Caenorhabditis elegans as a host for the study of host–pathogen interactions

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Recently, pathogenicity models that involve the killing of the genetically tractable nematode *Caenorhabditis elegans* by human pathogens have been developed. From the perspective of the pathogen, the advantage of these models is that thousands of mutagenized bacterial clones can be individually screened for avirulent mutants on separate petri plates seeded with *C. elegans*. The advantages of using *C. elegans* to study host responses to pathogen attack are the extensive genetic and genomic resources available and the relative ease of identifying *C. elegans* mutants that exhibit altered susceptibility to pathogen attack. The use of *Caenorhabditis elegans* as the host for a variety of human pathogens is discussed.

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Abbreviation
PCD programmed cell death

Introduction

An important question in the study of host–pathogen interactions is whether or not the underlying mechanisms of pathogenesis and host defense are highly conserved. Our laboratory and others have addressed this question by developing pathogenesis models that involve the infection of simple non-vertebrate hosts by human bacterial pathogens. For a given pathogen, if there is a large overlap between the virulence factors required to infect both the non-vertebrate and vertebrate hosts, then the basic mechanism of pathogenesis is most likely host-independent. Similarly, if a particular pathogen activates the same host-defense-related genes in both vertebrates and non-vertebrates, then the underlying mechanism of innate immunity is most likely highly conserved.

In this review, we shall focus on the use of *Caenorhabditis elegans* as the host for a variety of human pathogens. This organism's short 2–3-week life span and small (97 Mb), fully sequenced genome facilitate genetic and genomic analysis, offering an ideal compromise between complexity and tractability. Also, a wealth of data has demonstrated that a variety of developmental, neurological, cell biological and biochemical processes have been highly conserved between *C. elegans* and mammals.

C. elegans as a host for broad-host-range pathogens

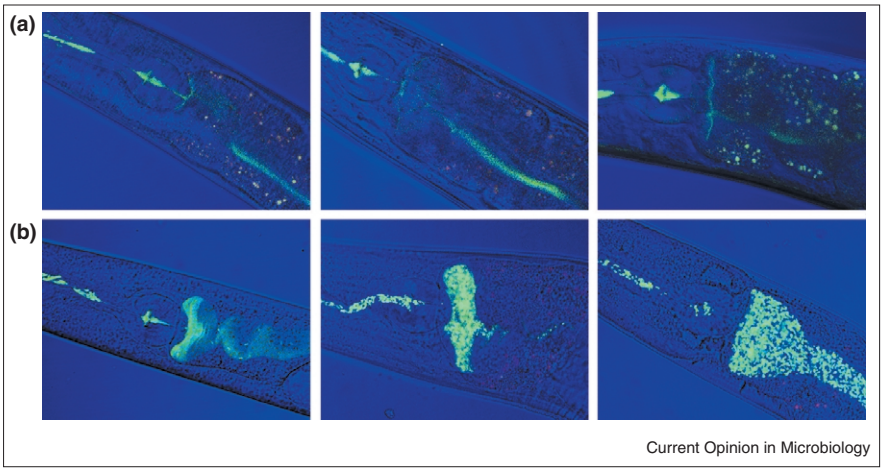
Pseudomonas aeruginosa, a ubiquitous environmental organism and important opportunistic human pathogen, kills *C. elegans* by at least three distinct mechanisms, depending on the *P. aeruginosa* strain and the medium on which *P. aeruginosa* is grown [1,2]. This illustrates a key aspect of the *C. elegans* model: the mode and extent of killing depends on a variety of genetic and environmental factors. Typically, *C. elegans* are propagated in the laboratory by feeding them *Escherichia coli* strain OP50 grown on relatively low-osmolarity nematode growth (NG) medium. *E. coli* is effectively disrupted by the *C. elegans* pharyngeal grinder and essentially no intact bacterial cells can be found in the intestinal lumen. In contrast, when *C. elegans* is fed *P. aeruginosa* strain PA14 grown on NG medium, PA14 accumulates within the lumen of the *C. elegans* intestine, killing worms relatively slowly over the course of 2–3 days ('slow killing') [1]. In contrast, PA14 grown on rich and high-osmolarity media kills worms quickly by excreting low-molecular-weight toxins ('fast killing') [1,3]. Grown on rich brain–heart infusion medium, the well-studied and sequenced strain of *P. aeruginosa*, PAO1, appears to kill *C. elegans* by a third mechanism that involves the generation of one or more neurotoxins, including hydrogen cyanide ([2,4].

Two other Gram-negative opportunistic human pathogens also kill *C. elegans*. *Serratia marcescens* kills by a toxin-mediated mechanism or by establishing an infection [5], and *Burkholderia pseudomallei* kills by a mechanism involving a neuromuscular endotoxin that targets Ca^{2+} signaling mediated by L-type Ca^{2+} channels [6].

C. elegans as a host for specialized pathogens

Some pathogens that have co-evolved or have had a long-standing association with their hosts, such as *Salmonella enterica*, appear to utilize finely tuned host-specific strategies to establish a pathogenic relationship [7]. It has been proposed that, when *Salmonella* are present in the vertebrate intestinal lumen, they respond to a number of relevant environmental conditions by producing protein effectors that are translocated into host cells by a type III secretory system and that alter specific signal-transduction pathways within host cells [8]. Although the type III secretion machinery is highly conserved among a variety of plant and animal bacterial pathogens, it is not clear if the translocated virulence factors (effector proteins) and their targets in the host are also broadly conserved. Despite the expectation that the host range of *S. enterica* would be limited to vertebrate species, several *S. enterica* serovars, including *Salmonella typhimurium*, kill *C. elegans* [9••,10••].

Figure 1



S. typhimurium proliferates in the *C. elegans* intestine. Young adult hermaphrodite worms were observed after 5, 24 and 48 hours of continuous feeding **(a)** on *E. coli*/green fluorescent protein (GFP) or **(b)** *S. typhimurium*/GFP lawns. *S. typhimurium* proliferates in the worm intestine and, after 48 hours, the intestinal lumen is distended and full of intact bacteria. In contrast, no intact bacteria are observed when the worms are fed with *E. coli*/GFP.

This killing, which takes place over the course of several days, is significantly slower than the so-called 'slow killing' described above for *P. aeruginosa* strain PA14 (Figure 1). An important point is that *S. typhimurium*-mediated killing of *C. elegans* was initially overlooked, apparently because the parental hermaphrodite worms initially exposed to *S. typhimurium* produce large numbers of progeny that grow to full-sized adults before they are also killed, obscuring the killing of the parental worms. To avoid this problem, it is necessary to transfer the initially exposed nematodes to fresh *S. typhimurium* lawns each day or to use temperature-sensitive *C. elegans* mutants, such as *glp4*, that do not produce progeny.

One feature that distinguishes the *P. aeruginosa* and *S. typhimurium* models is the establishment of a persistent infection, by *S. typhimurium* in the *C. elegans* intestine, that cannot be displaced by transferring the worms from a *S. typhimurium* lawn to an *E. coli* lawn. Following transfer, a high titer of *S. typhimurium* persists in the intestinal lumen, ultimately killing the worms [9••]. In contrast, when worms feeding on a *P. aeruginosa* lawn are transferred to *E. coli*, the *P. aeruginosa* that accumulated in the intestine is rapidly expelled by the normal rhythmic defecation process and the worms live a normal life span. These observations suggest that *S. typhimurium* may adhere to intestinal receptors that have been conserved between nematodes and mammals.

Gram-positive human pathogens also kill *C. elegans*

Recent work from our laboratory shows that several Gram-positive human pathogens, including *Streptococcus pneumoniae* (pneumococcus), *Staphylococcus aureus* and *Enterococcus faecalis*, also kill adult *C. elegans* when the bacterial lawns are grown on brain–heart infusion medium, whereas other Gram-positives, including *Streptococcus pyogenes* (Group A streptococci) and *Enterococcus faecium*, do not kill. The failure of *S. pyogenes* and *E. faecium* to kill, however, must be evaluated in the context that killing in the *C. elegans* model is determined, in part, by the medium on which the bacterial lawn is grown. *S. aureus*, for example, fails to kill *C. elegans* when grown on standard L-broth agar (R Feinbaum, F Ausubel, unpublished data), whereas *E. coli* OP50 kills when grown on brain–heart infusion medium [11•].

E. faecalis and *E. faecium* were selected among the Gram-positive human pathogens for initial characterization because both *E. faecalis* and *E. faecium* kill *C. elegans* eggs and newly hatched worms. This simplifies the pathogenesis model because, like *S. typhimurium*, the Gram-positive pathogens (with the exception of *S. pneumoniae*) do not kill the worms quickly enough to prevent the generation of a brood. Interestingly, even though *E. faecium* does not kill adult worms, *E. faecium* cells accumulate to high titers in the intestinal lumen. This is not a consequence of the inability of *C. elegans* to effectively grind Gram-positive bacteria, as no intact *Bacillus subtilis* cells can be found in the *C. elegans* intestine. Similar to *S. typhimurium*, *E. faecalis* (but not *E. faecium*) establishes a persistent infection in the *C. elegans* intestine [11•].

Microbacterium nematophilum, a natural *C. elegans* pathogen

A specific *C. elegans* pathogen, *Microbacterium nematophilum*, has been recently discovered. These bacteria adhere to the anal region of the nematodes and induce localized swelling of the underlying hypodermal tissue. Although the *C. elegans–M. nematophilum* interaction is non-lethal for the worm, it has been suggested to be parasitic, owing to the morphological changes in and lack of obvious benefits for the host [12].

Pathogen virulence factors involved in *C. elegans* killing

Both forward and reverse genetic analyses have been used to identify bacterial virulence factors that are required for *C. elegans* killing as well as mammalian pathogenesis,

thereby validating the use of *C. elegans* as a model host. Interestingly, the bacterial virulence factors identified in studies of this type will most likely depend on the environmental conditions used for screening for mutant phenotypes. For example, in the case of *P. aeruginosa* PA14, screening of a random transposon insertion library identified five genes (out of 3300 screened) involved in 'fast killing' on high-osmolarity medium, and eight genes (out of 2400 screened) involved in slow killing on low-osmolarity medium [1,3]. There was no overlap between the two sets of genes, and the mutations that affected fast killing did not affect slow killing and vice versa. Nevertheless, most of the genes identified in both screens were also shown to be required for maximum pathogenicity in a mouse thermal injury model. Several of these genes were previously known virulence factors, including the following: a locus (*hrpM*) that controls pathogenicity in the plant pathogen *P. syringae* and is involved in fast killing; a pair of two-component regulators (*gacA* and *gacS*) that is involved in slow killing; and a regulator of the quorum-sensing cascade (*lasR*) that is involved in slow killing. Novel genes not previously related to mammalian virulence were also identified in this screen [3,13–15], indicating that the *C. elegans* model is an efficient way to scan the entire *P. aeruginosa* genome for previously unknown non-essential genes involved in mammalian pathogenesis.

Surprisingly, given the importance of type III secretory systems in a variety of pathogenic mechanisms, *P. aeruginosa* PA14-mediated fast or slow killing of *C. elegans* does not appear to require type III pathway-secreted effector proteins (S Miyata, F Ausubel, unpublished data). Similarly, lipopolysaccharide (LPS) O-antigen, aminoglycoside/macrolide efflux pumping and type-II-pathway-secreted effector proteins — all of which are key virulence components in other systems — are not required for *B. pseudomallei*-mediated killing of *C. elegans* [6]. It is possible that the effects of translocated protein effectors may be masked by diffusible toxins that seem to be involved in the killing caused by *P. aeruginosa* and *B. pseudomallei*.

In contrast to *P. pneumoniae* and *B. pseudomallei*, both reverse and forward genetic approaches have shown that not only several genes located in the *S. typhimurium* pathogenicity island-1 (SPI-1), which encodes a type III secretory system, but also at least two type-III-pathway-secreted effector proteins are important for virulence in *C. elegans* (A Aballay, E Drenkard, F Ausubel, unpublished data). These results were somewhat surprising, given the prevailing view in the field that the specific interactions between *Salmonella* effector proteins and their host-cell targets reflect a long evolutionary arms race between pathogen and host.

In the case of *E. faecalis*, several previously characterized virulence factors, including cytolysin, gelatinase and the *fsrABC* regulatory system (which controls gelatinase production) were shown to be required for full pathogenicity in *C. elegans* [11•]. In addition, at least one previously unknown *E. faecalis* virulence-related gene, *scrB* (which encodes a sucrose-6-phosphate hydrolase, an enzyme involved in sucrose catabolism), was identified by screening of a transposon Tn*917* mutant library.

Reverse genetic analysis of the *C. elegans* innate immune response

Essentially nothing is known about the *C. elegans* innate immune response to bacterial pathogens. Although *C. elegans* and *Drosophila melanogaster* have been placed in sister phyla, *C. elegans* does not appear to have an intact Toll signaling pathway, a central feature of the insect and mammalian innate immune responses. In insects and mammals, a set of Toll-like receptors appears to be involved in the recognition of pathogen-associated molecular patterns (PAMPS), defined as components of pathogen-specific macromolecules such as lipopolysaccharide (LPS) and bacterial flagella [16]. The nematode genes encoding proteins homologous to several components of the Toll signaling pathway — Toll, IRAK, Traf1 and IκB — were identified and the corresponding deletion mutants generated. However, none of these mutants exhibited enhanced susceptibility to several pathogens, compared to a non-pathogenic *E. coli* control [17•]. On the other hand, the mutants had a shorter life span than wild-type worms when feeding on *E. coli*, a phenotype that may be expected for an 'immunocompromised' worm. This points out a major experimental difficulty in studying innate immunity in *C. elegans*. No biochemical or molecular markers, such as pathogen induction of defense-related genes, have been correlated with *C. elegans* immunity. Indeed, it is not known whether *C. elegans* mounts a specific immune response to pathogenic bacteria. Hopefully, genomic technologies, such as global transcription profiling analysis, will identify specific genes that can be used as reporters of induced innate immunity. Initial transcriptional profiling analysis has been used to identify *C. elegans* genes induced by *S. marcescens* and, among several nematode-specific genes, lectin-encoding genes where shown to be upregulated (J Ewbank, personal communication). Lectins are known to play a role in innate immunity of both vertebrates [18] and invertebrates [19].

One candidate for a marker of an innate immune response that is observed in evolutionarily disparate species is programmed cell death (PCD). Interestingly, *S. typhimurium* (but not *P. aeruginosa*) triggers somatic signals that induce PCD in *C. elegans* germline cells. Because the germline cells are contained in the gonads and because there is no evidence that *S. typhimurium* invades *C. elegans* cells, these data suggest that *Salmonella* may generate signals that affect cells not in direct contact with *Salmonella*. Using a variety of *C. elegans* mutants in which cell death is blocked, it was shown that *S. typhimurium*-induced germline cell death is dependent on the well-characterized CED-9/CED-4/CED-3 pathway homologous to the BCL2/APAF-1/CASPASE pathway in mammalian cells. Moreover, *ced-3* and *ced-4* mutants are hypersensitive to *S. typhimurium*-mediated killing, suggesting that PCD (or the CED-9/CED-4/CED-3

signal transduction pathway) may be involved in a *C. elegans* defense response to pathogen attack [20•]. Such a pathway could operate in the *C. elegans* intestine, for example, which is in direct contact with potential bacterial pathogens.

C. elegans defense mechanisms against bacterial toxins

In addition to Toll and PCD pathway components, several other *C. elegans* genes have been tested for their roles in pathogen resistance. For example, certain *C. elegans* P-glycoprotein (*pgp*) mutants that have defective efflux pumps are hypersusceptible to *P. aeruginosa* P14-mediated fast killing, but not slow killing. This was shown to be due to the synthesis by PA14 of pyocyanin, a tricyclic secondary metabolite that reacts with oxygen to form active oxygen species, including superoxide and hydrogen peroxide [21]. These data suggested that the ability to pump diffusible toxins out of cells is an important resistance strategy for *C. elegans*. Consistent with the conclusion that pyocyanin is an important toxin involved in fast killing, two *C. elegans* mutants (*mev-1(kn1)* and *rad-8(mn163)*) that are more susceptible to oxidative stress were found to be more susceptible to fast killing also, whereas a *C. elegans* mutant (*age-1(hx546)*) that is more resistant to oxidative stress was found to be more resistant to *P. aeruginosa* killing [3].

By forward genetic analysis, Darby *et al.* [2] isolated *C. elegans* mutants that are resistant to killing, on brain–heart infusion medium, mediated by toxin produced by *P. aeruginosa* strain PAO1. Worms carrying mutations in the previously described *egl-9* gene involved in egg laying were found to be resistant to the lethal paralysis caused by *P aeruginosa*. It was hypothesized that the toxin aberrantly activates muscle contraction by acting either directly on EGL-9 or on a pathway that includes it. Given that the *C. elegans* killing phenotype exhibited by *B. pseudomallei* or *Burkholderia thailandensis* resembles that of *P. aeruginosa* PAO1, the effect of *egl-9* on the infection by these two pathogens was tested. Although the *egl-9* mutation confers some resistance to *Burkholderia*, the effect was not as dramatic as that obtained for *P. aeruginosa* PAO1. Interestingly, loss-of-function mutations in genes for L-type voltage-gated Ca^{2+} channel subunits (*egl-19(n582)* and *unc-36(n251)*) resulted in enhanced susceptibility to *B. pseudomallei* or *B. thailandensis*, whereas gain-of-function mutations (*egl-19(n2368)sd* and *egl-19(ad695)*) enhanced survival, suggesting that the neuromuscular intoxication caused by *Burkholderia* acts, in part, through a disruption of normal Ca^{2+} signal transduction [6]. Since *egl-19* and *unc-43* act in the same neuromuscular signaling pathway, *unc-43* worms were also studied. It was found that *Burkholderia* intoxication of *C. elegans* is capable of suppressing the lethargic phenotype of *unc-43(n498)* gain-of-function mutations. The mammalian counterpart of Unc-43 is CDPKII, the most abundant kinase in nerve cells, which is also expressed in B and T cells (J Jeddeloh, personal communication). The Bt toxin of *Bacillus thuringiensis* has also been found to be toxic to *C. elegans*, and 10 mutants that resist the intoxication were isolated [22]. These mutants correspond to five genes, one of which, called *bre-5* (for *B*t *re*sistance) encodes a putative galactosyltransferase that may be required to form a carbohydrate structure necessary for toxin binding [23].

C. elegans defense mechanisms against nematode-specific pathogens

In the case of the nematode-specific pathogen *M. nematophilum*, about 200 *C. elegans* mutants have been examined for enhanced susceptibility or resistance [12]. Certain mutants with altered surface antigenicity (*srf-2*, *srf-3* and *srf-5*) [24,25] were found to be resistant to infection by *M. nematophilum*. Resistance in this case appears to be due to a change in the surface properties of the cuticle that blocks adherence of the bacteria. Interestingly, the three mutants that are more resistant to *M. nematophilum* exhibit increased susceptibility to a nematode fungal pathogen, *Duddingtonia flagrans* [26].

Forward genetic analysis to identify *C. elegans* defense mechanisms against bacterial infections

As described above, the power of the *C. elegans* genetic system can be readily exploited to identify mutants that are either more susceptible or more resistant to bacterial killing. Hopefully, some of these mutants will correspond to components of a general *C. elegans* innate immune response. For example, by using *P. aeruginosa* as the pathogen, several *C. elegans* mutants (called *e*nhanced *s*usceptibility to *p*athogens [*esp*] mutants) that exhibit hypersusceptibility to pathogen-mediated killing have been isolated (M-W Tan, G Alloing, R Feinbaum, D Kim, J Villanueva, FM Ausubel, unpublished data). Three *esp* mutants that have been studied in depth have the same life span as wild-type worms and do not have any obvious phenotype, such as a defective pharyngeal grinder, that could make them more susceptible to pathogen-mediated killing. Studies are currently under way to clone the genes corresponding to these mutants.

Conclusions

C. elegans has proven to be an efficient host model for both broad-host-range and specialized human pathogens. As validation of the relevance of the *C. elegans* model, a variety of studies have shown that there is a significant amount of overlap between the pathogen genes involved in *C. elegans* killing and mammalian pathogenesis. Also, several genes involved in host defense responses have been identified. Further studies using the power of *C. elegans* genetic and genomic analyses are expected to provide insights into the understanding of what makes bacteria pathogenic and hosts resistant.

Update

Recent work has demonstrated that pertussis toxin expression in transgenic *C. elegans* produces a phenotype that suggests that the toxin target is conserved between mammals and nematodes [27••]. Moreover, it has been reported that hydrogen cyanide is the primary toxic

component that mediates the paralytic killing of *C. elegans* caused by *P. aeruginosa* PAO1 [4]. Hydrogen cyanide exerts its effect on *C. elegans* through EGL-9 or on a pathway that includes it. Intriguingly, EGL-9 was recently identified as a dioxygenase that is part of a complex that plays a central role in mammalian oxygen homeostasis [28].

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