Microreview

Caenorhabditis elegans as a model for intracellular pathogen infection

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Summary

The genetically tractable nematode Caenorhabditis elegans is a convenient host for studies of pathogen infection. With the recent identification of two types of natural intracellular pathogens of C. elegans, this host now provides the opportunity to examine interactions and defence against intracellular pathogens in a whole-animal model for infection. C. elegans is the natural host for a genus of microsporidia, which comprise a phylum of fungal-related pathogens of widespread importance for agriculture and medicine. More recently, C. elegans has been shown to be a natural host for viruses related to the Nodaviridae family. Both microsporidian and viral pathogens infect the C. elegans intestine, which is composed of cells that share striking similarities to human intestinal epithelial cells. Because C. elegans nematodes are transparent, these infections provide a unique opportunity to visualize differentiated intestinal cells in vivo during the course of intracellular infection. Together, these two natural pathogens of C. elegans provide powerful systems in which to study microbial pathogenesis and host responses to intracellular infection.

Introduction

An intracellular lifestyle offers benefits as well as challenges for microbial pathogens (Casadevall, 2008). By invading and replicating inside of host cells, intracellular pathogens can bypass antimicrobial defences in the extracellular environment, and gain access to intracellular resources. However, these pathogens must traverse cell membranes to enter and exit the cell, navigate the host cytoskeleton and also evade intracellular defences. Despite these challenges, many pathogens have adopted an intracellular lifestyle. Intracellular pathogens are classified as either facultative intracellular pathogens, which are microbes that can replicate both intracellularly and extracellularly, or obligate intracellular pathogens, which are microbes that replicate exclusively inside of host cells. Obligate intracellular pathogens are widespread throughout nature and cause significant disease, but can be challenging to study in the laboratory because they often are not culturable outside their hosts.

Epithelial cells provide major routes of entry for intracellular pathogens, such as epithelial cells of the skin, and epithelial cells of the intestine (Gallo and Hooper, 2012). Visualizing and studying infections in these cells in vivo is important for understanding the mechanisms of disease pathogenesis and transmission. In this review we will describe the use of the nematode Caenorhabditis elegans as a simple transparent host for studies of intestinal infection by intracellular pathogens in vivo. In particular, we will focus on the recent identification of two natural, obligate intracellular pathogens of the nematode that infect the intestine.

The C. elegans body plan, defence system and ecology

Caenorhabditis elegans is an important genetic model system that has been the platform for many discoveries in biology, including key findings in the fields of neurobiology, development and small RNAs (Fire et al., 1998; The C. elegans Sequencing Consortium, 1998; Marx, 2002). Most of these studies have been performed with the standard laboratory strain of C. elegans called N2, which was isolated from Bristol, England decades ago (Brenner, 1974). In the last few years, the ecology of C. elegans in the wild has gained more attention due to the increased isolation of wild-caught nematodes and their associated microbes (Félix and Duveau, 2012). C. elegans is commonly found in rotting fruits and stems, and appears to feed on a variety of different microbes, which can be nutritious or pathogenic. These studies have provided a
global collection of wild-caught nematodes, and have identified some of the natural pathogens of *C. elegans* (see below).

*Caenorhabditis elegans* has a simple transparent body plan, which has facilitated many studies in the worm. For example, this transparency allowed *C. elegans* development to be described in great detail, with every cell division from a fertilized egg to a 1000-cell adult placed in a cell lineage map that illustrates the formation of all tissues in the animal (Sulston *et al*., 1983). The *C. elegans* tissues that are in regular contact with microbes are the hypodermis and the intestine. The hypodermis is a single layer epithelium that surrounds the animal and is a multinucleate syncytium that secretes collagen to form a tough outer cuticle (Chisholm and Hardin, 2005). *C. elegans* feeds on microbes that are ingested by the pharynx, which helps degrade food sources before they reach the intestine (Fig. 1A, B). The intestine is comprised of 20 epithelial cells that are mostly in pairs of cells that form a tube that runs the length of the animal (McGhee, 2007). *C. elegans* intestinal cells share many morphological similarities with human intestinal epithelial cells, including actin-rich microvilli on the apical side of cells that absorb nutrients from the lumen (Fig. 1C, D). These microvilli are anchored into a cytoskeletal structure called the terminal web, which spans the cell and connects into apical cell–cell junctions. Endocytosis and exocytosis in the worm intestine operate through conserved cytoskeletal and trafficking proteins, such as tubulin and small GTPases. Polarized vesicular trafficking occurs in this tissue with distinct sorting to apical and basolateral sides, similar to trafficking in epithelial cells of humans. The ability to visualize these structures and processes in a live animal provides a powerful system in which to examine pathogen infection of the intestine.

How does *C. elegans* defend itself against pathogen infection? *C. elegans* does not appear to have professional immune cells, such as phagocytes (Irazoqui *et al*., 2010a). Consequently, in terms of cellular defence, *C. elegans* appears to rely exclusively on epithelial cell immunity, which is described in more detail below. Although *C. elegans* does not have a canonical adaptive immune system using specialized immune cells, it will avoid pathogens (Pujol *et al*., 2001). This avoidance is learned over time, constituting a form of adaptive immunity using behaviour instead of specialized immune cells (Zhang *et al*., 2005). Induction of these aversive responses is presumably driven by signals emanating from the intestine to signal an infected or ‘sick’ state. RNA interference (RNAi) is another adaptive immune mechanism employed by *C. elegans* in defence, which protects against viral re-infection after exposure during the life of the animal and can even be passed to its progeny (Rechavi *et al*., 2011). Thus, with a combination of epithelial immune
defences and aversive learning, *C. elegans* has been able to thrive globally despite widespread microbial challenges (Félix and Braendle, 2010; Félix and Duveau, 2012). Some of the mechanisms employed by *C. elegans* during pathogenic encounters are described below.

### *C. elegans* infection by facultative intracellular pathogens and host defence

Most infection studies in *C. elegans* thus far have focused on clinically relevant pathogens, which have provided a strong framework for studying infections in this host (Irazoqui *et al.*, 2010a; Pukkila-Worley and Ausubel, 2012). Feeding-based infections of *C. elegans* are relatively straightforward to perform, which aids studies of intestinal infections in this host. The primary food source in a laboratory setting is the *E. coli* strain OP50, but other bacteria and fungi also support growth and reproduction (Brenner, 1974; Mylonakis *et al.*, 2002). Several feeding-based infection models have been developed, which involve simply substituting a microbial pathogen in place of the normal food source of *E. coli*. The lifespan of *C. elegans* on *E. coli* is only about 2 weeks, which makes it easy to assess whether pathogens cause a shortening of lifespan. The wide variety of microbial pathogens that have been shown to infect and kill *C. elegans* have been described in other reviews (e.g. see table 1 in Sifri *et al.*, 2005), so we will only briefly point out here the infections with facultative intracellular pathogens of humans, and then highlight some features of *C. elegans* immunity.

There are several facultative intracellular pathogens of clinical importance that have been shown to infect *C. elegans* (Table S1). These microbes include the human fungal pathogen *Cryptococcus neoformans*, and the human bacterial pathogens *Shigella* spp., *Salmonella enterica* and *Listeria* spp. (Mylonakis *et al.*, 2002; Burton *et al.*, 2006; Kesika and Balamurugan, 2012). Unlike in mammals, these pathogens remain extracellular throughout infection of *C. elegans*, with the exception of *Salmonella enterica* Serovar Typhimurium. Early reports of *S. typhimurium* infection indicated that it remained extracellular (Aballay *et al.*, 2000; Labrousse *et al.*, 2000), but a more recent report has indicated that *S. typhimurium* can be found intracellularly and kills more efficiently when autophagy genes are disrupted (Jia *et al.*, 2009). Autophagy may therefore constitute one of the conserved mechanisms employed by *C. elegans* to defend against intracellular pathogens. *Listeria* species have been studied in *C. elegans* models of infection, but there are conflicting reports of their pathogenicity and no intestinal invasion has been observed (Thomsen *et al.*, 2006; Forrester *et al.*, 2007; Guha *et al.*, 2013). Part of the difficulty in modelling intracellular infections in *C. elegans* with human pathogens may be due to intrinsic differences in tissue architecture. For instance, *Listeria monocytogenes* invades mammalian intestinal cells specifically at junctions during cell extrusion or through the lumenal side of goblet cells, neither of which are features of the *C. elegans* intestine (Pentecost *et al.*, 2006; Nikitas *et al.*, 2011). In order to use *C. elegans* as a host to model intracellular intestinal infection by human pathogens, it may be necessary to use pathogens that invade the luminal side of epithelial cells.

*Caenorhabditis elegans* responds to bacterial and fungal pathogens by robust transcriptional upregulation of many effector genes (Shivers *et al.*, 2008; Irazoqui *et al.*, 2010b). Effectors include those that appear to provide intracellular defence, such as UDP-glucoronyl transferases and cytochrome P450s that could inactivate intracellular toxins, and P-glycoprotein pumps that could excrete toxins out of the cell. *C. elegans* also has a vast repertoire of genes that could provide extracellular defence. Indeed about 17% of the genes in the *C. elegans* genome are predicted to encode secreted proteins, and a third of those are upregulated by exposure to pathogens (Suh and Hutter, 2012). These factors often are part of expanded gene families and include some proteins conserved with mammals, such as secreted C-type lectins, which have anti-microbial activity, but most of these secreted factors are nematode-specific. Two notable examples include the *C. elegans* nips or neuro-peptide-like proteins and caenacins, both of which are secreted peptides that have anti-fungal activity.

Effector gene expression is controlled by several distinct signalling pathways in *C. elegans*. For example, the conserved PMK-1 p38 MAP kinase pathway controls induction of nips and other subsets of effectors, and provides defence against bacterial and fungal infection. What induces activation of this and other defence pathways? Classical pattern recognition receptors have not yet been described in *C. elegans*. Instead, recent studies indicate that defensive responses can be induced by pathogen-directed perturbation of core processes, so-called effector-triggered immunity (ETI). ETI was originally described in plants, and is increasingly appreciated to be important in animal immunity as well (Stuart *et al.*, 2013). For example, *Pseudomonas aeruginosa*-directed block of host translation was shown to induce expression of some PMK-1 dependent genes, and also activate the ZIP-2 bZIP transcription factor defence pathway in *C. elegans* (Dunbar *et al.*, 2012; McEwan *et al.*, 2012). Interestingly, some of the other pathogen-induced transcriptional responses in *C. elegans* appear to be controlled non-cell-autonomously by the nervous system. For example, fungal infection induces expression of a TGF-beta ligand in neurons, which then activates a hypodermal pathway to induce expression of antimicrobial caenacins (Zugasti and Ewbank, 2009). This interplay of tissues has parallels
with mammalian immunity, for which the nervous system has increasingly been recognized as playing a regulatory role (Chiu et al., 2012). Altogether, these findings provide insights into how _C. elegans_ can integrate signals throughout the body to sense and respond to microbial infection, and provide a foundation for making progress with the recent discoveries of _C. elegans_ infection by natural, obligate intracellular pathogens, which are the subject for the rest of the review.

**C. elegans as a host for intracellular infection by viruses**

Natural viral infection models, as well as models using non-natural viruses of _C. elegans_, have been established. Viral infections of _C. elegans_ can be followed from the stages of invasion through to transmission. Furthermore, _C. elegans_ shares a conserved antiviral RNA interference (RNAi) defence system with other animals. These advantages make _C. elegans_ a flexible platform for studying host–virus interactions.

**Non-natural virus models**

Models of Flock House virus (FHV), vesicular stomatitis virus (VSV) and vaccinia virus (VV) infections have been established in _C. elegans_ (Lu et al., 2005; Schott et al., 2005; Wilkins et al., 2005; Liu et al., 2006). FHV has been studied in _C. elegans_ by introducing a genetically encoded viral replicon in transgenic animals, while VSV infections have been modelled by adding recombinant virus particles to primary embryonic _C. elegans_ cell cultures. Experiments with these models identified a prominent role for RNAi in defence against viruses, which is in agreement with data from host–virus interactions modelled in plants and insects. _C. elegans_ mutants defective for RNAi had higher levels of viral infection, while mutants with hyperactive RNAi responses had lower levels of viral infection. Thus, FHV and VSV subvert the wild-type _C. elegans_ antiviral responses enough to replicate, but can replicate more successfully when host defences are defective. Further work in the FHV model identified host factors that are conserved with mammalian antiviral proteins (Lu et al., 2009). Among the genes that were involved in viral interactions were Dicer-related helicases. Homologous proteins in mammals have been shown to both positively and negatively regulate immune responses to viruses. Likewise, _drh-1_ was found to positively regulate immunity to FHV in _C. elegans_, while _drh-2_ exhibits negative regulation. These conserved RNAi responses are hypothesized to have evolved in part to limit viruses that have double-stranded RNA genomes or that generate double-stranded RNA intermediates during replication within host cells. VV is a double stranded DNA virus that may generate double stranded RNA, but mutants in the RNAi pathway did not affect the outcome of infection in _C. elegans_. Instead, core members of the programmed cell death pathway appear to limit VV replication, as mutant animals have higher pathogen load. Interestingly, mutants in the programmed cell death pathway were not more susceptible to killing, suggesting that alternative mechanisms of resistance likely exist.

The experimental viral models discussed above have provided valuable tools, and further work with these models will likely reveal additional insight in to the mechanisms of antiviral defence pathways. And with the recent discovery of natural viral infections of _C. elegans_ described below, researchers should also be able to address other key aspects of host–viral interactions, such as transmission among live animals and the identification of genetic substrates participating in co-evolutionary processes in the wild.

**Natural virus models**

Natural viral infections in _C. elegans_ have recently been identified, providing powerful models for investigating co-evolution of intracellular interactions. Field surveys carried out by Félix and colleagues led to the identification of two infected _Caenorhabditis_ isolates, a _C. elegans_ strain called JU1580 isolated from a rotting apple from Orsay, and a _Caenorhabditis briggsae_ strain called JU1264 isolated from a snail found on a rotting grape from Santeuil (Félix et al., 2011). Both strains exhibited a range of intestinal pathologies observable by microscopy, which overall appear to compromise the integrity of intestinal cells (Fig. 2A). These phenotypes were not observed in the progeny of bleached parents, arguing against the possibility that the viruses were vertically transmitted. Furthermore, the phenotypes could be reinitiated in bleached progeny by adding a 0.2 µm filtrate from affected animals, suggesting viral infection. Electron micrographs of affected animals revealed the ultrastructural details of these cell pathologies, and detected electron dense particles that were reminiscent of virions. Sequencing of affected animals identified two novel viruses most closely related to the _Nodaviridae_ family as the causes of infection. And now a third virus infecting a wild-caught _C. briggsae_ from Le Blanc, France, has recently been reported, which is also related to the _Nodaviridae_ family (Franz et al., 2012). Altogether, these are the first models of natural host–virus infections in _Caenorhabditis_.

Nodaviruses are well studied due to their small genome sizes and ability to induce and suppress host RNAI defences. The Orsay and Santeuil viruses were identified as positive-sense RNA viruses with small bi-partite genomes. Phylogenetic analyses positioned the

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Orsay and Santeuil viruses as distantly related to all other characterized nodaviruses, and most similar to each other. A predicted open reading frame unique to the Orsay and Santeuil viruses was identified, but no functional role has been attributed. No obvious homologue to the RNAi suppression protein found in FHV was located in the Orsay or Santeuil genomes, but several experiments suggest that an antiviral RNAi response is initiated during infection. Small RNAs from the Orsay genome were identified in infected JU1580 animals. These small RNAs had range of nucleotide lengths for the sense strand RNAs, which is suggestive of non-specific Dicer products. Antisense RNAs showed length and nucleotide biases, favouring 22 nucleotide transcripts beginning with a guanidine residue. The significance of this pattern is not fully understood, but is generally indicative of a regulated antiviral RNAi response. Additional support for the antiviral response mounted during Orsay virus infection is the observation that the N2 strain, while normally 100-fold more resistant to infection (i.e. has 100-fold less viral transcript) than JU1580, becomes just as susceptible when the RNAi pathway is disrupted, such as in \rde-1 Argonaute mutants. Thus, like nodaviruses, the Orsay virus of \textit{C. elegans} is genomically small and provokes RNAi defences.

In the Félix \textit{et al.} study, the authors also provide evidence for natural variation among strains in their ability to resist viral infection. They tested a small collection of genetically diverse \textit{C. elegans} strains for responses to exogenously added double-stranded RNA and the Orsay virus, and found variation in the efficiency of endogenous and antiviral RNA responses. A loose trend emerged, suggesting that strains with compromised endogenous RNAi pathways showed more symptoms of viral infection. However, this hypothesis could not explain the data from all the strains, pointing towards the likelihood that independent mechanisms can regulate antiviral defences. The phenotypes scored for antiviral responses were the intestinal pathologies observed by microscopy. This method could allow for detection of variation in the cell biology of viral responses among strains. Expanding these studies with additional strains is expected to permit genetic mapping of loci that have evolved in \textit{C. elegans} to cope with natural viruses.

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**C. elegans** as a host for intracellular infection by microsporidia

The first natural intracellular pathogen identified in *C. elegans* defined a new genus and species of microsporidia (Troemel *et al.*, 2008). It was named *Nematocida parisii* (strain ERTm1), or nematode-killer from Paris, because it was found in wild-caught *C. elegans* from a compost pit near Paris, and causes a lethal intestinal infection in its host. Microsporidia comprise a phylum of obligate intracellular parasites with over 1200 species, which are most closely related to fungi (Texier *et al.*, 2010). They parasitize all animal phyla, infecting single-celled ciliates to mammals, with some microsporidian species able to infect only one host, and other species able to infect a broad range of hosts. Microsporidia infections in humans and agriculturally important animals can be lethal, but the mechanisms underlying these interactions are not well understood (Didier and Weiss, 2011; Troemel, 2011).

The *N. parisii* life cycle inside the *C. elegans* host shares overall similarities with other microsporidia infections (Fig. 2B). First, an ingested spore encounters a host environment that triggers firing of an infection apparatus called a polar tube, which serves to inject a nucleus and sporoplasm into an intestinal cell. This invasion leads to formation of a membrane-bound intracellular stage called a meront that often replicates as a multi-nucleate meront. These meronts eventually differentiate into spores, which exit the cell and propagate infection. The details of these processes may differ in other host/microsporidia pairs, but the overall developmental structure appears to be well conserved. As with many other intestinal pathogens, *N. parisii* is transmitted via a faecal–oral route, whereby spores are defecated out by the animal and subsequently consumed by new hosts to continue the life cycle.

Little is known about the genetic and molecular details of these steps in the microsporidia lifecycle in any system, because it is not possible to manipulate microsporidia genetically, and they can only grow inside of host cells. The discovery of natural pathogens of *C. elegans* has provided a tractable model to study microsporidia infection. In addition to *N. parisii* (ERTm1), microsporidia have been isolated from nematodes around the world, indicating that these pathogens are a common cause of infection for nematodes in the wild (Troemel *et al.*, 2008; M.-A. Félix, unpubl. data).

*Nematocida parisii* infection of *C. elegans* has recently been used to illuminate key features of the exit strategy of this species of microsporidia, which can be divided into two main phases (Fig. 2B) (Troemel *et al.*, 2008; Estes *et al.*, 2011). The first phase involves a restructuring of the terminal web, which is a conserved structure of intestinal cells. Intestinal actin is normally restricted to the apical side of intestinal cells in the microvilli and terminal web. However, as meronts develop, actin is ectopically redistributed to the basolateral side of the cell where it forms filaments. Soon after this relocalization, there are gaps that appear in intermediate filaments at the terminal web. These gaps may be triggered by actin redistribution away from the apical side, as reducing levels of actin caused gap formation in the absence of infection. Other pathogens of *C. elegans* do not appear to cause gaps to form in the terminal web, indicating that gaps are due to a specific perturbation caused by *N. parisii* infection. This terminal web restructuring may serve to remove a barrier to exit, so that spores can exit out the apical side of cells back into the intestinal lumen.

In the second phase of exit spores are released from the cell and defecated by the animal, with thousands of spores being shed per hour. Spores begin to form shortly after the appearance of holes in the terminal web, and then exit the cell in a non-lytic manner. No host membrane is visible around spores that have exited to the intestinal lumen, suggesting that non-lytic exit does not occur via budding. Spore exit appears to be highly directional – spores only exit from the intestine on the apical side of cells and not on the basolateral side. Thus, spores likely use a directional cue from the host in order to escape into the lumen, which is required for its faecal–oral life cycle. The precise molecular mechanisms of this directional escape by *N. parisii* remain to be determined.

To learn more about the pathogenic mechanisms of *N. parisii*, as well as other microsporidian species, the Microsporidian Genomes Consortium was recently formed with the goal of sequencing several species of microsporidia. The genomes of three *Nematocida* strains were recently sequenced as part of this consortium, including *N. parisii* (ERTm1), a related *N. parisii* strain (ERTm3) isolated from wild-caught *C. elegans* in Santeuil, France, and a divergent strain called *Nematocida* sp1 (ERTm2) isolated from a *C. briggsae* host in India (Cuomo *et al.*, 2012). Microsporidia have the smallest known eukaryotic genomes (Keeling and Corradi, 2011), and in keeping with that, *Nematocida* genomes are extremely reduced. Genome sizes and sequence were highly similar between *N. parisii* ERTm1 and ERTm3, sharing approximately 4.1 Mb of sequence that is 99.8% identical. *Nematocida* sp1 ERTm2 was slightly larger at 4.6 Mb and shared only 68.3% average identity with *N. parisii*. The high divergence between ERTm1 and ERTm2 does not appear to be due solely to differential host specification, as both are infective to *C. elegans* and *C. briggsae*. Over 70% of the *N. parisii* genome is predicted to be coding and intronless, with non-coding intergenic regions that are short. Thus, *Nematocida* genomes are compact like some other microsporidia genomes. This reduction is thought to reflect a dependency on host factors, which can be redirected to facilitate parasitic growth.
How does a genomically reduced eukaryotic pathogen succeed in a complex host environment? Phylogenomic characterization of Nematocida, together with several other microsporidia species, uncovered some of the potential evolutionary strategies used by these pathogens for obligate intracellular growth (Cuomo et al., 2012). RNA-seq data of N. parisi expression over the course of ERTm1 infection in C. elegans demonstrated a dramatic takeover of intestinal cells, growing within infected cells at an estimated doubling time of approximately three hours. This rate is similar to yeast growing in rich media, indicating that N. parisi is well-adapted to grow in this intracellular environment. Common features identified in microsporidian genomes that may explain their rapid growth include the loss of the retinoblastoma (Rb) cell cycle inhibitor, which is lost in most human cancers. Because Rb serves as quality control in DNA replication, loss of Rb may lead to a higher mutation rate and explain the high sequence divergence among microsporidian genomes. Another interesting observation that came out of this study with potential parallels with cancer is that microsporidia appear to secrete the metabolic enzyme hexokinase into host cells early during infection. It is unclear if this secreted hexokinase would benefit pathogen or host, but one possibility is that it could benefit the pathogen by driving the host to upregulate glycolysis and the pentose phosphate pathway to provide more building blocks for growth like lipids, nucleotides and amino acids, similar to the Warburg effect in cancer. Genome studies have also revealed that microsporidia have horizontally acquired nucleoside transporters (Katinka et al., 2001; Cuomo et al., 2012), which presumably could import these host-derived building blocks to favour pathogen growth. Finally, genetic diversity in Nematocida and other microsporidia may be increased through mating and recombination, both of which were hypothesized from Nematocida genome data. Together with the diversity driven by rapid and mistake-prone growth, mating and recombination may allow Nematocida to keep up with the ongoing host–pathogen evolutionary arms race likely taking place between hosts and their microsporidian parasites.

Summary and future directions

Caenorhabditis elegans is an especially convenient whole-animal model for obligate intracellular pathogen infection, made possible by the recent identification of natural virus and microsporidian pathogens that infect its intestine. Although these pathogens infect internal tissues, the transparency of C. elegans makes it possible to visualize and study pathogenic mechanisms of entry, replication and exit, as well as host defence mechanisms within whole animals. The progression of infection can be observed in real-time by light microscopy; a technique that has been utilized to describe some of the basic cell biology phenotypes associated with viral and microsporidian infections (Fig. 2). Powerful new optogenetic techniques may facilitate further dissection of these infections. For example, specific protein–protein interactions between host and pathogen could be observed and manipulated by tagging with a genetically encoded mini singlet oxygen generator, allowing for visualization by fluorescence microscopy, specific and localized protein destruction by over-production of reactive oxygen species, and high-resolution imaging by electron microscopy (Tour et al., 2003; Shu et al., 2011). These kinds of experiments are ideally suited to the small, transparent, genetically tractable C. elegans model.

Intracellular infections of C. elegans by natural pathogens are simple to propagate in the lab, providing a convenient system in which to study the mechanisms of intracellular pathogen transmission. Horizontal transmission is commonly observed among intracellular pathogens, and was observed for the Orsay and Santeuil viruses in Caenorhabditis. Similarly, only horizontal transmission was observed during Nematocida infections, and recent studies indicate that Nematocida exit occurs in a directional manner into the intestinal lumen. This polarized exit is crucial for all pathogens transmitted via a faecal–oral route, but the underlying mechanisms are unknown. Neither the virus nor microsporidia infections of C. elegans characterized so far appear to be transmitted vertically. The mechanisms that prevent this kind of transmission are not well understood. Studying transmission strategies in C. elegans may clarify how and why one strategy may be favoured over another. Additionally, transmission of the Orsay virus was only successful in strains of C. elegans, while the Santeuil virus could only be propagated in the C. briggsae isolate JU1264. In contrast, all three Nematocida strains could infect both C. elegans and C. briggsae. These models may therefore be informative for understanding the factors that specify the transmission strategies and host range of intracellular pathogens.

Defences against intracellular pathogens seem to be distinct from those defined for extracellular pathogens. The RNAi pathway is a well-characterized regulator of antiviral immunity in both C. elegans and other organisms (Ding, 2010). Data from the first natural viral infections of C. elegans indicate that other pathways will be involved in regulating viral pathology. These may be elucidated in both natural and artificial models of viral infection. Resistance mechanisms that limit microsporidia infections are not well understood for any host. In mammals, resistance is known to involve T cells of adaptive immunity, but the molecular mechanisms are unknown (Didier and Weiss, 2011). In C. elegans, the major immune defence...
pathways regulated by p38 MAP kinase and insulin signalling pathways do not appear to be involved in defence against *N. parisi*i infection (Troemel et al., 2008), indicating that new pathways will be found to regulate defence against this intracellular pathogen.

Other promising future directions for these models will exploit the fact that the host and pathogens have presumably co-evolved. In other systems, studies of natural host/pathogen pairs have provided insights into the multi-layered interactive networks that can occur through coevolving bouts of adaptation and counter adaptation (Woolhouse et al., 2002). One outcome of these interactions can be mimicry by both host and pathogen of each other (Elde and Malik, 2009). Efforts towards understanding selective pressures that have shaped immunity and pathogenesis will be greatly bolstered by the ongoing progress in identifying the ecology and global diversity of *C. elegans* and its pathogens (Félix and Braendle, 2010; Andersen et al., 2012; Cuomo et al., 2012; Félix and Duveau, 2012). Recent work in *C. elegans* has provided experimental support for pathogens being a driving force behind sexual reproduction in their hosts, and has demonstrated that co-evolutionary dynamics generate genetic changes in host and pathogen that affect fitness within a relatively brief time frame (Morran et al., 2011). Combining the increasing genetic data from wild isolates with experimental co-evolution approaches should facilitate the discovery of the specific molecular interactions that determine the evolutionary trajectory of *C. elegans* and its pathogens. While still quite new, the discovery of two natural intracellular infections in *C. elegans* is expected to provide new insights into the logic underlying the structure of the *C. elegans* immune response and more generally, the functional interactions between hosts and their intracellular pathogens.

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**Supporting information**

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Pathogens tested for intracellular infection in *C. elegans* and features of immune response.