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Signaling in Parasitic Nematodes: Physicochemical Communication Between Host and Parasite and Endogenous Molecular Transduction Pathways Governing Worm Development and Survival

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Abstract

Signaling or communication between host and parasite may occur over relatively long ranges to enable host finding and acquisition by infective parasitic nematode larvae. Innate behaviors in infective larvae transmitted from the soil that enhance the likelihood of host contact, such as negative geotaxis and hypermotility, are likely mediated by mechanoreception and neuromuscular signaling. Host cues such as vibration of the substratum, elevated temperature, exhaled CO_2, and other volatile odorants are perceived by mechanosensory and chemosensory neurons of the amphidial complex. Beyond this, the molecular systems that transduce these external cues within the worm are unknown at this time. Overall, the signal transduction mechanisms that regulate switching between dauer and continuous reproductive development in *Caenorhabditis elegans*, and doubtless other free-living nematodes, have provided a useful framework for testing hypotheses about how the morphogenesis and development of infective parasitic nematode larvae and the lifespan of adult parasites are regulated. In *C. elegans*, four major signal transduction pathways, G protein-coupled receptor signaling, insulin/insulin-like growth factor signaling, TGFβ-like signaling and steroid-nuclear hormone receptor signaling govern the switch between dauer and continuous development and regulate adult lifespan. Parasitic nematodes appear to have conserved the functions of G-protein-coupled signaling, insulin-like signaling and steroid-nuclear hormone receptor signaling to regulate larval development before and during the infective process. By contrast, TGFβ-like signaling appears to have been adapted for some other function, perhaps modulation of the host immune response. Of the three signal transduction pathways that appear to regulate development in parasitic nematodes, steroid-nuclear hormone signaling is the most straightforward to manipulate with administered small molecules and may form the basis of new chemotherapeutic strategies. Signaling between parasites and their hosts' immune systems also occurs and serves to modulate these responses to allow chronic infection and down regulate acute inflammatory responses. Knowledge of the precise nature of this signaling may form the basis of immunological interventions to protect against parasitism or related lesions and to alleviate inflammatory diseases of various etiologies.

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Keywords

Parasitic nematode; Signal transduction; Insulin; TGFβ; Nuclear receptor; G-protein

Introduction

On a global scale, parasitic nematodes exact enormous tolls on human health and on animal agriculture and animal welfare [1] Currently there are no effective vaccines against parasitic nematodes, and as a group the armamentarium of drugs that can be brought to bear on parasitic nematode infections is relatively small. Especially in the veterinary field, this panel of drugs is threatened by burgeoning, genetically-based resistance in parasite populations [2, 3]. Thus, discovery of novel drug and vaccine targets in pathogens is imperative. Existing anthelmintic drugs against parasitic nematodes may be broadly categorized as neuromuscular agents affecting cholinergic neurotransmission [4], hyperpolarizers of neuromuscular synapses [5], tubulin-binding agents and metabolic inhibitors [6]. It is likely that refinements in knowledge about these classes of drugs and the molecular structures of their targets will enable new drugs within those categories to be brought on line. However, it is also significant that there is increasing appreciation of the potential for essential parasite-specific signaling mechanisms to act as chemotherapeutic targets, particularly those mechanisms that involve interaction of small molecule ligands with cellular receptors [7]. Thus, the practical rationale for investigating signaling mechanisms within parasitic nematodes is strong. Also crucial to basic scientific importance is an understanding of how parasitic nematodes have adapted key signaling mechanisms from free-living ancestors to the evolution of animal parasitism.

Contemporary biologists tend to equate signaling with molecular mechanisms and pathways of cellular signal transduction. However, this review will define signaling broadly to include known mechanisms by which parasitic nematodes perceive environmental factors that aid in host finding and acquisition and by which they communicate with other parasites and with their hosts, in addition to the molecular pathways that transduce these signals within and between cells of the parasite. This distinction is crucial given the reliance of molecular studies of parasitic nematodes on science pertaining to *Caenorhabditis elegans* as a source of hypotheses for testing. Of the active areas of signal transduction research in *C. elegans*, TGFβ [8], insulin and insulin-like growth factor [9] nuclear receptor [10] and G-protein coupled receptor (GPCR) [11] signaling have received the greatest attention from parasitologists owing to their essential functions in regulation of dauer development in *C. elegans*. Dauer development is regarded as a paradigm for regulation of infective larval development before and during host invasion in parasitic nematodes [••12, 13]. As tools for functional genomic study in parasitic nematodes become more sophisticated and widely used, the extensive knowledge of chemoreceptors in sensory neurons of *C. elegans* will likely be applied to parasite biology based on the likely functions of their homologs in host finding by soil-transmitted parasitic nematodes [14]. This review will examine the adaptation of these relevant signaling mechanisms from *C. elegans* to the requirements for the evolution of parasitism in nematodes, and it will examine other external cues, many originating from the host, that are likely essential for parasitic nematode life cycles,

indicating, or hypothesizing where possible, the mechanisms by which these cues are processed within the worms at the neuronal and molecular levels. The article will conclude with a critique of these pathways as chemotherapeutic targets.

Physico-chemical signals from host to free-living infectious stages – host finding

Prior to encountering a host, infective third-stage larvae of soil dwelling parasitic nematodes (iL3) undertake innate behaviors that increase their chances of contacting a host (Fig. 1, Table 1). One such behavior is crawling upwards in water films on stationary objects. Such behaviors are common in skin penetrating iL3, such as *Strongyloides stercoralis* and various hookworms, which climb to the apices of vegetation or prominences in the soil, and in passively ingested iL3 such as those of *H. contortus*, which crawl to the ends of grass blades [15, 16]. Skin penetrating *S. stercoralis* and *Ancylostoma caninum* (hookworm) iL3 clearly exhibit true negative geotaxes whereas those of the passively ingested *H. contortus* do not [17]. Interestingly, iL3 of *Oesophagostomum dentatum*, which are also passively ingested by their porcine hosts, exhibit an age-dependent shift in geotactic behavior in which four-day old iL3 show no geotaxis while eight-day old larvae exhibit a positive geotaxis, tending to crawl downwards [18]. This may represent a behavioral adaptation by which older larvae position themselves in the moist soil where they are protected from desiccation and more liable to be ingested by rooting pigs. The neuronal and molecular bases for these crucial taxes in host finding are currently unknown, but it might be inferred from *C. elegans* science that they are mediated by touch receptors. Neurons called microtubule cells, which are lodged between the cuticle and underlying epidermis, detect tactile and seismic stimuli in *C. elegans. C. elegans* also uses these microtubule cells to receive delocalized mechanical signals such as substrate vibration. This system may also provide a model of mechanosensory aspects of the infective process in skin penetrating nematode parasites. L3i of some species appear to use soil vibration to detect the approach of a host [19]. Identifying mechanoreceptors in parasitic nematodes and ablating them by microlaser surgery [20, 21] or by cell-specific expression of recombinant lytic factors [22] in an appropriate parasitic nematode model may confirm the role of mechanosensation in innate behaviors that foster host acquisition.

Another innate behavior that increases the likelihood of skin penetrating L3i establishing contact is shift from rest to motile or hypermotile behavior at the approach of a host. It is assumed that the requisite change in motility would have to occur prior to when contact is imminent in response to host-associated cues, such as vibration of the substratum [23, 19], volatile chemicals [14] and elevated temperature [23, 19], that act over relatively long ranges (Fig. 1B, Table 1). It is likely that responsiveness to seismic cues of host approach is mediated by mechanosensory neurons as discussed above. Orientation towards mammalian hosts by migrating up temperature gradients appears to be conserved in diverse skin-penetrating iL3 that dwell in contaminated soil such as *Ancylostoma caninum* [23] and *Strongyloides stercoralis* [24]. Results of cell ablation reveal that ALD class neurons in the amphids are necessary for thermotaxis by *S. stercoralis* [24]. Exhaled CO_2 has long been considered a candidate for such a long-range host odorant cue, and its status as a stimulus

for motility shift on host approach has been confirmed for skin penetrating iL3 of *S. stercoralis* and *A. caninum*, and the passively ingested iL3 of *H. contortus* [25]. L3i of the skin penetrating species respond to CO_2 at a concentration approximating that found in exhaled breath (3%) with increased but non-directional crawling activity. By contrast, *H. contortus* iL3 virtually cease progressive movement in the presence of physiological CO_2 levels, remaining motile but tightly coiled. Both of these responses are saturable at high concentrations of CO_2, and *H. contortus* iL3 that become non-motile at high concentrations of CO_2 regain progressive motility within approximately 30 seconds of transfer to ambient air. The molecular mechanisms underlying the CO_2 responses of these infective larvae are currently unknown, but their saturability and reversibility are consistent with mediation by a cellular receptor [25]. Thus, CO_2 constitutes a long-range chemical cue for specific behavioral modifications that would enhance host contact in skin-penetrating L3i and in passively ingested ones. Presumably, hypermotility would serve to increase host contacts by the former, which must actively acquire the host, and reduction or loss of motility by the latter would serve to maintain their positions at the apices of grass blades and other plant structures where ingestion by grazing herbivores would then be likely. This overall interpretation of host-elicited behaviors has been upheld by more comprehensive comparative study of parasitic nematodes from diverse phyla, including species infecting arthropods and mammals [•26].

As an interesting example of convergent evolution, it appears that host preference is a stronger factor than phylogeny in determining sensitivity to specific olfactory cues. For example, L3i of the rodent parasite *Strongyloides ratti* is more similar to iL3 of the distantly related *Nippostrongylus braziliensis* in its profile of responses to a large panel of olfactory cues than it is to iL3 of its cogener *S. stercoralis* [•26], a parasite of humans and dogs. In an even more striking example, it appears that parasitic nematodes and blood-sucking arthropods exploit similar volatile odorants to help them orient to their hosts. iL3 of *S. stercoralis* are attracted to diverse human associated odorants, and among the most active of these are 2- and 3-methyl-1-butanol, 3-heptanol, 1-nonanol, which are all potent attractants for host seeking mosquitoes (Table 1) [•26].

Orientation on the host once acquired

Once contact with the host has been established, orientation of larvae in the pelt onto the skin and location of optimal sites for penetration may be mediated by continued thermotaxis, and orientation in response to both aqueous and volatile chemicals (Fig. 1B, Table 1). Transduction of aqueous host cues by specific chemosensory neurons in the amphids appears largely conserved between the free-living *C. elegans* and the phylogenetically distant parasite *S. stercoralis*. Cell ablation studies in *S. stercoralis* confirm that neuron pair ASE mediates attraction of L3i to optimal NaCl concentrations and that neuron pair ASH mediates repulsion from high salinity [27]. Notably, the positional homologs of these neurons in *C. elegans*, ASE and ASH, also mediate attraction to NaCl and repulsion from high osmolality, respectively (Fig. 1B, C; Table 1) [28]. Most putative aqueous attractants from host skin such as NaCl have been selected for study based on the premise that they are known components of skin or secretions therefrom. By contrast, an unbiased scheme for fractionation of a crude extract of canine skin that was attractive for iL3 of *S. stercoralis*

revealed that urocanic acid, a common component of human and other mammalian skin, accounted for the majority of this attractancy [29].

Avoiding noxious or harmful chemicals is also advantageous for parasitic iL3 in the environment and on the host. Responses to sodium dodecyl sulfate (SDS) have been used as a model for this type of behavior in *Ancylostoma caninum* whose iL3, migrating on agar layers, avoid droplets of 1% SDS, quickly reversing the direction of movement and migrating away. Ablation of neuron pairs ASH and ADL eliminates this rearward avoidance response by *A. caninum* iL3, actually increasing the speed of forward migration in the presence of SDS [30]. In *C. elegans*, amphidial neuron pairs ASH and ASK mediate chemical avoidance [31], indicating at least partial conservation of neuronal control of noxious chemical avoidance by free-living and parasitic nematodes.

Environmental cues governing morphogenesis and development of infective parasitic nematode larvae

Morphological and behavioral similarities between the iL3 of parasitic nematodes, particularly the soil-transmitted species, and dauer third-stage larvae of *C. elegans* have been recognized for decades [13, 32]. The majority of parasitic iL3 are like *C. elegans* dauers in that they are radially constricted in overall body profile, have narrow pharynxes, are non-feeding and have modified, chemically resistant cuticles. More importantly, parasitic iL3, like *C. elegans* dauers, are in a state of developmental arrest and require entry into the definitive host for re-activation (Fig. 1). Moreover, theoretical parallels drawn between molecular regulation of iL3 morphogenesis and development and *C. elegans* dauer regulation have been largely substantiated in parasitic nematodes by genomic and transcriptomic evidence and, in the few instances where direct interrogation of gene or protein function is possible, by experimentation [••12]. Dauer development in *C. elegans* is a conditional response to environmental stressors including overcrowding with con-specific nematodes, declining food levels and elevated temperature [33, 32].

The majority of parasitic nematode species develop through an invariant pattern of first- through third larval stage development in the environment extrinsic to the host, infection of the host as iL3 and development through two additional molts to the adult stage and then localization to some predilection site within the host body (Fig. 1A). In these life histories, development to the iL3 is fixed and there are no equivalents of the life cycle switching between developmental fates exemplified by the checkpoint governing dauer versus continuous development in *C. elegans*. By contrast, first-stage larvae (L1) of members of the Strongyloidoidea, including the genera *Strongyloides* and *Parastrongyloides* are capable of switching between direct development to iL3 or development to one or more generations of free-living males and females (Fig. 1A). Most species of *Strongyloides* execute only one generation of free-living development, with the progeny of free-living males and females developing exclusively to the iL3. However, *S. planiceps* can undertake up to nine sequential generations of free-living development, with increasing attrition to the parasitic cycle in each [34] and *Parastrongyloides* spp. can develop through an indefinite number of free-living generations as a free-living nematode but switch to formation of iL3 to infect a marsupial

host in response to changing environmental conditions [35]. The switch between free-living and parasitic alternatives in the Strongyloidoidea appears to be governed by factors similar to those governing dauer switching in *C. elegans*, but in ways that are frequently contrary to the *C. elegans* responses. For example, while high temperatures predispose *C. elegans* towards dauer development, the majority of *Strongyloides* spp. L1 exhibit the opposite response, developing more frequently to free-living adults at elevated temperature [36, 37]. The exception to this generalization is *S. stercoralis*, where temperatures approaching host body temperature (34–37° C) promote direct development to iL3 while lower temperatures promote free-living development. As did chemotaxis, this temperature dependent developmental switch in *S. stercoralis* requires signaling by ALD-class amphidial neurons [38]. Additional neuronal inputs from amphidial neurons ASF and ASI are required to regulate this switch (Fig. 1F) [20]. Consistent with dauer switching in *C. elegans*, depletion of nutrients in the environments of post-parasitic larvae promotes direct development to iL3 and enrichment of these in culture media of promotes development to free-living adults in *Strongyloides* spp. [39–41].

Cellular signaling pathways mediating morphogenesis and development of iL3 in parasitic nematodes

Four cellular signal transduction pathways regulate dauer development and the associated property of lifespan extension in *C. elegans*. Thermal cues and chemical signals of food level and population density in the local environment are received by G-protein coupled receptors (GPCRs) in chemo- and thermosensory neurons in the amphids [42–45]. Dauer regulatory signals from GPCRs in the amphids are transduced downstream by parallel insulin/insulin-like growth factor (IIS) [33, 9] and TGFβ pathways [8, 33], and these pathways converge on a steroid-nuclear hormone receptor (NHR) pathway involving the DAF-12 NHR and its dafachronic acid ligands [46–51]. In general, experimental evidence, discussed in the following paragraphs, supports that GPCR, IIS and steroid-NHR signaling function to regulate the infective process in parasitic nematodes in ways that are consistent with their functions in regulating dauer development in *C. elegans* (Fig. 1D, E; Table 2). By contrast, the limited data available suggest that TGFβ has been adapted for functions in parasitic nematodes that are fundamentally different from the dauer regulatory role of this signaling pathway in *C. elegans* (Fig. 1G, Table 2) [••12].

Genomic and transcriptomic studies support that elements of GPCR signaling that regulate dauer development in *C. elegans* are conserved in parasitic nematodes (Table 2) [52, 53]. Gene homologs of *gpa-2* and *gpa-3*, whose products mediate dauer pheromone signals in *C. elegans* have been cloned from the genome of *S. stercoralis* [54], and the transcriptome of *S. stercoralis* contains homologs of mRNAs encoding key elements of GPCR signaling in *C. elegans* including *Ce-daf-11* (encoding a guanylyl cyclase), *Ce-tax-2* and *Ce-tax-4* (encoding α and β subunits of a neuronal cyclic nucleotide-gated ion channel). These homologs were designated *Ss-gyc-11, Ss-tax-2* and *Ss-tax-4*, respectively [53]. Experimental evidence that GPCR signaling governs development by iL3 of parasitic stems from pharmacological studies in which a cell-permeable analog of cGMP, 8-bromo-cyclic GMP, stimulates resumption of development by cultured iL3 of *A. caninum* [55], *Nippostrongylus*

braziliensis [56] and *S. stercoralis* [57]. In *C. elegans*, dauer-regulatory GPCR signaling is coupled to downstream IIS and TGFβ signaling pathways as indicated by the fact that increasing cGMP levels during continuous development elicit production of DAF-7, the TGFβ-like ligand of the heterodimeric receptor comprising DAF-1 and DAF-4. Likewise, increasing cGMP stimulates production of INS-7 and DAF-28, insulin-like peptides (ILPs) and agonists of the insulin-like receptor kinase DAF-2. Strikingly, in addition to stimulating resumption of development, administration of 8-bromo-cGMP to resting iL3 of *S. stercoralis* also upregulates production of several TGFβ-like ligands in that parasite and of the ILPs *Ss*-ILP-1 and *Ss*-ILP6, putative agonists of *Ss*-DAF-2, strongly supporting that GPCR signaling acts upstream of both IIS and TGFβ-like signaling (Fig 1D, E) [57].

An increasing body of descriptive and experimental data supports a role for IIS in regulating the iL3 of several parasitic nematodes during their infective processes. Genomic and transcriptomic data indicate that the key elements of IIS in *C. elegans* are conserved in *S. stercoralis*, including insulin-like peptides (ILPs), strikingly reduced to seven from the 40 ILPs in *C. elegans* [58, 59, 53], *Ss-daf-2*, encoding an insulin-like receptor kinase [60, 53], *Ss-age-1, Ss-pdk-1* and *Ss-akt-1* encoding cytoplasmic insulin regulated signaling kinases [61, 53] and phosphatases or their regulatory subunits encoded in *Ss-pten-1* and *Ss-pten-2* and in *Ss-pptr-1* that modulate the activities of *Ss*-AGE-1 and *Ss*-AKT-1 (Table 2). Similarly, key insulin-like signaling intermediates are conserved in the agriculturally important trichostrongyle *Haemonchus contortus* [62]. Finally, the homologs of genes encoding the insulin-regulated forkhead transcription factor DAF-16 have been discovered and characterized in *S. stercoralis* [63], the hookworms *A. caninum* and *A. ceylanicum* [64] and *H. contortus* [65]. A body of evidence obtained by administering putative inhibitors of insulin-regulated signaling kinases supports that, consistent with its requirement for dauer exit in *C. elegans*, insulin signaling is necessary for resumption of development by iL3 of parasitic nematodes in the host (Fig. 1D). The nominal PI3 kinase inhibitor LY294002 blocks resumption of development by iL3 of *A. caninum, A. ceylanicum* [66], *N. brasiliensis* [56] and *S. stercoralis* [61] cultured in permissive host-like conditions. Furthermore, AKT Inhibitor IV blocks resumption of development by cultured *N. brasiliensis* iL3 [56]. Absent robust functional genomic methods in most parasitic nematodes, evidence of the developmental regulatory capabilities of genes encoding insulin signaling elements in parasitic nematodes has been gathered from studies in which the ability these parasite genes to rescue loss-of-function mutations in their *C. elegans* homologs has been assessed. Genomic response elements and some downstream target genes of the hookworm homolog *Aca-daf-16* have been defined [•67, 68]. These parasite DAF-16 homologs are all able to rescue the dauer arrest when expressed as transgenes in *C. elegans* carrying the null mutation mu86 in *daf-16* (Table 2) [69, 65, 70]. A system for transgenesis in *S. stercoralis* enabled a more direct interrogation of *Ss-daf-16* function in that worm [71]. Here, expression patterns of a GFP::*Ss*-DAF-16 fusion protein in *S. stercoralis* were virtually identical to those of a similar GFP::Ce-DAF-16 fusion protein in C. elegans [72], and regulation of subcellular localization of Ss-DAF-16 by AKT-mediated phosphorylation was confirmed by introducing putative phospho-null and phospho-mimetic mutations in sequences encoding AKT phosphorylation sites in the transgene. Most significantly, a *gfp::Ss-daf-16* transgene construct with phospho-null mutations in all AKT phosphorylation

sites and a deletion in the C-terminal domain, which encodes the transactivating functions of forkhead transcription factors, prevented normal iL3 morphogenesis, blocking normal accumulation of vesicles in intestinal cells of post free-living L1 of *S. stercoralis* and promoting initiation of an aberrant L3-L4 molt as well as retention of rhabditiform pharyngeal structure in post free-living L3 [71]. These are characteristics, which in contrast to normal developmentally arrested iL3 with filariform pharynxes, are reminiscent of larvae developing to free-living adults. These phenotypes underscore the requirement for *Ss-daf-16* function in morphogenesis of iL3 (Fig. 1E).

Whereas IIS in parasitic nematodes appears to regulate the morphogenesis and development of iL3 during the infective process in a manner consistent with its function in dauer development in *C. elegans*, homologous TGFβ-like signaling appears to have been adapted for different functions in parasitic nematodes (Fig. 1G, Table 2). In *C. elegans*, favorable environmental conditions stimulate production and secretion of the TGFβ-like ligand DAF-7 from amphidial neurons and its binding to the type I and type II receptors DAF-1 and DAF-4 in membranes of target cells. Ligation of DAF-1/DAF-4 results in phosphorylation of receptor SMADs DAF-8 and DAF-14. These rSMADs repress the coSMAD DAF-3 and thus confer a pattern of gene expression leading to continuous reproductive development. Under dauer inducing conditions, production of DAF-7 is downregulated. In the absence of DAF-1/DAF-4 signaling, the coSMAD DAF-3 is upregulated and, in concert with the Sno/Ski-like transcription factor DAF-5, represses synthesis of *daf-7* and *daf-8* conferring a dauer-specific pattern of gene expression. DAF-7-related TGFβ homologs and some or all of their downstream signaling elements have been discovered in parasitic nematodes representing diverse phylogenetic clades, including hookworms (*A. caninum*) [73, 74] and trichostrongyles (*Heligmosomoides polygyrus, N. brasiliensis, Teladorsagia circumcincta* and *H. contortus*) [75] in Clade V, *S. stercoralis* [76, 53] *S. ratti* and *Parastrongyloides trichosuri* [77] in Clade IV and *Brugia malayi* [78, 79] in Clade III (Table 2). It was postulated early on that in keeping with the dauer hypothesis or “daf-c paradigm” [13], TGFβ-like signaling would regulate formation of parasitic iL3 and that the majority of parasitic nematodes develop in a manner similar to dauer-constitutive (daf-c) mutants in *C. elegans* DAF-7 [80]. However, although the functions of *daf-7* homologs have not been directly interrogated in any parasitic nematode to date, patterns of these homologs’ expression in diverse species feature a peak in the iL3 in the majority of species [••12, 73, 78, 75, 53] or in another developmentally arrested stage, the microfilaria of *B. malayi* (Table 2) [78]. This is in direct opposition to patterns of *daf-7* expression in *C. elegans*, which is minimal during dauer arrest [8]. Furthermore, *Pt-daf-7* from *P. trichosuri* does not complement a null mutation in *C. elegans daf-7* (e1372) [81]. Overall, these findings indicate a function for TGFβ signaling in parasitic nematodes that is fundamentally different from that of DAF-7 signaling in *C. elegans*. Among those postulated is a host immunomodulatory function for DAF-7 homologs that facilitates invasion and establishment of infective and post-infective larvae (Fig. 1G) [78, 75].

In *C. elegans*, dauer regulatory insulin-like and TGFβ-like signaling outputs converge to regulate a steroid-NHR signaling pathway involving dafachronic acids [48] and their nuclear receptor, DAF-12. In the presence of ligand, DAF-12 promotes continuous development by *C. elegans* [48] and reverses lifespan extension [47]. In the absence of ligand, DAF-12, in

concert with the co-repressor DIN-1 promotes dauer arrest [48] and confers lifespan extension. Genetic epistasis analysis [82], reverse genetic [83] and biochemical [48] evidence confirm that DAF-12 signaling operates downstream of ILS and TGFβ-like signaling, and likewise, both ILS and TGFβ-like signaling positively regulate biosynthesis of dafachronic acids, principally by upregulating expression of the cytochrome P450 DAF-9. Like GPCR signaling and ILS, regulatory elements constituting DAF-12 signaling appear to be conserved in parasites and, based on current evidence, to undertake functions that are consistent with their dauer regulatory functions in *C. elegans*. Homologs of *daf-12* have been discovered in a number of parasitic nematodes including *A. caninum* [7, 84], *S. stercoralis* [85], *Strongyloides papillosus* (Table 2), and it is noteworthy that the natural ligands of DAF-12, 03944- and Δ7-dafachronic acids can signal through the *A. caninum* and *S. stercoralis* homologs of this NHR [7]. Moreover, sequence homologs of other key DAF-12 regulatory elements, among them the enzymes catalyzing dafachronic acid synthesis, are conserved in *S. stercoralis* [53]. Abundance profiles of transcripts encoding DAF-12 signaling elements in *S. stercoralis*, do not strictly conform to expression patterns of their homologs in *C. elegans* [53]. Nevertheless, there is ample evidence from functional studies that DAF-12 signaling, in some form, regulates iL3 morphogenesis and development during the infective process (Fig. 1 D, E). Most strikingly, administration of Δ7-dafachronic acid, and to a lesser degree Δ4-dafachronic acid, produces phenotypes in cultured parasitic nematode larvae that are consistent with the ability of these ligands to promote continuous reproductive development and suppress or reverse dauer development in *C. elegans*. Dafachronic acids promote resumption of development by cultured iL3 of *A. caninum* and *S. stercoralis* in the absence of host-like cues [57, 7, 84]. Administered Δ7-dafachronic acid also suppresses formation of iL3 in the post free-living generations of *S. stercoralis* and *S. papillosus*, giving rise to aberrant post-free living L4 in *S. stercoralis* and reproductively competent free-living females in *S. papillosus* [•86, 87, 7]. Similarly, administered Δ7 dafachronic acid can regulate developmental switching by female larvae of *S. stercoralis* in the post-parasitic generation, suppressing direct development to the iL3 and promoting development to free-living females [•86]. Studies with pharmacologic inhibitors provide evidence of endogenous DAF-12 signaling. The cytochrome P450 inhibitor ketoconazole suppresses resumption of feeding by diverse species of parasitic nematode iL3, including *A. caninum, S. stercoralis* and *N. brasiliensis*, in permissive in vitro culture systems [•86, 56, 7]. Care is warranted in interpreting these findings, given the limited specificity of ketoconazole and the multiplicity of cytochrome P450s in the parasites studied. In this regard it is noteworthy that administered Δ7-dafachronic acid partially rescues the developmental blockade imposed by ketoconazole in *S. stercoralis* iL3 [•86], lending support to a conclusion of a requirement for endogenous synthesis of a steroid ligand for DAF-12 to stimulate resumption of iL3 development on infection (Fig. 1D).

Signaling between parasitic nematodes and the host immune system

Parasitic nematodes secrete biologically active proteins into their surroundings in host tissues or gastrointestinal tract, and contemporary bioinformatic and proteomic methods have proven powerful in analyzing these “secretomes” [88–93]. Some of the proteins parasitic nematodes elaborate within the host appear capable of directing the host immune

response in a manner that is adaptive for the parasite. Exemplary studies in this regard are of the secretome of *Strongyloides ratti*, [91, 94], which stressed secretory proteins from iL3 and parasitic females that are capable of modulating immune function in host intestinal mucosa. The combined secretomes of *S. ratti* stages investigated to date comprise some 586 proteins [91]. Seventy nine proteins are secreted by parasitic females of *S. ratti*, among them a prolyl oligopeptidase that appears to be necessary for survival and a number of small heat shock proteins that are immunogenic to the host, interact with the host epithelium or are capable of shifting cytokine production by cultured monocytes to IL-10 in favor of TNFα. This suggests that these proteins are capable of directing polarity of the host immune response [91].

Likewise there is evidence that a protective host immune response may affect subsequent developmental fates in the progeny of parasitic adult worms. In a striking example of this, *S. ratti* larvae exiting a host that is mounting an immune response to the parasite are more likely to develop to free-living males and females, as opposed to iL3, than larvae exiting naïve hosts [95, 96]. Given the roles that amphidial neuron pairs ASF and ASI play in developmental switching by *S. stercoralis*, it is logical to hypothesize that these neurons receive signals of mounting immunity that contribute to commitment to free-living development [95, 96]. However, in view of the fact that a history of dauer development is imprinted in the form of epigenetic marks on subsequent generations of developing *C. elegans* larvae [97], the possibility that the effect of host immunity on development by subsequent generations of parasitic nematode larvae is epigenetically controlled should also be considered.

Conclusion and future directions

Currently, the mechanisms governing the innate behaviors that orient infective parasitic nematode larvae can only be surmised based on knowledge of the interplay between mechanosensory neurons and muscles involved in progressive motility in *C. elegans* [98]. However, the technology now exists to test relevant hypotheses using microlaser cell ablation and transgene-mediated lysis of specific cells in appropriate parasite models such as *Strongyloides* spp. [20, 99]. By contrast, neuronal control of taxes by infective larvae that bring them in contact with a host is better understood thanks to careful studies of neuroanatomy in larvae of soil transmitted parasitic nematodes and the microlaser ablation studies that these enable [20, 21]. It may again be surmised from *C. elegans* science that impulses within crucial sensory neurons in parasites are transduced by GPCR signaling, but this has not been tested. Overall, molecular mechanisms that are required for host finding and acquisition could theoretically become the bases for preventative medications, but it is unlikely that such interventions would be practical in combatting medically important nematode parasitisms, which are generally infections associated with extreme poverty. On the other hand, specific signal transduction mechanisms governing the infective process that are adapted from those regulating dauer larval recovery and adult lifespan in *C. elegans* have supported this “dauer hypothesis” in part [••12] and have revealed points of regulation by small molecules that could constitute novel chemotherapeutic targets. The most obvious of these is signaling through homologs of the DAF-12 nuclear receptor by dafachronic acid-related steroids [•86, 7, 84]. Cell based reporter assays incorporating DAF-12 homologs

from parasitic nematodes have already been developed [7] and could constitute probes to identify natural ligands of parasite DAF-12 homologs and form the bases for high throughput screens for both agonistic and antagonistic ligands of these NHRs. “Hits” from such high capacity screening could constitute leads in the search for new drugs to prevent establishment by infective larvae and hasten expulsion of adult worms in a wide range of parasitic nematode infections or prevent potentially fatal hyperinfection by *S. stercoralis*. Finally, thanks to efforts to define the secretomes of parasitic nematodes within their definitive hosts and to assess the roles of these in modulating the host immune response, a picture of how nematodes establish and maintain chronic infection on the one hand, but also contribute to immunological homeostasis in the host, is now emerging with greater clarity [91]. The molecular mechanisms involved in signaling between nematode parasites and their hosts’ immune systems could constitute new immunotherapies, not only for nematode parasitosis, but also for inflammatory diseases of diverse etiology [100].

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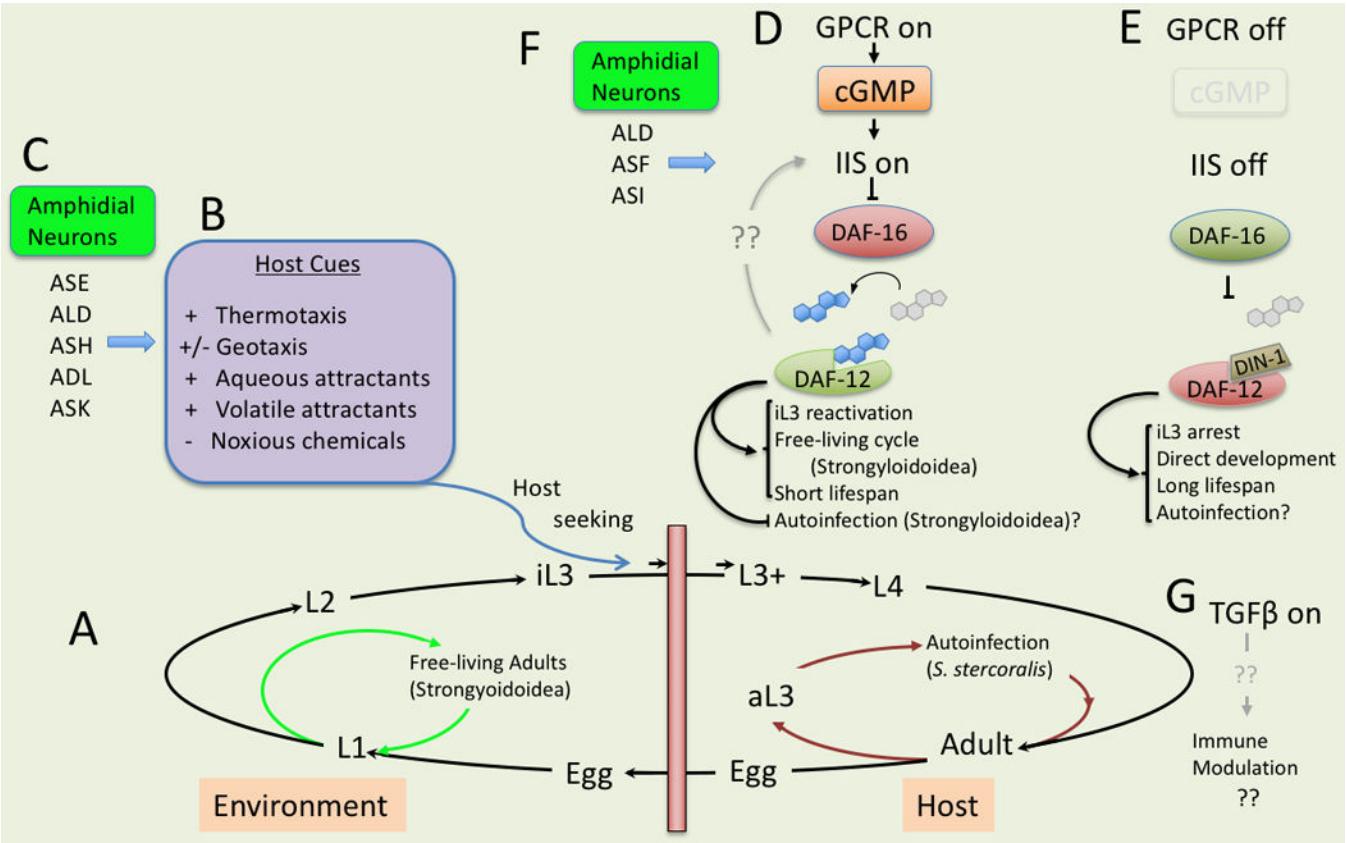

Fig. 1.

Control of host finding and development by infective third-stage parasitic nematode larvae (iL3). (A) Generalized developmental diagram for parasitic nematodes. Includes free-living and autoinfective alternatives in the Strongyloidoidea. (B) Innate behaviors and host-associated cues mediating host finding and acquisition by iL3. (C) Amphidial sensory neurons mediating innate behaviors and responsiveness to host cues. (D) Coordinated G-protein coupled (GPCR), insulin/insulin-like growth factor (IIS) and DAF-12 nuclear hormone receptor signaling. Note: active GPCR and IIS (signaling “on”) negatively regulate the forkhead transcription factor DAF-16 and promote synthesis of DAF-12 ligands (blue steroid icon) from dietary cholesterol (gray steroid icon). Upregulation of putatively agonistic insulin-like peptides by the DAF-12 ligand suggests possible feedback of IIS by DAF-12 signaling in *S. stercoralis*. This confers resumption of development by iL3, free-living development in Strongyloidoidea and may shorten adult lifespan and suppress autoinfection in *Strongyloides stercoralis*. (E) GPCR, IIS and DAF-12 signaling in the “off” state confer iL3 arrest, direct development of L1 to iL3 and autoinfection in *S. stercoralis*. (F) Amphidial neurons mediating resumption of iL3 development and switching between parasitic and free-living alternatives in *S. stercoralis*. (G) Although TGFβ-like signaling is conserved in parasitic nematodes, expression patterns of ligands during development are inconsistent with conservation of *C. elegans*-like TGFβ function in parasitic nematodes. Alternative functions hypothesized include modulation of host immunity.

Table 1

Summary of behaviors associated with host finding and acquisition, their mediation by host cues and, where known, the amphidial neurons required for their perception.

Behavior	Host cue category	Parasites studied	Active compounds	Amphidial neurons involved	References
Geotaxis (positive)	None – innate behavior	*S. stercoralis*	NA	??	15, 16
		H. contortus			
Geotaxis (negative)	None – innate behavior	*O. dentatum*	NA	??	18
Hypermotility	Vibration of substratum	*A. caninum*	NA	??	19, 23
	Elevated temperature	*A. caninum*	NA	??	19, 23
		S. stercoralis	NA	ALD	24
Nictation *	Elevated temperature	*S. stercoralis*	NA	ALD	14, 24, 26
		S. ratti	NA	??	26
		N. brasiliensis			
		H. contortus			
Approaching the host	Volatile odorants	*S. stercoralis*	CO_2	??	25
		S. stercoralis	Numerous host skin-associated cues	??	26
		S. ratti			
		N. brasiliensis			
		H. contortus			
	Thermal gradient	*S. stercoralis*	NA	ALD	23, 24, 26
		A. caninum	NA	??	
Orientation on the host	Aqueous attractants and repellants	*S. stercoralis*	NaCl	ASE, ASH	27, 28
			Urocanic acid	??	29
			SDS – noxous stimuli - avoidance	ASH, ADL	30, 31

*Nictation is a behavior in which nematodes stand on their tails, often in aggregates, thus further elevating themselves above the substratum to promote host contact

Abbreviation of parasite genera: *A.* – *Ancylostoma, H.* – *Haemonchus*, O. Oesophagostomum, *N.* – *Nippostrongylus, S.* – *Strongyloides*,

Table 2

Summary of parasitological findings on structural and functional conservation of dauer-like signaling pathways in parasitic nematodes

Pathway	Parasite	Phylogenetic Clade	Genes conserved?	Genes complement *C. elegans* mutants?	Expression consistent with dauer hypothesis?	Function Conserved?	References
GPCR	*A. caninum*	V	Yes	??	??	Yes	12, 53–57
	N. brasiliensis	V	Yes	??	??	Yes	
	S. stercoralis	IV	Yes	??	Yes	Yes	
IIS	*A. caninum*	V	Yes	Yes – *daf-16*	??	Yes	12, 53, 60–71
	A. ceylanicum	V	Yes	??	??	Yes	
	N. brasiliensis	V	Yes	??	??	Yes	
	H. contortus	V	Yes	Yes – *daf-16*	Yes	Yes	
	S. stercoralis	IV	Yes	Yes – *daf-16*	Yes	Yes	
TGFβ-like	*A. caninum*	V	Yes	??	No	??	12, 53, 73–79, 81
	N. brasiliensis	V	Yes	??	No	??	
	He. polygyrus	V	Yes	??	No	??	
	T. circumcincta	V	Yes	??	No	??	
	H. contortus	V	Yes	??	No	??	
	S. stercoralis	IV	Yes	??	No	??	
	S. ratti	IV	Yes	??	No	??	
	P. trichosuri	IV	Yes	No – *daf-7*	No	No	
	B. pahangi	III	Yes	??	No	??	
	B. malayi	III	Yes	??	No	??	
Steroid-NHR	*A. caninum*	V	Yes	??	??	Yes	7, 12, 53, 84–87
	A. ceylanicum	V	Yes	??	??	Yes	
	S. stercoralis	IV	Yes	??	Yes/No	Yes	

Abbreviations: GPCR – G protein coupled receptor, IIS – Insulin/Insulin-like growth factor signaling, TGFβ - Transforming Growth Factor β, NHR – Nuclear hormone receptor. **Parasite genera**: *A.* – *Ancylostoma, N.* – *Nippostrongylus, He.* – *Heligmomosoides, T.* – *Teladorsagia, H.* – *Haemonchus, S.* – *Strongyloides, P.* – *Parastrongyloides, B.* – *Brugia.*