72] A. Aballay, P. Yorgey, F.M. Ausubel, Salmonella typhimurium proliferates and establishes a persistent infection in the intestine of Caenorhabditis elegans, Curr Biol 10(23) (2000) 1539-42.
[73] C.L. Kurz, S. Chauvet, E. Andres, M. Aurouze, I. Vallet, G.P. Michel, M. Uh, J. Celli, A. Filloux, S. De Bentzmann, I. Steinmetz, J.A. Hoffmann, B.B. Finlay, J.P. Gorvel, D. Ferrandon, J.J. Ewbank, Virulence factors of the human opportunistic pathogen Serratia marcescens identified by in vivo screening, EMBO J 22(7) (2003) 1451-60.
[74] C.D. Sifri, J. Begun, F.M. Ausubel, S.B. Calderwood, Caenorhabditis elegans as a model host for Staphylococcus aureus pathogenesis, Infect Immun 71(4) (2003) 2208-17.
[75] A.L. O'Quinn, E.M. Wiegand, J.A. Jeddeloh, Burkholderia pseudomallei kills the nematode Caenorhabditis elegans using an endotoxin-mediated paralysis, Cell Microbiol 3(6) (2001) 381-93.
[76] A.K. Simon, G.A. Hollander, A. McMichael, Evolution of the immune system in humans from infancy to old age, Proc Biol Sci 282(1821) (2015) 20143085.
[77] A. Scharf, F. Pohl, B.M. Egan, Z. Kocsisova, K. Kornfeld, Reproductive Aging in Caenorhabditis elegans: From Molecules to Ecology, Front Cell Dev Biol 9 (2021) 718522.

[78] T.J. Little, A.R. Kraaijeveld, Ecological and evolutionary implications of immunological priming in invertebrates, Trends Ecol Evol 19(2) (2004) 58-60.
[79] R.S. Moore, R. Kaletsky, C.T. Murphy, Piwi/PRG-1 Argonaute and TGF-beta Mediate Transgenerational Learned Pathogenic Avoidance, Cell 177(7) (2019) 1827-1841 e12.
[80] N.O. Burton, C. Riccio, A. Dallaire, J. Price, B. Jenkins, A. Koulman, E.A. Miska, Cysteine synthases CYSL-1 and CYSL-2 mediate C. elegans heritable adaptation to P. vranovensis infection, Nat Commun 11(1) (2020) 1741.
[81] A.R. Willis, W. Zhao, R. Sukhdeo, L. Wadi, H.T. El Jarkass, J.M. Claycomb, A.W. Reinke, A parental transcriptional response to microsporidia infection induces inherited immunity in offspring, Sci Adv 7(19) (2021).
[82] C. Ma, R. Niu, T. Huang, L.W. Shao, Y. Peng, W. Ding, Y. Wang, G. Jia, C. He, C.Y. Li, A. He, Y. Liu, N6-methyldeoxyadenine is a transgenerational epigenetic signal for mitochondrial stress adaptation, Nat Cell Biol 21(3) (2019) 319-327.
[83] A. Ashe, A. Sapetschnig, E.M. Weick, J. Mitchell, M.P. Bagijn, A.C. Cording, A.L. Doebley, L.D. Goldstein, N.J. Lehrbach, J. Le Pen, G. Pintacuda, A. Sakaguchi, P. Sarkies, S. Ahmed, E.A. Miska, piRNAs can trigger a multigenerational epigenetic memory in the germline of C. elegans, Cell 150(1) (2012) 88-99.
[84] E.L. Greer, T.J. Maures, D. Ucar, A.G. Hauswirth, E. Mancini, J.P. Lim, B.A. Benayoun, Y. Shi, A. Brunet, Transgenerational epigenetic inheritance of longevity in Caenorhabditis elegans, Nature 479(7373) (2011) 365-71.
[85] M.G. Sterken, L.B. Snoek, K.J. Bosman, J. Daamen, J.A. Riksen, J. Bakker, G.P. Pijlman, J.E. Kammenga, A heritable antiviral RNAi response limits Orsay virus infection in Caenorhabditis elegans N2, PLoS One 9(2) (2014) e89760.
[86] A. Ashe, P. Sarkies, J. Le Pen, M. Tanguy, E.A. Miska, Antiviral RNA Interference against Orsay Virus Is neither Systemic nor Transgenerational in Caenorhabditis elegans, J Virol 89(23) (2015) 12035-46.
[87] W. Lei, W. Ren, M. Ohmoto, J.F. Urban, Jr., I. Matsumoto, R.F. Margolskee, P. Jiang, Activation of intestinal tuft cell-expressed Sucnr1 triggers type 2 immunity in the mouse small intestine, Proc Natl Acad Sci U S A 115(21) (2018) 5552-5557.
[88] M.S. Nadjsombati, J.W. McGinty, M.R. Lyons-Cohen, J.B. Jaffe, L. DiPeso, C. Schneider, C.N. Miller, J.L. Pollack, G.A. Nagana Gowda, M.F. Fontana, D.J. Erle, M.S. Anderson, R.M. Locksley, D. Raftery, J. von Moltke, Detection of Succinate by Intestinal Tuft Cells Triggers a Type 2 Innate Immune Circuit, Immunity 49(1) (2018) 33-41 e7.
[89] C. Schneider, C.E. O'Leary, J. von Moltke, H.E. Liang, Q.Y. Ang, P.J. Turnbaugh, S. Radhakrishnan, M. Pellizzon, A. Ma, R.M. Locksley, A Metabolite-Triggered Tuft Cell-ILC2 Circuit Drives Small Intestinal Remodeling, Cell 174(2) (2018) 271-284 e14.
[90] C.H. Kim, Immune regulation by microbiome metabolites, Immunology 154(2) (2018) 220-229.
[91] A. Price-Whelan, L.E. Dietrich, D.K. Newman, Rethinking 'secondary' metabolism: physiological roles for phenazine antibiotics, Nat Chem Biol 2(2) (2006) 71-8.
[92] D. Mao, L.B. Bushin, K. Moon, Y. Wu, M.R. Seyedsayamdost, Discovery of *scmR* as a global regulator of secondary metabolism and virulence in *Burkholderia thailandensis* E264, Proceedings of the National Academy of Sciences 114(14) (2017) E2920-E2928.

[93] L.E. Dietrich, A. Price-Whelan, A. Petersen, M. Whiteley, D.K. Newman, The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of Pseudomonas aeruginosa, Mol Microbiol 61(5) (2006) 1308-21.
[94] S.H. Saunders, E.C.M. Tse, M.D. Yates, F.J. Otero, S.A. Trammell, E.D.A. Stemp, J.K. Barton, L.M. Tender, D.K. Newman, Extracellular DNA Promotes Efficient Extracellular Electron Transfer by Pyocyanin in Pseudomonas aeruginosa Biofilms, Cell 182(4) (2020) 919-932 e19.
[95] L. Houri-Zeevi, G. Teichman, H. Gingold, O. Rechavi, Stress resets ancestral heritable small RNA responses, Elife 10 (2021).

Figure 1. Detection of patterns of pathogenesis and immune inheritance in *C. elegans*. Upon infection, *C. elegans* detects pathogens through several mechanisms, including 1) infection-induced damage, 2) microbial secondary products, and 3) surveillance of core cellular processes through expanded gene families. These signals induce transcription of immune response genes in the parents and 4) confer immunity to their progeny. Image was generated with BioRender.


lumen
intestinal cells
gonad
DAMPs
1
2
sRNA
3
toxin
4
clec, irg, nlp, cyp, spp, cnc
damage-associated molecular patterns (DAMPs)
non-pathogenic microbe and their secondary products
pathogenic microbes and their secondary products
transcription of immune genes
immuno-primed embryos
transcription factor