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Insights from *C. elegans* into Microsporidia Biology and Host-Pathogen Relationships

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Abstract

Microsporidia are poorly understood, ubiquitous eukaryotic parasites that are completely dependent on their hosts for replication. With the discovery of microsporidia species naturally infecting the genetically tractable transparent nematode *C. elegans*, this host has been used to explore multiple areas of microsporidia biology. Here we review results about microsporidia infections in *C. elegans*, which began with the discovery of the intestinal-infecting species *Nematocida parisii*. Recent findings include new species identification in the *Nematocida* genus, with more intestinal-infecting species, and also a species with broader tissue tropism, the epidermal and muscle-infecting species *Nematocida displodere*. This species has a longer polar tube infection apparatus, which may enable its wider tissue range. After invasion, multiple *Nematocida* species appear to fuse host cells, which likely promotes their dissemination within host organs. Localized proteomics identified *Nematocida* proteins that have direct contact with the *C. elegans* intestinal cytosol and nucleus, and many of these host-exposed proteins belong to expanded, species-specific gene families. On the host side, forward genetic screens have identified regulators of the Intracellular Pathogen Response (IPR), which is a transcriptional response induced by both microsporidia and the *Orsay virus*, which is also a natural, obligate intracellular pathogen of the *C. elegans* intestine. The IPR constitutes a novel immune/stress response that promotes resistance against microsporidia, virus, and heat shock. Overall, the *Nematocida/C. elegans* system has provided insights about strategies for microsporidia pathogenesis, as well as innate defense pathways against these parasites.

Keywords

C. elegans; Microsporidia; Nematocida; Tissue tropism; Syncytium; Intracellular Pathogen Response; Host-exposed proteins

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Conflict of Interest The authors declare that there is no conflict of interest.

Ethical Approval The chapter is a review of previously published accounts; as such, no animal or human studies were performed.

5.1 Introduction

Study of the genetically tractable nematode *Caenorhabditis elegans* has resulted in seminal discoveries ranging from RNA interference (RNAi) to apoptotic signaling pathways (Singh 2021). *C. elegans* has several advantages for research in the lab including reproduction by selfing of hermaphrodites, which allows for propagation without crossing, a 3-day reproductive life cycle, and a 1-mm-long transparent body plan that facilitates imaging. Over 20 years ago, *C. elegans* was established as a powerful system to study innate immune responses in a whole animal model, using bacterial and fungal pathogens that cause lethal infections in this host (Darby et al. 1999; Mahajan-Miklos et al. 1999; Pujol et al. 2001). The majority of infection studies in *C. elegans* have been performed with clinically relevant bacterial pathogens, but these studies have now broadened to include natural pathogens, including microsporidia infections as described below.

The Microsporidia phylum contains over 1400 species of obligate intracellular pathogens related to fungi, and they can infect a wide range of hosts, from single-celled protists to humans (Han and Weiss 2017). Historical observations of microsporidia species that infect non-*C. elegans* nematodes date back to the early 1900s, but it was not until 2008 that a microsporidia species was identified that infects *C. elegans*. This pathogen was found in a wild *C. elegans* from a compost pit outside Paris, France, and causes a lethal intestinal infection. Based on ribosomal sequence, it was given a new genus and species name *Nematocida parisii* (in Latin *Nematocida* means nematode killer and *parisii* means from Paris) in the Microsporidia phylum (Troemel et al. 2008). Since that time, the *Nematocida* genus has grown to include eight species, which have been identified from wild *Caenorhabditis* and related nematodes across the globe (Zhang et al. 2016). Based on these studies, microsporidia are a common cause of infection for *C. elegans* in the wild, and *N. parisii* infection is the most common microsporidia species to infect *C. elegans*. This chapter will describe the isolation and characterization of these microsporidia species, their impact on host physiology, and the *C. elegans* innate immune response to these naturally occurring intracellular infections.

5.2 Identification of Microsporidia That Infect *C. elegans*

Species in the *Caenorhabditis* genus are free-living, bacterivorous nematodes that belong to the family Rhabditidae. Among the fifty-plus species in the *Caenorhabditis* genus, *C. elegans* and *C. briggsae* are the best studied (Stevens et al. 2019). The standard laboratory strains of each of these species are “domesticated” in that they have been grown for decades in a controlled laboratory environment. Driven by the interest to describe the natural ecology and biology of these species, researchers increased efforts to sample wild worms from across the world in the early 2000s, and there are now hundreds of wild-caught isolates of these two species, as well as several other *Caenorhabditis* species (Kiontke et al. 2011; Lee et al. 2021; Schulenburg and Felix 2017). In the course of isolating wild-caught nematodes from their natural habitats including rotting fruits, decaying plant stems, and compost heaps, co-occurring natural microbes have also been isolated, including pathogens like microsporidia.

Of the eight *Nematocida* species isolated from wild *Caenorhabditis* and related nematodes, six have so far been shown to infect *C. elegans*. These are *N. parisii*, *Nematocida ironsii*, *Nematocida ausubeli*, *Nematocida displodere*, *Nematocida major*, and *Nematocida homosporus* (Table 5.1) (Zhang et al. 2016). In addition, one non-*Nematocida* species called *Pancytospora epiphaga* can infect *C. elegans*. *N. parisii* has been isolated from wild *C. elegans* and wild *C. briggsae* hosts from various regions in France. Notably, the ERTm5 strain originally isolated from *C. briggsae* in Kauai (Hawaii, USA) was named *N. parisii* based on large subunit ribosomal sequence (Balla et al. 2015) but was later re-named *N. ironsii* based on whole genome sequencing and molecular divergence (Reinke et al. (2017) and see below). This finding indicates that other strains assigned as *N. parisii* based on large subunit ribosomal sequence may be reassigned once their genomes are fully sequenced. After *N. ironsii*, the species with closest sequence identity to *N. parisii* is *N. ausubeli*. This species was originally called *Nematocida* sp. 1 and is described with this name in several publications (Bakowski et al. 2014b; Balla et al. 2016; Reinke et al. 2017; Troemel et al. 2008). It subsequently was named *N. ausubeli* for Fred Ausubel, a pioneer in the field of *C. elegans* host/pathogen interactions (Zhang et al. 2016). *N. ausubeli* was originally isolated from *C. briggsae* in Kerala, India, and has subsequently been isolated in wild-caught *C. elegans* from France and from Portugal; in wild-caught *C. briggsae* from Cape Verde; and in wild-caught *C. remanei* from Germany. *N. major* has been isolated from *C. briggsae* in Guadalupe and Thailand and from *C. tropicalis* in Guadalupe. This species has not yet been isolated from *C. elegans* in the wild, but it is capable of infecting *C. elegans* in the lab. Similarly, *N. homosporus* and *P. epiphaga* have both been isolated from non-*C. elegans* nematode species in the wild, but can infect *C. elegans* in the lab. The fourth species so far shown to infect *C. elegans* in the wild is *N. displodere*, which was isolated from a wild *C. elegans* in France. This species is distinct from other *Nematocida* species in that it can infect multiple tissues, in addition to the intestine, providing an opportunity to learn more about what governs tissue tropism ((Luallen et al. 2016) and see below).

While infection by all *Nematocida* species appear capable of killing *C. elegans* hosts, different species have varying levels of virulence. Side-by-side comparisons between *N. ausubeli* and *N. parisii* demonstrate that *N. ausubeli* is more virulent, as assessed by *N. ausubeli* causing more severe reduction in body size and a greater reduction in the number of eggs laid by infected hosts (Balla et al. 2016). Furthermore, *N. ausubeli* replicates more quickly inside *C. elegans* and forms spores earlier than *N. parisii*. Side-by-side comparisons of *N. displodere* and *N. parisii* virulence have not been described, but *C. elegans* begins shedding *N. parisii* spores earlier than *N. displodere* spores, likely because *N. parisii* spores are actively shed via host exocytosis from the intestine, whereas *N. displodere* spores gradually build up inside the animal until it bursts (Luallen et al. 2016; Szumowski et al. 2014). Further distinctions among the different *Nematocida* species genomes, life cycles, and interactions with hosts are described below.

5.3 *Nematocida* Genomes, Transcriptomes, and Proteomes

Next-generation sequencing has provided whole genome assembly for the four microsporidia species found to infect *C. elegans* in the wild (*N. parisii*, *N. ausubeli*, *N. ironsii*, and *N. displodere*). Microsporidia have the smallest known genomes among

eukaryotes, with the human-infecting species *Encephalitozoon intestinalis* as one of the smallest at only 2.3 Mb (Corradi et al. 2010). The first *Nematocida* genome sequences were obtained as part of the Microsporidian Genome consortium and included two strains of *N. parisii* (ERTm1 from Paris, France, and ERTm3 from Santeuil, France) (Cuomo et al. 2012) and two strains of *N. ausubeli* (ERTm2 from Kerala, India, and ERTm6 from the Cape Verde Islands) (Bakowski et al. 2014b; Cuomo et al. 2012). *N. parisii* ERTm1 and ERTm3 each have genomes of about 4.1 Mb, with 2661 predicted genes for ERTm1 and 2770 for ERTm3. Transcriptome analysis aided annotation of the *N. parisii* genome and indicated that 2546 of the ERTm1 genes were expressed during at least one of five stages of the *N. parisii* intracellular replicative life cycle and/or during the spore stage (Cuomo et al. 2012). Genome size was more divergent between the two *N. ausubeli* sequenced strains, at 4.7 Mb with 2770 predicted genes for ERTm2 and 4.3 Mb with 2433 genes for ERTm6. Both *N. parisii* and *N. ausubeli* appear to be diploid, with the highest degree of heterozygosity seen in *N. ausubeli* ERTm2 at 1 SNP every 82 bases (Cuomo et al. 2012). Large regions in *N. parisii* and *N. ausubeli* genomes have lost heterozygosity, which is suggestive of a recent or rare recombination event as part of a sexual or parasexual cycle. Morphological evidence of sexual cycles had previously been described for microsporidia species infecting other hosts (Becnel et al. 2005).

These genome sequencing studies were part of a larger phylogenomics effort to compare diverse microsporidia genomes (Cuomo et al. 2012). This effort identified gene gains and losses specific to the Microsporidia phylum and elucidated possible strategies used for the unique life cycle of these parasites. For example, compared to other eukaryotes, microsporidia have lost the cell cycle inhibitor retinoblastoma, which appears to be one of the earliest steps in the evolution of the Microsporidia phylum and may have facilitated their rapid replication inside host cells (Cuomo et al. 2012; Haag et al. 2014). Furthermore, these studies and others at the same time (Capella-Gutierrez et al. 2012) firmly established microsporidia as being most closely related to fungi, which had previously been a topic of controversy (Keeling and McFadden 1998).

Following the efforts of the Microsporidian Genomes Consortium, the genomes of one strain each of *N. displodere* and *N. ironsii* have also been described. As mentioned earlier, *N. ironsii* ERTm5 has the same large subunit ribosomal sequence as *N. parisii*, but whole genome sequencing revealed only 92.3% identity between ERTm5 and ERTm1 DNA, thus leading to its definition as a distinct species (Reinke et al. 2017). For comparison, *N. parisii* strains ERTm1 and ERTm3 share 99.8% genome sequence identity (Cuomo et al. 2012). The *N. ironsii* ERTm5 genome spans 4.4 Mb with 2709 predicted genes and is the closest to that of *N. parisii*. At 3 Mb, the *N. displodere* strain JU2807 has a smaller genome than either *N. parisii*, *N. ironsii*, or *N. ausubeli* (Luallen et al. 2016). This reduction is due in part to a decrease in intergenic regions: 85.8% of *N. displodere*'s genome is protein coding, while 69.2% and 63.7% are protein coding in *N. parisii* and *N. ausubeli*, respectively. In addition, the *N. displodere* genome has only 2278 predicted genes, which is fewer than *N. parisii*, *N. ironsii*, or *N. ausubeli*. *N. displodere* proteins share only 48% amino acid sequence similarity with *N. parisii* or *N. ausubeli* proteins, supporting the assignment of this species as an outgroup in the *Nematocida* genus compared to *N. parisii* and *N. ausubeli* and also compared to other species. Of note, genome sequencing indicated that all

Nematocida genomes contain expanded species-specific gene families, with the largest being a family with 235 members found in *N. displodere* (Lualien et al. 2016; Reinke et al. 2017). Thus, this family comprises about 10% of the protein-coding potential in the *N. displodere* genome. This gene family is characterized by the presence of a RING domain, which is a protein-protein interaction domain found in most E3 ubiquitin ligases.

To determine which microsporidia proteins might enable its obligate intracellular life cycle, localized proteomics was performed to identify *Nematocida* proteins that are “host-exposed” to the *C. elegans* host cytosol or nucleus (Reinke et al. 2017). Specifically, spatially restricted enzymatic tagging was performed by expressing the promiscuous biotin ligase APX in either the *C. elegans* intestinal cytosol or intestinal nucleus, which allows APX to attach a biotin tag to lysine residues on any nearby proteins in these cellular compartments. APX-expressing transgenic animals were then infected with either *N. parisii* or *N. ausubeli*. To identify *Nematocida* proteins located in either the intestinal cytosol or intestinal nuclei, biotin-tagged proteins were isolated by streptavidin affinity pull-down, followed by liquid chromatography and tandem mass spectrometry. Overall, this analysis identified 82 *Nematocida* proteins (72 from *N. parisii* and 10 from *N. ausubeli*) with high confidence of interacting with intestinal cell tissue, including several proteins found in the nucleus. These host-exposed proteins were enriched for those that contained transmembrane domains and/or signal sequences. In addition, most of these host-exposed proteins were found to be rapidly evolving and species-specific. In fact, only 12% of *N. parisii* host-exposed proteins have orthologues outside of the “clade” of *N. parisii*, *N. ironsii*, and *N. ausubeli*, whereas 63% of proteins as a whole in the *N. parisii* genome have orthologs outside of this clade. These host-exposed proteins appear to not just be species-specific, but also belong to large gene families. Such families appear to be a general feature of microsporidia genomes. The size and divergence of these gene families may reflect the outcome of host/pathogen arms races that drive both sides to expand and diversify the sequence of proteins directly involved in the battle for survival during intracellular replication (Lazetic and Troemel 2020).

5.4 *Nematocida* Life Cycles and Their Impact on *C. elegans* Cell Biology

The life cycles of *Nematocida* species share many similarities with other microsporidia species, including the spore stage being the transmissible form, and all parasite replication and differentiation occurring inside of host cells, before spores exit to infect new hosts (Fig. 5.1). As *N. parisii* strain ERTm1 is the best characterized of the microsporidia species infecting *C. elegans*, we will first describe its life cycle and then compare it to other species below.

N. parisii is transmitted fecal orally, with infection starting after *C. elegans* ingests infectious spores that enter the lumen of the intestine (Fig. 5.1) (Troemel et al. 2008). The *C. elegans* intestine is composed of 20 polarized epithelial cells with structural and functional similarity to intestinal epithelial cells in mammalian hosts, except that these intestinal cells are non-renewable (Dimov and Maduro 2019). While in the intestinal lumen, *N. parisii* spores fire an infection apparatus common to all microsporidia species called a polar tube, which is coiled inside of the spore before receiving a cue to fire. As the polar tube fires, a small parasite cell called a sporoplasm is injected through the tube and delivered into a *C. elegans*

intestinal cell. The exact cues and mechanisms used by *N. parisii* to fire the polar tube and invade host cells are poorly understood, but a recent report describes an intestinally secreted host factor that promotes microsporidia invasion, perhaps through enabling more efficient polar tube firing (El Jarkass et al. 2022). Notably, the process of intestinal cell invasion is fast, as sporoplasms inside intestinal cells can be observed within minutes after feeding *C. elegans* infectious *N. parisii* spores (Balla et al. 2019). Like other microsporidia species, *N. parisii* sporoplasms develop into multinucleated, replicating cells called meronts, which then differentiate back into spores that exit into the lumen. From there, spores can be defecated out of the animal to infect new hosts. The intestine is the only tissue where *N. parisii* replicates, and spore exit appears to be directional. Specifically, all spores exit apically from intestinal cells into the lumen, and have not been seen to exit basolaterally into the pseudocoelomic space of the animal (Estes et al. 2011).

N. parisii sporoplasms and meronts appear to replicate in direct contact with host intestinal cytosol. In particular, they have their own cellular plasma membrane, but do not appear to have a separate host membrane surrounding them at this stage (Szumowski et al. 2014). Further, *N. parisii* causes several restructuring events in the host while going through its life cycle. Here we will only briefly summarize such events related to spore exit, as they have been previously reviewed (El Jarkass and Reinke 2020; Troemel 2016). One restructuring event is a partial loss of polarity, with apically restricted cytoskeletal component actin appearing on the basolateral side of intestinal cells (Estes et al. 2011). This relocation may trigger another restructuring event, which is the appearance of gaps in the terminal web. The terminal web is a conserved actin and intermediate filament-rich structure found in the apical region of most polarized epithelial cells, including epithelial cells of the *C. elegans* intestine. These gaps in the terminal web appear precisely when *N. parisii* meronts differentiate into spores, and thus formation of gaps may remove a barrier to enable spores to exit into the lumen (Estes et al. 2011). As meronts differentiate into spores, they enter a separate membrane-bound compartment that feeds into the host recycling endocytosis pathway. Near the apical membrane, these compartments get coated with the *C. elegans* small GTPase RAB-11, which is a host trafficking factor that facilitates fusion of these spore-containing compartments with the apical membrane to direct non-lytic exit of spores back into the intestinal lumen (Szumowski et al. 2014; Szumowski et al. 2016). Overall, these restructuring events appear to enable a large number of *N. parisii* spores to be shed from worms while minimizing damage to the host intestine.

In contrast to the intestinal-specific tropism described for *N. parisii* and other *Nematocida* species, the more recently described species *N. displodere* is able to replicate in several tissues in *C. elegans*. *N. displodere* replicates most robustly in muscle and epidermis cells, with rare replication seen in coelomocytes (phagocytic cells with no known role in immunity), neurons, and intestinal cells (Fig. 5.1) (Luallen et al. 2016). *N. displodere* bursts out of worms (*displodere* in Latin means to cause to explode), which may occur because it is not possible to non-lytically exit out of the non-intestinal tissues where *N. displodere* replicates and differentiates most efficiently. After *N. displodere* replicates in the epidermis and muscle, it differentiates into spores that exit from the animal by bursting through the *C. elegans* vulva, or egg-laying structure. *N. displodere* infection begins through feeding, and thus the pathogen appears to access all of these diverse tissues by starting from the

intestinal lumen. Interestingly, the *N. displodere* polar tube is longer than that of *N. parisii* (12.6 μm vs. 4.0 μm). These observations, together with the observation that *N. displodere* sporoplasms are found inside non-intestinal tissues less than 2 minutes after feeding spores to worms, suggest that *N. displodere* spores fire their polar tubes from the intestinal lumen all the way through both the apical and basal side of intestinal cells to invade non-intestinal tissue like the epidermis and muscle, although this event has not been directly visualized (Fig. 5.1). Another notable characteristic of *N. displodere* is having only one spore size, while *N. parisii* has two distinct spore sizes. The functional significance of these spore differences is unknown. It should be noted that the morphological and life cycle differences between *N. displodere* and *N. parisii* are accompanied by differences in genome content as described above, including the strikingly expanded gene family that has 235 members in *N. displodere*. This family has been named NemLGF2 and has only 1–3 members each in *N. parisii*, *N. ironsii*, and *N. ausubeli* genomes (Reinke et al. 2017). Perhaps this family is responsible for the distinct lifestyle and tissue tropism of *N. displodere*, which has the broadest tissue tropism described so far for any *C. elegans* microbial pathogen.

One pathogen-driven restructuring event seen in common with infections by *N. displodere*, *N. parisii*, and *N. ausubeli* is the fusion of host cells (Balla et al. 2016). This fusion occurs while meronts are replicating, with *N. parisii* able to spread across several intestinal cells after first invading only a single intestinal cell. Imaging experiments with photoconvertible GFP demonstrated that *N. parisii* growth across intestinal cells causes sharing of cytoplasmic contents, indicating that what once was 20 separate intestinal cells becomes a large syncytial organ during infection. Similar results were found with *N. ausubeli*, which also only grows in the intestine. In contrast, *N. displodere* did not spread across intestinal cells in the rare instances where it replicated there, but instead spread across muscle cells after invading a single cell. These results indicate that the ability to fuse host cells is conserved across *Nematocida* species, with each species triggering syncytial formation in the host organ where it grows best. This cell-to-cell spread by *Nematocida* meronts likely enables faster, more efficient dissemination than if they were to differentiate back into spores, exit the infected cell, and then invade uninfected host cells using the polar tube invasion strategy. These findings provide a new perspective on how microsporidia can invade host cells, using a strategy independent of spores and polar tubes.

What regulates the differentiation of microsporidia meronts into spores? Experiments using differing doses of *N. parisii* spores and differing sizes of *C. elegans* host animals indicated that sporulation is triggered when meronts reach a critical density inside the intestine (Balla et al. 2016). An RNAi screen identified predicted *C. elegans* transcription factors as host factors that regulate sporulation of *N. parisii* inside intestinal cells. These host factors include members of the Myc family of transcription factors (Botts et al. 2016). *N. parisii* replication appears to proceed normally in *C. elegans* animals defective for these factors, but *N. parisii* differentiation is compromised. The exact role for *C. elegans* Myc transcription factors in regulating *N. parisii* sporulation is unclear, but one possibility is that they regulate expression of *C. elegans* metabolites or other cues that are sensed by *N. parisii* upon high meront density within the intestine to enter the sporulation program.

5.5 *C. elegans* Natural Variation in Resistance to *Nematocida* Infections

The original *N. parisii* isolate ERTm1 came from a wild-caught *C. elegans* near Paris, France, but ERTm1 is able to infect the N2 laboratory strain and other strains of *C. elegans* as well (Troemel et al. 2008). A comparison of wild-caught *C. elegans* strains found that the *C. elegans* Hawaiian isolate CB4856 displays higher resistance to infection with a Hawaiian isolate of *Nematocida* called *N. ironsii* ERTm5 (Balla et al. 2015). There is a lower initial colonization of *N. ironsii* inside CB4856 intestinal cells compared to N2 intestinal cells, and CB4856 intestinal cells can also clear *N. ironsii* infection over time, unlike N2, which always succumbs to infection by *N. ironsii*, as well as by other microsporidia species (Balla et al. 2015; Balla et al. 2019). Competition experiments showed that this increased resistance of CB4856 provided a fitness advantage over N2 in the presence of *N. ironsii*. Interestingly, the increased resistance and clearance (the reduction in parasite load over time) ability of CB4856 is restricted to the first larval (L1) stage of development, with no difference between N2 and CB4856 at later larval stages. Of note, the L1 stage is the only stage where infection reduces progeny production, with a greater reduction of progeny production caused by infection of N2 compared to infection of CB4856. While infection by *N. ironsii* shortens life span of N2 and CB4856 at any stage of development, this pathogen somewhat surprisingly does not reduce progeny production in older animals, despite establishing a similarly robust infection. Therefore, L1 may be the only stage where increased resistance against *N. ironsii* can promote the evolutionary success of *C. elegans*.

Quantitative genetics using recombinant-inbred lines identified four regions in the CB4856 genome associated with resistance (Balla et al. 2015). Introgressing two of these regions from the CB4856 genome into the N2 genome conferred resistance, and introgressing them from the N2 genome into the CB4856 genome conferred susceptibility, thus confirming the role of these two regions in resistance to *Nematocida* infection, acting additively. While the causative alleles in these genomic regions have not been identified, they are enriched for genes encoding components of cullin-ring E3 ubiquitin ligases. Ubiquitin ligases are enzymes that add ubiquitin tags to proteins and other targets to alter their fates, which can include autophagy-mediated degradation of intracellular pathogens in a process called xenophagy (Kuo et al. 2018). For this reason, the role of ubiquitin and the downstream process of autophagy was further analyzed in the ability of the CB4856 strain to clear *N. ironsii* (Balla et al. 2019). Ubiquitin and LGG-1/ATG8, a common marker for autophagy, were previously shown to target *N. parisii* sporoplasms in N2 animals. The level of targeting was relatively low, and ubiquitin-mediated autophagy played a modest but significant functional role in resistance against infection, although clearance was not examined in this study (N2 is not able to clear infection) (Bakowski et al. 2014a). In support of the possibility that more efficient xenophagy could explain the increased resistance of CB4856 hosts, this strain was found to have much higher levels of ubiquitin targeting sporoplasms compared to N2. For example, 84% of parasite cells were targeted at 15 hours post-inoculation (hpi) in CB4856 animals, and only 11% targeted at 15 hpi in N2 animals (Balla et al. 2019). Ubiquitin targeting correlated with parasite clearance in terms of parasite species, as analysis in CB4856 hosts showed there was high levels of targeting to *N. ironsii* cells, which are cleared, and little targeting to *N. parisii* and *N. ausubeli* parasite cells, which are not cleared.

Furthermore, increased ubiquitin targeting was seen in infections of CB4856 L1 but not L4 animals, again showing a correlation between ubiquitin targeting to parasite cells and their subsequent clearance.

Despite this strong correlation between host targeting of ubiquitin to *N. ironsii* parasite cells and their subsequent clearance, a functional role for ubiquitin and the downstream process of autophagy has not yet been shown. Ubiquitin is an essential gene, and *C. elegans* has an enormous number of ubiquitin ligases, making it difficult to test their roles. Ubiquitin ligase-mediated autophagy converges on core components however, including the ATG8 homolog LGG-1 mentioned above, which is commonly used as a marker for autophagy in *C. elegans*, as well as the other *C. elegans* ATG8 homolog LGG-2, which is most similar to LC3 in mammals. Interestingly, it was seen that LGG-2 targeting parasite cells correlated with clearance, with higher levels of LGG-2 targeting *N. ironsii* cells in CB4856 compared to *N. ironsii* cells in N2 hosts. There was no difference in LGG-1 targeting *N. ironsii* cells in CB4856 compared to N2. However, clearance still occurred normally in *lgg-2* mutants in CB4856, indicating this gene is not required for clearance. One possibility is that when LGG-2 is absent, LGG-1 can take its place. Of note, LGG-1 has redundant functions with LGG-2 for other processes in the N2 background. However, this model is difficult to test in CB4856 animals, because *lgg-1* is an essential gene in N2 (and thus likely also in CB4856), and RNAi knockdown is relatively ineffective in CB4856 animals.

While the host factors that cause clearance of *N. ironsii* over time are yet undefined, *lgg-2* was found to be required for the 50% decrease in *N. ironsii* initial colonization inside CB4856 intestinal cells compared to N2 intestinal cells (Balla et al. 2019). Deletion of *lgg-2* had no effect in N2 animals, but in CB4856 animals, it led to an increase in intracellular colonization by *N. ironsii* to the same levels as N2 animals. This result indicates a specific role for *lgg-2* in the increased resistance to intracellular colonization by *N. ironsii* in CB4856 animals. Thus, the role of different defense components can vary depending on the host strain background. Given the numerous roles for autophagy components in host defense outside of xenophagic clearance of intracellular pathogens, it will be interesting to define how *lgg-2* controls intracellular colonization of microsporidia infection.

5.6 *C. elegans* Host Transcriptional Response to Microsporidia Infection: The Intracellular Pathogen Response

To determine the *C. elegans* transcriptional response to microsporidia infection, RNA-seq transcriptomic profiling was performed on *N. parisii*-infected N2 animals isolated at five different points of infection representing *N. parisii* invasion, growth, and sporulation (Bakowski et al. 2014a). At each infection time point, there was a significant enrichment of differentially expressed genes associated with the intestine, as compared to uninfected controls. Through comparisons with other gene sets, the genes regulated by *N. parisii* infection showed little similarity to genes regulated by bacterial extracellular pathogens or known immunity signaling pathways. However, there was a significant similarity between genes upregulated by *N. parisii* and genes upregulated by *Orsay virus* infection. *Orsay virus* is a three-gene, positive-sense, single-stranded RNA virus isolated from wild *C. elegans* in

France (Felix et al. 2011). Despite their vast molecular differences, the *Orsay virus* and *N. parisii* are both obligate intracellular pathogens of the *C. elegans* intestine, suggesting they trigger a previously undefined immune response. The common gene set induced by both pathogens was termed the Intracellular Pathogen Response, or IPR (Fig. 5.2) (Bakowski et al. 2014a; Reddy et al. 2017). Analysis of a subset of IPR genes by qRT-PCR indicated that *N. ausingensis* is a weaker IPR activator than *N. parisii* in *C. elegans*, despite having a higher negative impact on host fitness than *N. parisii* (Balla et al. 2016; Zhang et al. 2016). One possible explanation is that *N. ausingensis* actively inhibits the IPR to enable a more virulent infection compared to *N. parisii*, or perhaps *N. ausingensis* is not as easily recognized by the host to induce the IPR.

What is the function of IPR genes? IPR genes are enriched for those that encode components of multi-subunit cullin-ring ubiquitin ligases (Bakowski et al. 2014a). Ubiquitin localizes to about 4–7% of *N. parisii* sporoplasms in the N2 host, and this localization was shown to be reduced in half by RNAi knockdown of the cullin *cul6*, a core component of cullin-ring ubiquitin ligases. Almost no ubiquitin localizes to parasite cells at later stages of *N. parisii* development, such as multinucleate meronts and spores. However, large clusters of conjugated ubiquitin appear throughout *C. elegans* intestinal cells, while *N. parisii* meronts are replicating and differentiating into spores, including sites distant from the parasite. Such ubiquitin clusters are a hallmark of perturbations in protein homeostasis (proteostasis), indicating that intracellular growth of microsporidia impairs proteostasis. Interestingly, IPR genes can also be induced by perturbations to proteostasis, such as genetic or pharmacological inhibition of the proteasome. RNA-seq analysis demonstrated that proteasome blockade induces almost all IPR genes (Reddy et al. 2019). Importantly, IPR genes are distinct from genes regulated by other stress response pathways, such as the HSF-1/heat shock response and the SKN-1/proteotoxic stress response, indicating the IPR constitutes a novel response pathway (Reddy et al. 2017, 2019).

In addition to ubiquitin ligase components, IPR genes include the *pals* genes, which are defined by a loosely conserved *pals* protein signature (Leyva-Diaz et al. 2017). The biochemical functions of *pals* genes are unknown, but *pals* stands for protein containing ALS2CR12 signature, which is found in the single *pals* gene in humans called ALS2CR12, of unknown function. There is also a single *pals* gene in mouse and none found in *Drosophila melanogaster*. The *pals* gene family expanded in the *C. elegans* genome to over 39 members, and these genes are found in clusters in the genome (Leyva-Diaz et al. 2017). 26 *pals* genes are IPR genes in that they are upregulated by both *N. parisii* and viral infection, and phylogenetic analysis indicates sequence similarity among upregulated genes (Fig. 5.3). One of these genes is *pals-5*, which is highly induced in the intestine upon infection and is used as a robust readout for the IPR through the use of a *pals-5p::GFP* reporter (Bakowski et al. 2014a).

Several forward genetic screens monitoring *pals-5p::GFP* expression have identified *C. elegans* regulators of the IPR and have provided insight into the physiological function of this pathogen response. The first regulator of IPR gene expression identified in *C. elegans* is called *pals-22*, which is a negative regulator found through a genetic screen for mutants with constitutively high *pals-5p::GFP* expression (Reddy et al. 2017). Expression of *pals-22*

is not regulated by infection; rather it acts as a negative regulator of induced *pals* and all other IPR genes. *pals-22* mutants have several phenotypes, including (1) increased resistance to natural pathogens of the intestine such as *N. parisii* and the *Orsay virus*; (2) increased resistance to the natural oomycete pathogen *Myzocytiopsis humicola*, a eukaryotic pathogen that infects the epidermis; (3) increased transgene silencing; (4) increased susceptibility to *Pseudomonas aeruginosa* bacterial infection; (5) shortened life span; and (6) increased tolerance of proteotoxic stress (Fasseas et al. 2021; Leyva-Diaz et al. 2017; Reddy et al. 2017; Reddy et al. 2019). It is this last phenotype that has been characterized in the most detail, using the phenotype of thermotolerance as a readout. Here, a combination of genetics and biochemistry demonstrated that the increased thermotolerance in *pals-22* mutants is dependent on *cul-6*, acting together with other components in a multi-subunit ubiquitin ligase complex (Panek et al. 2020; Reddy et al. 2017). The target(s) of this ubiquitin ligase remain to be defined, but their identification may provide novel insights into maintenance of proteostasis, especially during intracellular infection.

To better understand how *pals-22* regulates the IPR and defense against natural pathogens, suppressor screens were performed in a *pals-22* mutant background to find mutants with loss of constitutive *pals-5p::GFP* expression. These screens identified eight alleles in the gene *pals-25*, which is immediately downstream of *pals-22* in the genome, and is transcribed together with *pals-22* as part of an operon (Reddy et al. 2019). Like *pals-22*, *pals-25* expression is not induced by intracellular infection. All *pals-22* mutant phenotypes, including increased IPR gene expression, increased thermotolerance, and altered immunity, as well as reduced fitness in other contexts, are reversed to wild-type levels by mutations in *pals-25*. Because *pals-22* and *pals-25* direct opposite phenotypes and are in the same gene family, they are termed “antagonistic paralogs,” with *pals-22* acting as a negative regulator of *pals-25*, which is a downstream activator of the IPR. As PALS-22 and PALS-25 proteins are not enriched in the nucleus, they do not appear to be transcription factors, and it is not known how they regulate IPR gene transcription. By determining the overlap between genes upregulated by *N. parisii* infection, those upregulated in *pals-22* mutants, and also reversed back to wild-type levels in *pals-22 pals-25* double mutants, the IPR has been refined to include approximately 80 genes, including induced *pals* genes. Overall, these results show that *pals-25* is a global activator of *pals-5* and other IPR gene expression, but it is still unclear which induced PALS protein or other IPR proteins function to restrict microsporidia growth downstream of *pals-22/25*. Answering this question could provide better insight into how *C. elegans* controls intracellular pathogens in the intestine.

Genome analysis of 330 wild *C. elegans* strains suggests that there may be balancing selection acting at the *pals-22* and *pals-25* locus, with distinct alleles being maintained in *C. elegans* populations in the wild. Interestingly, the CB4856 Hawaiian *C. elegans* strain has predicted loss-of-function mutations in both *pals-22* and *pals-25* (Thompson et al. 2015). There are no obvious orthologs of *pals-22* and *pals-25* in the 27 other nematode genomes examined, including *C. inopinata*, the recently described sister species of *C. elegans* (Leyva-Diaz et al. 2017; Reddy et al. 2019). The *pals* gene family is smaller in other *Caenorhabditis* species compared to *C. elegans*, with eight *pals* genes in *C. briggsae*, eight in *C. brenneri*, and 18 in *C. remanei* (Leyva-Diaz et al. 2017). It is difficult to confidently assess orthology among *pals* genes in different *Caenorhabditis* species, because of the high

level of sequence divergence within this gene family. For example, *C. elegans* PALS-22 and PALS-25 proteins only have 19.4% identity on the amino acid level. However, the induction of *pals* genes by intracellular infection appears to be conserved between *C. elegans* and *C. briggsae* (Chen et al. 2017). While *C. briggsae* cannot be infected by the *Orsay virus*, it can be infected by the related Santeuil virus, found naturally infecting *C. briggsae* (Felix et al. 2011). Comparison of the genes expressed upon *Orsay virus* infection in *C. elegans* and those expressed upon Santeuil virus infection in *C. briggsae* identified 58 homologous genes differentially regulated by both viruses. These genes included all 8 *C. briggsae pals* genes, with one of them only modestly induced. Taken together, these results suggest that the IPR may be an evolutionarily conserved immune response to intracellular pathogens in *Caenorhabditis* nematodes.

While *pals-22* and *pals-25* control IPR gene expression and rewire *C. elegans* physiology, they are dispensable, as their deletion from the genome results in animals with wild-type phenotypes. For example, *pals-22 pals-25* double mutants still upregulate IPR gene expression upon exposure to all known triggers of the IPR, including *N. parisii* infection, virus infection, proteotoxic stress, and chronic heat stress, among other triggers (Reddy et al. 2019). Thus, *pals-22* and *pals-25* appear to have been inserted in the *C. elegans* genome as an ON/OFF switch for the IPR, but act in parallel to other factors that mediate induction (Fig. 5.2). Induction of the IPR is best understood for viral infection, which is sensed by DRH-1, a *C. elegans* homolog of mammalian RIG-I-like receptors that sense double-stranded viral RNA and other viral replication products. DRH-1 is required for induction of IPR genes upon viral infection, but not upon other triggers (Sowa et al. 2020). How microsporidia infection and other triggers induce the IPR is unknown.

While there appear to be many independent upstream triggers of the IPR, there does appear to be a common downstream regulator through which these triggers induce gene expression. The predicted bZIP transcription factor ZIP-1 was identified in two independent RNAi-based screens as being required for inducing *pals-5p::GFP* expression, and it appears to be important for induction of this reporter upon all known IPR triggers (Lažeti et al. 2022). Interestingly, RNA-seq analysis demonstrated that ZIP-1 is required for induction of less than half of the IPR genes. This finding indicates there are other transcription factors required for regulating these *zip-1*-independent IPR genes. Despite only regulating some IPR genes, *zip-1* is required to promote resistance to *Orsay virus* and *N. parisii* infection and is particularly important in a mutant background where IPR genes are constitutively expressed. These results indicate that ZIP-1 controls IPR genes that protect against intracellular infection.

Recently, forward genetic screens identified *pnp-1* as another negative regulator of the IPR, functioning in parallel to *pals-22* and upstream of *zip-1* (Teclé et al. 2021). *pnp-1* encodes a purine nucleoside phosphorylase (PNP), which is an enzyme that acts in the purine salvage pathway to recycle guanine and adenine nucleotides. RNA-seq profiling of *pnp-1* mutants demonstrated that they have constitutive upregulation of almost all IPR genes, including the induced *pals* genes. In keeping with this constitutive upregulation of IPR genes, *pnp-1* mutants are resistant to *Orsay virus* and *N. parisii* infection, and *zip-1* is important for this resistance. Restoring expression of *pnp-1* in *C. elegans* intestinal epithelial cells rescues

pnp-1 mutant phenotypes, including IPR gene expression and resistance against *N. parisii*, indicating that purine metabolism in the intestine regulates the IPR. It would be interesting to investigate if loss of *pnp-1* in *C. briggsae* results in resistance to *Nematocida* and/or Santeuil virus infection in this host.

The phenotypes of *pnp-1* highlight the relevance of purine metabolism to obligate intracellular infections like those caused by microsporidia. Purine metabolism pathways are conserved across all domains of life and consist of the energy-costly de novo pathway and the far less energy-costly salvage pathway. PNPs function only in the purine salvage pathway and are evolutionarily conserved from bacteria to man. In contrast to the lack of *pals-22* sequence conservation, *pnp-1* sequences are highly conserved, for example, 95% homology between *C. elegans* and *C. briggsae*. PNPs convert the purine nucleoside inosine into the purine base hypoxanthine, which is then submitted to the action of various enzymes to generate purine nucleotides. Metabolomics analysis confirmed that *pnp-1* mutants have increased levels of inosine and decreased levels of hypoxanthine, as expected if *pnp-1* functions as a PNP in *C. elegans*. Considering that microsporidia are completely dependent on the host for nucleotides, it seems plausible that perturbations in purine metabolism in the host could impair microsporidia growth. Moreover, buildup of purine metabolite precursors (such as inosine) or the absence of others (such as hypoxanthine) due to infection could be sensed by the host which then deploys defense mechanisms against infection, such as the IPR. Interestingly, human mutations in purine salvage enzymes like PNP and adenosine deaminase result in immunodeficiency due to T cell dysfunction, but little is known about the role of PNP and other purine salvage enzymes in epithelial cells, which are the common sites of virus and microsporidia infection, and where *pnp-1* functions in *C. elegans*. Perhaps purine metabolism enzyme mutations in humans provide an evolutionary advantage in the context of viral or microsporidia infection in epithelial cells, which are often the first cells to be infected.

The work with *pals-22* and *pnp-1* demonstrates that IPR induction promotes resistance within a generation. Recent work indicates that *N. parisii* infection and IPR induction can provide an immunity benefit to the progeny of infected animals (Willis et al. 2021). Thus, immunity against microsporidia can be maternally inherited in *C. elegans*. *N. parisii* infection itself is not required for inheritance of resistance, only IPR activation in the parental generation. Mothers with IPR activation, such as *pals-22* mutants, have progeny without IPR gene activation, but nonetheless these progeny are resistant to *N. parisii* infection. Several other IPR activators confer an immune benefit against *N. parisii* to the next generation, including viral infection, and mutations in another negative regulator of IPR gene expression, *lin-35*, which is a homolog of the cell cycle regulator retinoblastoma. This inherited immunity does come at a fitness cost however; progeny from infected parents are smaller, less fecund and display increased susceptibility to other types of stresses. Inherited resistance to *N. parisii* infection is observed in *C. briggsae*, indicating that this is an evolutionary conserved trait (Burton et al. 2021). It would be interesting to determine whether inherited immunity can be triggered by infection with other *Nematocida* species or by loss of *pnp-1*, and if *zip-1* is required for this process. It is not well understood how IPR activation leads to increased resistance either within a generation or across a generation,

but as more is uncovered, it will be interesting to compare whether similar or different mechanisms are at play.

5.7 Perspectives and Future Directions

The power of the *C. elegans* host system has facilitated progress on several fronts of microsporidia research. For example, the ease of imaging the transparent body plan of *C. elegans* has enabled visualization of *Nematocida* species fusing host cells together during replication to create syncytial structures that facilitate spread of this pathogen. These studies were notable because they described a polar tube-independent invasion mechanism of microsporidia. The ease of isolating wild-caught *C. elegans* and related nematodes has provided a growing collection of natural microsporidian pathogens of nematodes, which offer new models for studying important questions about microsporidia pathogenesis, such as what determines their host and tissue tropism. Localized proteomics in *C. elegans* intestinal cells highlighted how species-specific gene families from microsporidia encode proteins that directly interface with host tissue during the host/pathogen battle, which may drive the expansion and diversification of these families. Similarly, forward genetics has highlighted the expansion and diversification of species-specific gene families involved in host defense against these pathogens (Lazetic and Troemel 2020). Genetic and transcriptomic studies have identified the IPR, a shared immune/stress response pathway induced by natural microsporidian and viral infections. The IPR is regulated by metabolism of purine nucleotides, which are crucial factors stolen by both microsporidia and viruses to enable their replication. The short life cycle of *C. elegans* facilitated the discovery that activation of the IPR can confer an immune benefit to the next generation. However, many questions remain. For example, it is unknown how *C. elegans* senses microsporidia to trigger the IPR, how the IPR confers resistance to infection, and whether there is an analogous system in mammals, which lack obvious sequence orthologs of IPR regulators *pals-22/25* and many other IPR genes. In addition, the mechanisms by which *Nematocida* species fuse *C. elegans* organs into syncytia are unknown. Further elucidation of these findings and investigation of their potential roles in mammalian hosts will be exciting areas for future inquiry.

Despite such progress, research of microsporidia infections in *C. elegans* is hampered by the same problem that all microsporidia researchers face: manipulation of the microsporidia genome (Reinke and Troemel 2015). The lack of genetics or DNA transformation techniques makes it challenging to determine which *Nematocida* factors trigger the various restructuring events described above, cause disease, or activate the IPR. Heterologous expression of pathogen proteins provides one avenue to explore these questions. Here, the host-exposed *Nematocida* proteins identified through localized proteomics, including proteins from the species-specific expanded gene families, provide attractive candidates to explore (Reinke et al. 2017). Without genetic manipulation of microsporidia, we are like detectives who meticulously investigate a crime scene but never manage to specifically identify the perpetrators. Although genetics and transformation techniques are currently not available in microsporidia, the development of highly sensitive reporter systems such as nanoluciferase will provide useful tools for developing these techniques in the future (Sfarcic et al. 2019). Together with CRISPR-Cas9-mediated gene editing, novel genetic tools will help define the

microsporidia factors that enable its elaborate intracellular life and the host response to these ubiquitous, fascinating parasites.

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References

- Bakowski MA et al. (2014a) Ubiquitin-mediated response to microsporidia and virus infection in *C. elegans*. *PLoS Pathog* 10:e1004200. 10.1371/journal.ppat.1004200 [PubMed: 24945527]
- Bakowski MA, Priest M, Young S, Cuomo CA, Troemel ER (2014b) Genome sequence of the microsporidian species *Nematocida* sp1 strain ERTm6 (ATCC PRA-372). *Genome Announc* 2:e00905–14. 10.1128/genomeA.00905-14 [PubMed: 25237020]
- Balla KM, Andersen EC, Kruglyak L, Troemel ER (2015) A wild *C. elegans* strain has enhanced epithelial immunity to a natural microsporidian parasite. *PLoS Pathog* 11:e1004583. 10.1371/journal.ppat.1004583 [PubMed: 25680197]
- Balla KM, Luallen RJ, Bakowski MA, Troemel ER (2016) Cell-to-cell spread of microsporidia causes *Caenorhabditis elegans* organs to form syncytia. *Nat Microbiol* 1:16144. 10.1038/nmicrobiol.2016.144 [PubMed: 27782144]
- Balla KM, Lazetic V, Troemel ER (2019) Natural variation in the roles of *C. elegans* autophagy components during microsporidia infection. *PLoS One* 14:e0216011. 10.1371/journal.pone.0216011 [PubMed: 31013330]
- Becnel JJ, White SE, Shapiro AM (2005) Review of microsporidia-mosquito relationships: from the simple to the complex. *Folia Parasitol (Praha)* 52:41–50 [PubMed: 16004363]
- Botts MR, Cohen LB, Probert CS, Wu F, Troemel ER (2016) Microsporidia intracellular development relies on myc interaction network transcription factors in the host. *G3 (Bethesda)* 6:2707–2716. 10.1534/g3.116.029983 [PubMed: 27402359]
- Burton NO et al. (2021) Intergenerational adaptations to stress are evolutionarily conserved, stress-specific, and have deleterious trade-offs. *bioRxiv*. 10.1101/2021.05.07.443118
- Capella-Gutierrez S, Marcet-Houben M, Gabaldon T (2012) Phylogenomics supports microsporidia as the earliest diverging clade of sequenced fungi. *BMC Biol* 10:47. 10.1186/1741-7007-10-47 [PubMed: 22651672]
- Chen K, Franz CJ, Jiang H, Jiang Y, Wang D (2017) An evolutionarily conserved transcriptional response to viral infection in *Caenorhabditis* nematodes. *BMC Genomics* 18:303. 10.1186/s12864-017-3689-3 [PubMed: 28415971]
- Corradi N, Pombert JF, Farinelli L, Didier ES, Keeling PJ (2010) The complete sequence of the smallest known nuclear genome from the microsporidian *Encephalitozoon intestinalis*. *Nat Commun* 1:77. 10.1038/ncomms1082 [PubMed: 20865802]
- Cuomo CA et al. (2012) Microsporidian genome analysis reveals evolutionary strategies for obligate intracellular growth. *Genome Res* 22:2478–2488. 10.1101/gr.142802.112 [PubMed: 22813931]
- Darby C, Cosma CL, Thomas JH, Manoil C (1999) Lethal paralysis of *Caenorhabditis elegans* by *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 96:15202–15207. 10.1073/pnas.96.26.15202 [PubMed: 10611362]
- Dimov I, Maduro MF (2019) The *C. elegans* intestine: organogenesis, digestion, and physiology cell tissue res, p 377:383–396. 10.1007/s00441-019-03036-4 [PubMed: 31065800]
- El Jarkass HT, Reinke AW (2020) The ins and outs of host-microsporidia interactions during invasion, proliferation and exit. *Cell Microbiol* 22:e13247. 10.1111/cmi.13247 [PubMed: 32748538]

- El Jarkass HT, Mok C, Schertzberg MR, Fraser AG, Troemel ER, Reinke AW (2022, Jan 7) An intestinally secreted host factor promotes microsporidia invasion of *C. elegans*. *Elife* 11:e72458. 10.7554/eLife.72458. Epub ahead of print [PubMed: 34994689]
- Estes KA, Szumowski SC, Troemel ER (2011) Non-lytic, actin-based exit of intracellular parasites from *C. elegans* intestinal cells. *PLoS Pathog* 7:e1002227. 10.1371/journal.ppat.1002227 [PubMed: 21949650]
- Fasseas MK, Grover M, Drury F, Essmann CL, Kaulich E, Schafer WR, Barkoulas M (2021) Chemosensory Neurons Modulate the Response to Oomycete Recognition in *Caenorhabditis elegans* *Cell Rep* 34:108604. 10.1016/j.celrep.2020.108604
- Felix MA et al. (2011) Natural and experimental infection of *Caenorhabditis* nematodes by novel viruses related to nodaviruses. *PLoS Biol* 9:e1000586. 10.1371/journal.pbio.1000586 [PubMed: 21283608]
- Haag KL, James TY, Pombert JF, Larsson R, Schaer TM, Refardt D, Ebert D (2014) Evolution of a morphological novelty occurred before genome compaction in a lineage of extreme parasites. *Proc Natl Acad Sci U S A* 111:15480–15485. 10.1073/pnas.1410442111 [PubMed: 25313038]
- Han B, Weiss LM (2017) Microsporidia: obligate intracellular pathogens within the fungal Kingdom. *Microbiol Spectr* 5:97–113. 10.1128/microbiolspec.FUNK-0018-2016
- Keeling PJ, McFadden GI (1998) Origins of microsporidia. *Trends Microbiol* 6:19–23. 10.1016/S0966-842X(97)01185-2 [PubMed: 9481819]
- Kiontke KC, Felix MA, Ailion M, Rockman MV, Braendle C, Penigault JB, Fitch DH (2011) A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits. *BMC Evol Biol* 11:339. 10.1186/1471-2148-11-339 [PubMed: 22103856]
- Kuo CJ, Hansen M, Troemel E (2018) Autophagy and innate immunity: insights from invertebrate model organisms. *Autophagy* 14:233–242. 10.1080/15548627.2017.1389824 [PubMed: 29130360]
- Lazetic V, Troemel ER (2020) Conservation lost: host-pathogen battles drive diversification and expansion of gene families. *FEBS J*. 10.1111/febs.15627
- Lažeti V, Wu F, Cohen LB et al. (2022) The transcription factor ZIP-1 promotes resistance to intracellular infection in *Caenorhabditis elegans*. *Nat Commun* 13:17. 10.1038/s41467-021-27621-w [PubMed: 35013162]
- Lee D et al. (2021) Balancing selection maintains hyper-divergent haplotypes in *Caenorhabditis elegans* *Nat Ecol Evol* 5:794–807. 10.1038/s41559-021-01435-x [PubMed: 33820969]
- Leyva-Diaz E, Stefanakis N, Carrera I, Glenwinkel L, Wang G, Driscoll M, Hobert O (2017) Silencing of repetitive DNA is controlled by a member of an unusual *Caenorhabditis elegans* gene family. *Genetics* 207:529–545. 10.1534/genetics.117.300134 [PubMed: 28801529]
- Lualien RJ, Reinke AW, Tong L, Botts MR, Felix MA, Troemel ER (2016) Discovery of a natural microsporidian pathogen with a broad tissue tropism in *Caenorhabditis elegans*. *PLoS Pathog* 12:e1005724. 10.1371/journal.ppat.1005724 [PubMed: 27362540]
- Mahajan-Miklos S, Tan MW, Rahme LG, Ausubel FM (1999) Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa*-*Caenorhabditis elegans* pathogenesis model. *Cell* 96:47–56. 10.1016/s0092-8674(00)80958-7 [PubMed: 9989496]
- Panek J, Gang SS, Reddy KC, Lualien RJ, Fulzele A, Bennett EJ, Troemel ER (2020) A cullin-RING ubiquitin ligase promotes thermotolerance as part of the intracellular pathogen response in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 117:7950–7960. 10.1073/pnas.1918417117 [PubMed: 32193347]
- Pujol N et al. (2001) A reverse genetic analysis of components of the toll signaling pathway in *Caenorhabditis elegans*. *Curr Biol* 11:809–821. 10.1016/s0960-9822(01)00241-x [PubMed: 11516642]
- Reddy KC et al. (2017) An intracellular pathogen response pathway promotes Proteostasis in *C. elegans*. *Curr Biol* 27:3544–3553. 10.1016/j.cub.2017.10.009 [PubMed: 29103937]
- Reddy KC et al. (2019) Antagonistic paralogs control a switch between growth and pathogen resistance in *C. elegans*. *PLoS Pathog* 15:e1007528. 10.1371/journal.ppat.1007528 [PubMed: 30640956]

- Reinke AW, Troemel ER (2015) The development of genetic modification techniques in intracellular parasites and potential applications to microsporidia. *PLoS Pathog* 11:e1005283. 10.1371/journal.ppat.1005283 [PubMed: 26720003]
- Reinke AW, Balla KM, Bennett EJ, Troemel ER (2017) Identification of microsporidia host-exposed proteins reveals a repertoire of rapidly evolving proteins. *Nat Commun* 8:14023. 10.1038/ncomms14023 [PubMed: 28067236]
- Schulenburg H, Felix MA (2017) The natural biotic environment of *Caenorhabditis elegans*. *Genetics* 206:55–86. 10.1534/genetics.116.195511 [PubMed: 28476862]
- Sfarcic I, Bui T, Daniels EC, Troemel ER (2019) Nanoluciferase-based method for detecting gene expression in *Caenorhabditis elegans*. *Genetics* 213:1197–1207. 10.1534/genetics.119.302655 [PubMed: 31585955]
- Singh J (2021) Harnessing the power of genetics: fast forward genetics in *Caenorhabditis elegans*. *Mol Gen Genomics* 296:1–20. 10.1007/s00438-020-01721-6
- Sowa JN, Jiang H, Somasundaram L, Teclé E, Xu G, Wang D, Troemel ER (2020) The *Caenorhabditis elegans* RIG-I homolog DRH-1 mediates the intracellular pathogen response upon viral infection. *J Virol* 94. 10.1128/JVI.01173-19
- Stevens L et al. (2019) Comparative genomics of 10 new *Caenorhabditis* species. *Evol Lett* 3:217–236. 10.1002/evl3.110 [PubMed: 31007946]
- Zsumowski SC, Botts MR, Popovich JJ, Smelkinson MG, Troemel ER (2014) The small GTPase RAB-11 directs polarized exocytosis of the intracellular pathogen *N. parisii* for fecal-oral transmission from *C. elegans*. *Proc Natl Acad Sci U S A* 111:8215–8220. 10.1073/pnas.1400696111 [PubMed: 24843160]
- Zsumowski SC, Estes KA, Popovich JJ, Botts MR, Sek G, Troemel ER (2016) Small GTPases promote actin coat formation on microsporidian pathogens traversing the apical membrane of *Caenorhabditis elegans* intestinal cells. *Cell Microbiol* 18:30–45. 10.1111/cmi.12481 [PubMed: 26147591]
- Teclé E, Chhan CB, Franklin L, Underwood RS, Hanna-Rose W, Troemel ER (2021) The purine nucleoside phosphorylase *pnp-1* regulates epithelial cell resistance to infection in *C. elegans*. *PLoS Pathog* 17:e1009350. 10.1371/journal.ppat.1009350 [PubMed: 33878133]
- Thompson OA et al. (2015) Remarkably divergent regions punctuate the genome assembly of the *Caenorhabditis elegans* Hawaiian strain CB4856. *genetics*, p 200:975–989. 10.1534/genetics.115.175950 [PubMed: 25995208]
- Troemel ER (2016) Host-microsporidia interactions in *caenorhabditis elegans*, a model nematode host. *Microbiol Spectr* 4(5). 10.1128/microbiolspec.FUNK-0003-2016
- Troemel ER, Felix MA, Whiteman NK, Barriere A, Ausubel FM (2008) Microsporidia are natural intracellular parasites of the nematode *Caenorhabditis elegans*. *PLoS Biol* 6:2736–2752. 10.1371/journal.pbio.0060309 [PubMed: 19071962]
- Willis AR, Zhao W, Sukhdeo R, Wadi L, El Jarkass HT, Claycomb JM, Reinke AW (2021) A parental transcriptional response to microsporidia infection induces inherited immunity in offspring. *Sci Adv* 7(19):eabf3114. 10.1126/sciadv.abf3114 [PubMed: 33952520]
- Zhang G, Sachse M, Prevost MC, Luallen RJ, Troemel ER, Felix MA (2016) A large collection of novel nematode-infecting microsporidia and their diverse interactions with *Caenorhabditis elegans* and other related nematodes. *PLoS Pathog* 12:e1006093. 10.1371/journal.ppat.1006093 [PubMed: 27942022]

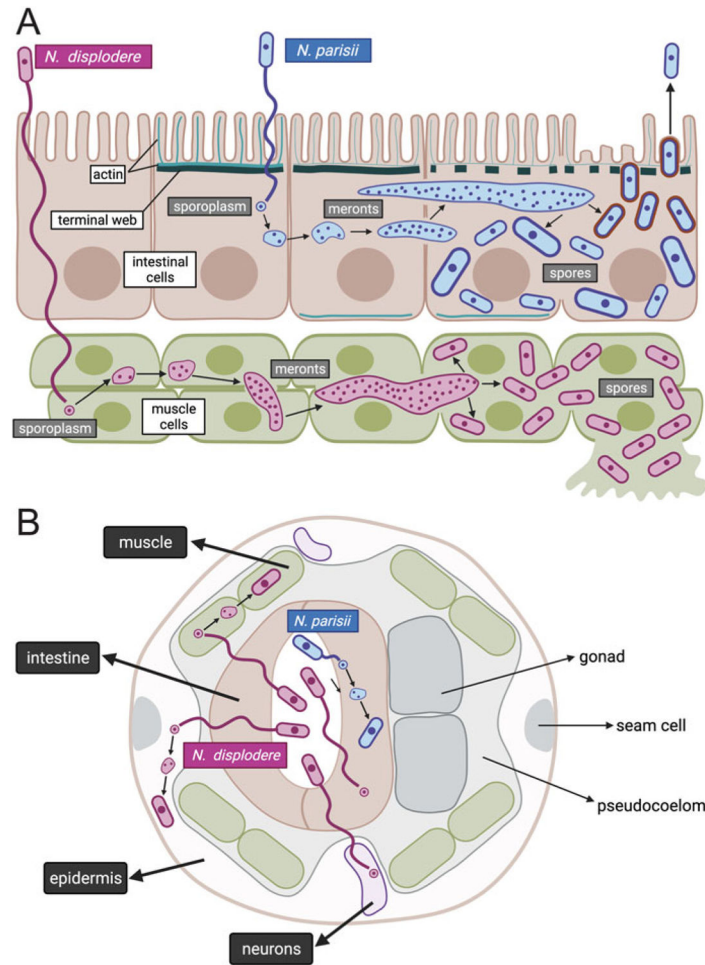


Fig. 5.1.

Life cycle of *N. parisii* and *N. displodere* inside the *C. elegans* host. Longitudinal (a) and cross-sectional (b) diagrams of *Nematocida* infection of the *C. elegans* intestine, muscle, epidermis, and neurons. *N. parisii* spore in blue is shown in the intestinal lumen, firing a polar tube that delivers a sporoplasm (a membrane-bound parasite cell) into the cytoplasm of *C. elegans* intestinal cells. This sporoplasm develops in direct contact with the host cytoplasm, replicating its nuclei without undergoing cell division, to develop into a multinucleate meront. This meront can spread across intestinal cells, causing the intestinal organ to become a syncytial structure with shared cytoplasmic contents. As *N. parisii* meronts develop into spores, gaps appear in the terminal web (made of actin and intermediate filaments), which is thought to remove a barrier to exit. Once *N. parisii* meronts develop into spores, they are found in separate membrane-bound compartments of unknown origin. These spores then become coated with the small GTPase RAB-11 (in red) and fuse with the apical membrane, to be released non-lytically back into the lumen. *N. displodere* spore in purple is also shown in the intestinal lumen, firing a polar tube that is longer than the polar tube of *N. parisii*. The *N. displodere* polar tube is hypothesized to reach all the way from the lumen into muscle cells, epidermal cells, and neurons to deliver sporoplasms inside of these cells. *N. displodere* sporoplasms develop into meronts, which can lead to fusion of

muscle cells to form a syncytium (the epidermis is already a syncytium). In contrast to the non-lytic exit of *N. parisii*, *N. displodere* spores appear to burst out of cells

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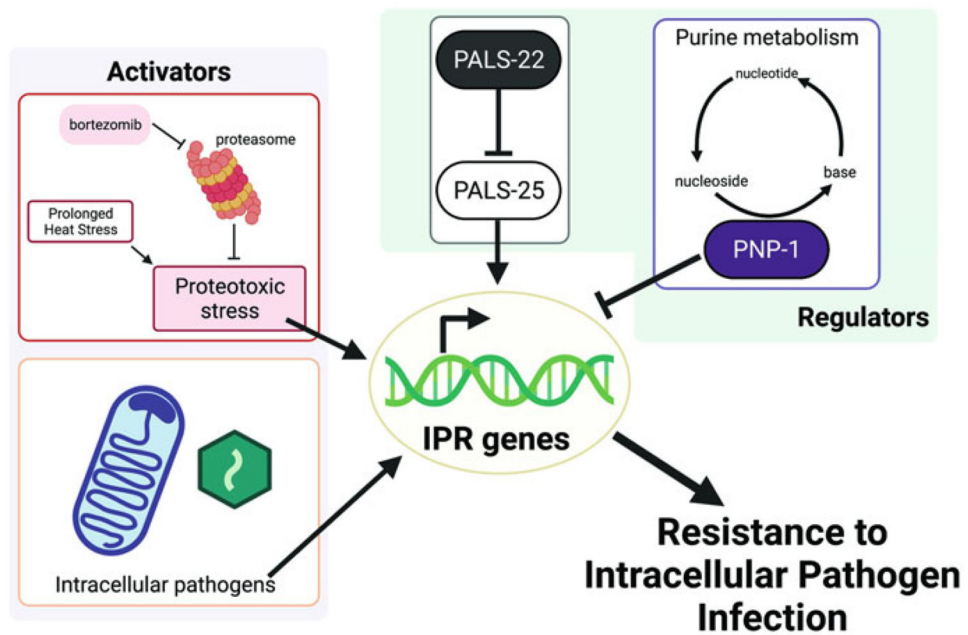


Fig. 5.2.

Model of the Intracellular Pathogen Response. Intestinal intracellular pathogens, like *N. parisii* and the *Orsay virus*, upregulate mRNA expression of a common set of *C. elegans* genes called the Intracellular Pathogen Response, or IPR genes. IPR genes can also be induced by proteotoxic stress, as well as by perturbation to purine metabolism. Independently of these triggers, they are also regulated by a pair of antagonistic paralogs called PALS-22 and PALS-25. Upregulation of IPR genes leads to increased resistance to pathogen infection

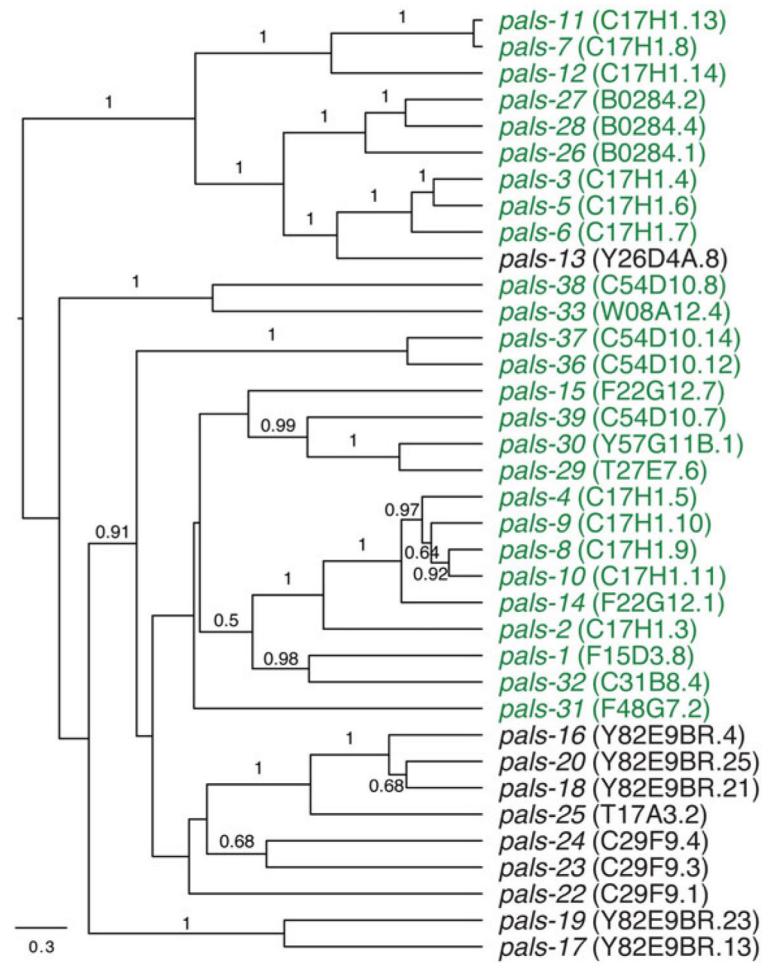


Fig. 5.3. Summary of *pals* genes. Phylogenetic tree of sequence relationships of *pals* genes. Green text indicates genes that are upregulated by both *N. parisii* and *Orsay virus* infection. Adapted with permission from Reddy et al. 2017

Table 5.1

List of microsporidia species that can infect *C. elegans* in the wild or the lab

Microsporidia species	Isolated from	Infects <i>C. elegans</i>	Number of published strains	Tissues infected	Genomes sequenced	Other names	References
<i>Nematocida parisii</i>	<i>Caenorhabditis elegans</i> , <i>Caenorhabditis briggsae</i> (France)	In wild, in lab	17	Intestine	Strains ERTm1, ERTm3		Troemel et al., PLOS Biology (2008); Cuomo et al., Genome Research (2012); Zhang et al., PLOS Pathogens (2016)
<i>Nematocida ironsii</i>	<i>Caenorhabditis briggsae</i> (Hawaii, USA)	In wild, in lab	1	Intestine	Strains ERTm5	<i>N. parisii</i>	Balla et al., PLOS Pathogens (2015); Zhang et al., PLOS Pathogens (2016); Reinke et al., Nature Communications (2017)
<i>Nematocida ausubeli</i>	<i>Caenorhabditis elegans</i> (France, Portugal); <i>Caenorhabditis briggsae</i> (India, Cape Verde, Germany); <i>Caenorhabditis remanei</i> (Germany)	In wild, in lab	10	Intestine	Strains ERTm2, ERTm6	<i>N. sp. 1</i>	Troemel et al., PLOS Biology (2008); Cuomo et al., Genome Research (2012); Bakowski et al., Genome Announcement (2014b); Zhang et al., PLOS Pathogens (2016)
<i>Nematocida displodere</i>	<i>Caenorhabditis elegans</i> (France)	In wild, in lab	1	Intestine, coelomocytes, neurons, epidermis, muscle	JUm2807		Luallen et al., PLOS Pathogens (2016); Zhang et al., PLOS Pathogens (2016)
<i>Nematocida major</i>	<i>Caenorhabditis briggsae</i> (Thailand, Guadeloupe); <i>Caenorhabditis tropicalis</i> (Guadeloupe)	In lab	3	Intestine			Zhang et al., PLOS Pathogens (2016)
<i>Nematocida homosporus</i>	<i>Oschelius tipulae</i> (France); <i>Rhabditella typhae</i> (Portugal)	In lab	2	Intestine			Zhang et al., PLOS Pathogens (2016)
<i>Pancytospora epiphaga</i>	<i>Caenorhabditis brenneri</i> (Colombia)	In lab	1	Epidermis			Zhang et al., PLOS Pathogens (2016)
<i>Nematocida minor</i>	<i>Oschelius tipulae</i> (Czech Republic, Armenia)	No	2	Intestine			Zhang et al., PLOS Pathogens (2016)
<i>Nematocida ciangi</i>	<i>Procephalobus</i> sp. (Spain)	No	1	Intestine			Zhang et al., PLOS Pathogens (2016)