The dauer hypothesis and the evolution of parasitism: 20 years on and still going strong

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Abstract

How any complex trait has evolved is a fascinating question, yet the evolution of parasitism among the nematodes is arguably one of the most arresting. How did free-living nematodes cross that seemingly insurmountable evolutionary chasm between soil dwelling and survival inside another organism? Which of the many finely honed responses to the varied and harsh environments of free-living nematodes provided the material upon which natural selection could act? Although several complementary theories explain this phenomenon, I will focus on the dauer hypothesis. The dauer hypothesis posits that the arrested third-stage dauer larvae of free-living nematodes such as *Caenorhabditis elegans* are, due to their many physiological similarities with infective third-stage larvae of parasitic nematodes, a pre-adaptation to parasitism. If so, then a logical extension of this hypothesis is that the molecular pathways which control entry into and recovery from dauer formation by free-living nematodes in response to environmental cues have been co-opted to control the processes of infective larval arrest and activation in parasitic nematodes. The molecular machinery that controls dauer entry and exit is present in a wide range of parasitic nematodes. However, the developmental outputs of the different pathways are both conserved and divergent, not only between populations of *C. elegans* or between *C. elegans* and parasitic nematodes but also between different species of parasitic nematodes. Thus the picture that emerges is more nuanced than originally predicted and may provide insights into the evolution of such an interesting and complex trait.

Keywords

Dauer hypothesis; Evolution; Co-option; TGF-β; Insulin signaling

1. Introduction

A remarkable range of hosts are parasitized by nematodes, to the extent that it is likely that every possible host species has been colonized. Nematode body size varies massively across the phylum and, although the basic nematode body plan is retained, many parasitic nematodes have specialized adaptations to allow the invasion and colonisation of their host(s). Prospective animal parasitic nematodes need to adapt to hostile conditions inside and outside the host, including high temperature, low oxygen potential, gut pH and enzymes, and the host immune response, with a different set of equally hostile conditions facing plant

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parasitic nematodes. However, phylogenetic analysis by rRNA sequencing shows that parasitism, be it of plants, insects or animals, has evolved from free-living ancestors at least five times (Blaxter et al., 1998). Thus not only is parasitism a successful strategy but the obstacles to becoming a parasitic nematode have been overcome multiple times. This then posits the following questions: how has parasitism evolved in nematodes and is the mechanism conserved across multiple occurrences of free-living to parasitic evolution?

These questions can be considered from two points of view, which are essentially different sides of the same coin; the evolutionary ecology of nematodes and the pre-adaptation to parasitism. From the evolutionary ecology viewpoint, the evolution towards parasitism proceeded via an intermediate phoretic association with another organism, such as that seen with multiple *Caenorhabditis* spp. (Baird, 1999; Barriere and Felix, 2005), towards endophoresy, necromeny (Hong and Sommer, 2006), facultative parasitism and finally obligate parasitism. However, it is most likely that the ecological changes towards parasitism and the role of existing pre-adaptations favouring parasitism are inseparable as each is unlikely to have occurred without the other. There is an expanding body of work on the ecological changes that may have lead towards parasitism, such as excellent work on host-nematode interactions by the Sommer laboratory (Hong et al., 2008).

From the pre-adaptation viewpoint, the physiological similarities between arrested dauer L3s of free-living nematodes and arrested infective L3s (iL3) of parasitic nematodes represent an underlying pre-adaptation to parasitism (Rogers and Sommerville, 1963; Hawdon and Schad, 1991; Hotez et al., 1993). Although the ability to develop directly to adulthood without entering larval arrest is known to occur in a limited number of parasitic nematode species, such as *Parastrongyloides trichosuri* (Grant et al., 2006) and *Strongyloides* spp. (Yamada et al., 1991; Viney, 1996), and has most likely been lost from obligate parasites, infective larvae of all parasitic and entomopathogenic nematodes must precisely control their activation upon infecting a suitable host. This review will focus on the pre-adaptation to parasitism perspective within the framework of the dauer hypothesis.

2. The dauer hypothesis

Dauers of free-living nematodes are arrested, non-feeding L3s that are resistant to a wide range of environmental insults and act as a dispersal stage to colonise distant high nutrient microenvironments. In *Caenorhabditis elegans*, dauer larvae are formed under conditions of low food availability, high con-specific population density sensed via a constitutively produced pheromone and high temperature (Riddle and Albert, 1997). In parasitic nematodes, infective larvae are also arrested, primarily L3s, non-feeding and able to survive under harsh conditions. Their role is also to act as a dispersal stage, in this case between hosts. For obligate parasitic nematodes such as *Haemonchus contortus*, formation of infective larvae is constitutive. For parasitic nematodes with free-living generations, infective larvae formation is influenced by host immune status and environmental temperature, e.g. in *Strongyloides ratti* (Harvey et al., 2000), or a secreted compound analogous to dauer pheromone, e.g. in *P. trichosuri* (Grant et al., 2006; Stasiuk et al., 2012). In addition, both dauer and infective larvae share a radial constriction and filariform, as opposed to rhabditiform, pharynx. These similarities in physiology and role between dauer larvae of free-living nematodes and infective larvae of parasitic nematodes have long been commented upon (Rogers and Sommerville, 1963; Hawdon and Schad, 1991) and were canonised as the dauer hypothesis; the prediction that dauer larvae are a pre-adaptation to parasitism and that the mechanisms used to control dauer developmental decisions have been co-opted to control infective larval development and reactivation (Hotez et al., 1993).

3. The control of entry into *C. elegans* dauer development

Entry into and exit from dauer formation in *C. elegans* is precisely controlled in response to temperature, food and population density, sensed as a constitutively produced ascaroside pheromone complex (Riddle and Albert, 1997; Jeong et al., 2005; Butcher et al., 2007). These environmental stimuli are collated by a defined set of sensory neurons, the ASI, ASG, ASJ and ADF plus, to a lesser extent, ASK, amphidial neurons (Bargmann and Horvitz, 1991; Schackwitz et al., 1996), and four molecular pathways, cGMP, insulin and TGF-β pathways, and dafachronic acid signaling via the nuclear hormone receptor, DAF-12 (Riddle and Albert, 1997; Motola et al., 2006) (Fig. 1.; see Stoltzfus et al. (2012b) for additional pathway components). Laser ablation of ASI, ASG and, to a lesser extent, ADF neurons results in constitutive dauer formation (Bargmann and Horvitz, 1991; Schackwitz et al., 1996), whereas laser ablation of ASJ neurons prevents recovery from dauer arrest (Bargmann and Horvitz, 1991). Although the amphidial neuron controlling entry into dauer development at high temperatures has not been described, ALD is a likely candidate, given its role in thermotaxis (Mori and Ohshima, 1995).

The four molecular pathways that control entry into dauer development have been similarly well characterised. Dauer pheromone, a complex of C3, C6, C7 and C9 side chain ascarosides (Jeong et al., 2005; Butcher et al., 2007; Butcher et al., 2008) is sensed in the ASK neuron with different affinities by at least four G-protein coupled receptors, SRBC-64, SRBC-66, SRG-36 and SRG-37 (Kim et al., 2009; McGrath et al., 2011). G-proteins GPA-2 and GPA-3 (Zwaal et al., 1997) transduce this signal to the guanylyl cyclase receptor DAF-11 and HSP-90 protein DAF-21 (Birnby et al., 2000), which then act via cyclic nucleotide-gated channel subunits TAX-2 and TAX-4 (Ailion and Thomas, 2000) to suppress *daf-7* and *daf-28* expression in response to pheromone.

A large family of insulin ligands, with agonists (*e.g. daf-28* (Li et al., 2003)) and antagonists (*e.g. ins-18* (Matsunaga et al., 2012) and *ins-1* (Pierce et al., 2001)), binds to the DAF-2 insulin receptor orthologue (Kimura et al., 1997), the summation of which determines the output of DAF-2. When activated, DAF-2 in turn activates the AGE-1 phosphoinositide 3-kinase (PI3K) and downstream protein kinases, PDK-1, AKT-1 and AKT-2 (Paradis and Ruvkun, 1998). AKT-1 then phosphorylates DAF-16, a FOXO orthologue, resulting in the cytoplasmic localisation of DAF-16 (Paradis and Ruvkun, 1998; Lee et al., 2001) and reproductive development. Under low food and high pheromone conditions, a lack of DAF-2 activation results in the nuclear localisation of DAF-16 and entry into dauer development (Paradis and Ruvkun, 1998).

DAF-7 is expressed in ASI neurons under low pheromone and high food conditions (Ren et al., 1996; Schackwitz et al., 1996; Crook et al., 2010). DAF-7 binding to the DAF-1/DAF-4 heterodimeric receptor results in phosphorylation of the R-SMADs DAF-8 and DAF-14 (Georgi et al., 1990; Estevez et al., 1993; Inoue and Thomas, 2000), which in turn inhibit Sno-like DAF-5 and SMAD DAF-3 binding to the promoters of genes involved in dauer development (Patterson et al., 1997; da Graca et al., 2004). DAF-3 also acts as part of a feedback loop by repressing DAF-7 and DAF-8 expression under dauer inducing conditions (Park et al., 2010). Mutations in pro-reproductive development genes, such as *daf-7* and *daf-1* (Estevez et al., 1993; Ren et al., 1996), result in constitutive dauer formation (*daf-c*), even under favourable conditions, whereas mutations in pro-dauer genes, such as *daf-3*, result in defective dauer formation, even under dauer inducing conditions (*daf-d*) (Patterson et al., 1997). In addition, *daf-7* expression is switched on when pheromone induced dauer larvae are transferred to freshly seeded plates without pheromone, which suggests a role for *daf-7* signaling during exit from the dauer stage (Ren et al., 1996).

Finally, outputs of these three pathways are collated by a nuclear hormone receptor, DAF-12, which is considered the master regulator for entry into dauer development (Inoue and Thomas, 2000; Snow and Larsen, 2000), for which the ligands are dafachronic acids, sterols produced by cytochrome p450 DAF-9 (Jia et al., 2002; Motola et al., 2006). Therefore under the dauer hypothesis, one would expect both the molecular and cellular control of dauer entry to be conserved in parasitic nematodes. I will first cover the conservation of the cellular control of dauer development, then the three pathways that control dauer development.

4. Cellular conservation of the control of arrested development

The identity of sensory neurons in parasitic nematodes has been determined by positional homology for *Strongyloides stercoralis*, *H. contortus* and *P. trichosuri* (Ashton et al., 1995; Li et al., 2000; Li et al., 2001; Zhu et al., 2011) (Fig. 2). To demonstrate functional homology, laser ablation of ASI and ADF (ASF) neurons in *S. stercoralis* L1s resulted in direct development into the dauer-analogous iL3 stage as opposed to development into free-living adults, showing that the role of ASI and ADF (ASF) neurons in controlling entry into arrested development is conserved between distantly related free-living and parasitic nematodes (Ashton et al., 1998). Further conservation of neuron function in developmental arrest was demonstrated by laser ablation of the ASJ cell pair in *S. stercoralis* and *Heterorhabditis bacteriophora*, which resulted in partial blocking of the resumption of iL3 development under appropriate conditions (Ashton et al., 2007; Hallem et al., 2007). Similarly, the functional homology of the ALD thermotaxis neuron was demonstrated in *S. stercoralis* by the failure to migrate along a thermal gradient and inability to form iL3s at high temperatures in larvae in which this neuron pair was ablated at the L1 stage (Lopez et al., 2000; Nolan et al., 2004). Thus the chemo- and thermosensory apparatus used by free-living nematodes and parasitic nematodes to control entry into and exit from L3 arrest is broadly conserved. Expansion of the analysis of the conservation of neuron function into other free-living and parasitic nematodes such as *P. trichosuri* (Zhu et al., 2011) would increase our understanding of the role of conservation of cellular control of larval arrest in the evolution of parasitism.

5. Co-option of molecular pathways

5.1. cGMP signaling

cGMP signaling has been investigated in only four parasitic nematodes, with conflicting results. A membrane permeable cGMP analogue, 8-bromo-cyclic GMP, is able to activate feeding in *Ancylostoma caninum* iL3s and exsheathment of *H. bacteriophora* infective juveniles, indicating that cGMP plays a role in the resumption of development (Hawdon and Datu, 2003; Hallem et al., 2007). However, in *Nippostrongylus brasiliensis*, where iL3 activation is dependent solely on a shift in host temperature, 8-bromo-cyclic GMP had no effect on activation of feeding (Huang et al., 2010). Although these species represent only three data points from which to draw conclusions, the response to 8-bromo-cyclic GMP does fit with the observation that host cues are needed for resumption of development in *A. caninum* and *H. bacteriophora*, a situation analogous to *C. elegans* dauer recovery, whereas *N. brasiliensis* relies only on host temperature cues. In addition, orthologues of *Ce-gpa-2* and *Ce-gpa-3* were cloned from *S. stercoralis* (Massey Jr et al., 2001) and other members of the cGMP signaling pathway in *S. stercoralis* were identified by RNAseq analysis, with a consistent expression peak in infective larvae (Stoltzfus et al., 2012b), suggesting a role for cGMP signaling in the maintenance of larval arrest.

5.2. Insulin signaling

In contrast to the guanylyl cyclase pathway, there has been considerably more research on the role of insulin signaling in parasitic nematode development. Early research using muscarinic agonists hinted at a conserved role for insulin signaling in recovery from dauer and iL3 arrest in *C. elegans* and *A. caninum* (Tissenbaum et al., 2000; Hawdon and Datu, 2003), which was subsequently confirmed by the inhibition of hookworm and *N. brasiliensis* infective larvae activation by a PI3-kinase inhibitor, LY294002 (Brand and Hawdon, 2004; Huang et al., 2010).

Subsequent research focused on *daf-16*, identifying homologues from *S. stercoralis* (Massey Jr et al., 2003), *A. caninum* (Gao et al., 2009), *H. contortus* (Hu et al., 2010) and the phoretic nematode *Pristionchus pacificus* (Ogawa et al., 2011). Conservation of function was demonstrated by complementation rescue of *C. elegans daf-16* mutants (Massey et al., 2006; Hu et al., 2010; Gelmedin et al., 2011) by DNA binding assays (Gao et al., 2009; Gao et al., 2010) and by AKT phosphorylation-dependent binding to the *A. caninum* 14-3-3 orthologue, FTT-2 (Kiss et al., 2009). The use of purified recombinant *Ac-daf-16* DNA binding domain as bait was especially interesting as it identified 24 *Ac-daf-16* binding sites, eight of which corresponded to genes differentially expressed on infective larvae activation (Gao et al., 2010).

However, conclusive evidence for the conservation of insulin signaling in parasitic nematodes was provided by the use of a dominant negative form of DAF-16 (Castelletto et al., 2009). Not only was the *Ss-daf-16b* expression pattern in *S. stercoralis* analogous to that of *Ce-daf-16b* in *C. elegans*, but modification of the phosphorylation sites to create phosphonull and phosphomimetic *Ss-daf-16b* transgenes reproduced the nuclear and cytoplasmic localisation, respectively, seen in *C. elegans* (Lee et al., 2001; Castelletto et al., 2009) (Fig. 3). Finally, *Ss-daf-16b* transgenes containing the DNA binding domains but lacking C-terminal transactivating domains produced a dominant negative phenotype, whereby L3s either transiently arrested and proceeded to an L4 stage or incompletely entered iL3 development (Castelletto et al., 2009). This is the first known reported case of affecting gene function in a parasitic nematode using a gene knock-in approach and holds huge potential.

Subsequent work by the same group has identified an orthologue of *daf-2*, the *C. elegans* insulin receptor, and *age-1*, part of the downstream kinase cascade that inhibits DAF-16 (Stoltzfus et al., 2012a; Massey et al., 2013), as well as confirming the requirement for PI3-kinase activity in the resumption of development (Stoltzfus et al., 2012a). Interestingly, the two *Ss-daf-2* splice isoforms are differentially expressed, with *Ss-daf-2a* expressed throughout the life-cycle and *Ss-daf-2b* expression peaks in infective and post-iL3, perhaps reflecting a role for this splice isoform in the control of the resumption of development (Massey et al., 2013). The flexible gene architecture of two key members of the insulin signaling pathway, with the demonstrably different roles and temporal expression patterns of their splice isoforms (Lee et al., 2001; Massey et al., 2006; Massey et al., 2013), suggests that this may be the material on which the co-option of this pathway in the evolution of parasitism occurred.

In addition to insulin signaling, three recent papers have explored the role of *daf-12* and its ligands, Δ4-dafachronic (Δ4-DA) and Δ7-dafachronic acid (Δ7-DA), in parasitic nematode development. Wang et al. (2009) succeeded in cloning full or partial *daf-12* sequences from *A. caninum*, *Ancylostoma ceylanicum*, *S. stercoralis* and *Necator americanus*, each with significant sequence identity in their ligand binding domains. Each DAF-12 orthologue, including that of *C. elegans*, demonstrated Δ4-DA and Δ7-DA binding in cell reporter assays, which was abolished when conserved DA binding pocket residues were altered

(Wang et al., 2009). Incubation of iL3s with either Δ4-DA or Δ7-DA resulted in resumption of feeding and, for *A. caninum*, the production of secreted proteins produced on infection of a host. Further supporting a role for *daf-12* signaling and dafachronic acids in the control of development in parasitic nematodes, Δ7-DA but not Δ4-DA prevented entry into iL3 development for *S. stercoralis* larvae. The ability of Δ7-DA to prevent entry into arrested larval development and promote recovery from arrest was also seen for *Strongyloides papillosus*, a parasite of ruminants, and *P. pacificus* (Ogawa et al., 2009). Another piece of evidence corroborating the role of Δ7-DA and Δ4-DA in mediating the resumption of development was their ability to overcome the inhibition of DAF-9 P450, which synthesizes Δ7-DA and Δ4-DA from 3-keto-sterols (Motola et al., 2006), by ketoconazole, demonstrating the role of DAF-9 in transducing environmental signals via Δ7-DA and Δ4-DA and *daf-12* (Wang et al., 2009). Inhibition of DAF-9 by ketoconazole also blocked the resumption of feeding and production of secreted proteins in *N. brasiliensis* (Huang et al., 2010). A review by Sommer and Ogawa (2011) elegantly summarises the current view on the role of hormone signaling in the evolution of novel traits in nematodes.

Thus, not only are the insulin signaling pathway and *daf-12* present in free-living and parasitic nematodes but their roles appear to be deeply conserved. This deep conservation in the role of two key parts of the molecular machinery in the control of the development of both dauer larvae and infective larvae lends considerable support to the dauer hypothesis.

5.3. daf-7 signaling

Given the clear conservation of the roles of insulin signaling and *daf-12* activation from free-living to parasitic nematodes, it was of considerable interest to determine whether the same degree of conservation existed for TGF-β signaling. The first *daf-7* orthologue discovered was *tgh-2* in *Brugia malayi*, where its expression fluctuated with the molting cycle (Gomez-Escobar et al., 2000). Subsequently, *daf-7* orthologues were characterized from *S. ratti*, *S. stercoralis* and *P. trichosuri*, as well as from the more distantly related *Heligomosomoides polygyrus*, *Teladorsagia circumcincta*, *A. caninum*, *N. brasiliensis* and *H. contortus* which are from the same phylogenetic clade as *C. elegans* (Brand et al., 2005; Crook et al., 2005; Freitas and Arasu, 2005; Massey et al., 2005; McSorley et al., 2010). With the exception of *H. polygyrus* and *T. circumcincta*, where *daf-7* expression is maximal in adult stages (McSorley et al., 2010), and *B. malayi* mentioned above, parasite *daf-7* expression peaks in the arrested L3. This observation lead to the hypothesis that, contrary to its role in controlling entry into the arrested state in *C. elegans*, *daf-7* signaling in parasitic nematodes acts to maintain the arrested state until a suitable host is found (Viney et al., 2005). A role for *daf-7* in the maintenance of infective larval arrest would predict that *daf-7* expression would drop in larvae that had infected a suitable host and resumed development. Indeed, for *H. contortus*, *N. brasiliensis*, *P. trichosuri* and *S. ratti daf-7* expression was low to barely detectable in post-infective L3s and L4s recovered from the host, and significantly lower in exsheathed *H. contortus* iL3s and *S. ratti* iL3s that had penetrated through host skin (Crook et al., 2005; McSorley et al., 2010). These results suggest that the role of *daf-7* signaling, to suppress dauer entry and promote dauer exit, has been reversed in a wide variety of parasitic nematodes.

Evolution via changes in the control of developmental genes is a central tenet of EvoDevo (Carroll, 2000, 2005), into which the apparent changes in control of *daf-7* expression in parasitic nematodes fit. To investigate this further Crook et al. (2010) tested the function of the *Pt-daf-7* promoter in *C. elegans*, driving both a GFP reporter and a copy of *Ce-daf-7*.. They found that, while the *Pt-daf-7* promoter was functional in *C. elegans*, GFP expression was both spatially and temporally expanded compared with that of *Ce-daf-7*, with expression predominantly in dauer larvae (Fig. 4). This expression pattern suggests that the

Ptdaf-7 promoter contains some as yet unidentified arrested larva-specific regulatory element(s) and that the acquisition of these elements may be the mechanism by which the role of *daf-7* changed in some parasitic nematodes. In addition, the *Cedaf-7* coding region under control of the *Pt-daf-7* promoter was unable to rescue a *daf-7* mutant, as expected given the differences in expression, further highlighting the divergence in the role of *daf-7* in *P. trichosuri* and *C. elegans* (Crook et al., 2010). Unfortunately, as with *Ss-age-1* (Stoltzfus et al., 2012a), the *Pt-daf-7* coding region, even just the ligand domain, was efficiently silenced in *C. elegans* so it was not possible to confirm its function (Crook et al., 2010).

What is the role of *daf-7* signaling in parasitic nematodes? Expression studies suggest a role in the maintenance of larval arrest, although this is by no means consistent across the phylum, and the activity of the *Pt-daf-7* promoter in *C. elegans* dauer larvae implies that this role change has occurred via changes in gene regulation. However, a definitive role in the maintenance of larval arrest requires modification of *daf-7* signaling levels. Lower levels of *daf-7* signaling would be predicted to result in transient larval arrest or inappropriate resumption of development; higher levels of *daf-7* signaling would be predicted to result in an inability to resume development. The use of dominant negative *daf-7* transgenes, as with *daf-16* and insulin signaling (Castelletto et al., 2009), offers one possible approach, although recent work in *C. elegans* suggests that this may not be straightforward (Crook and Grant, 2013). Another tantalizing approach would be the use of random insertional mutagenesis using the piggyBac transposon pioneered in *S. stercoralis* (Shao et al., 2012). Given that any *daf-7* mutants thus generated may be incapable of forming infective larvae, this approach would need to be undertaken in a parasitic nematode, such as *P. trichosuri*, that can undergo multiple free-living generations (Grant et al., 2006).

6. Advances from whole genome sequencing and microarray studies

The targeted approach of searching for parasitic nematode homologues of *C. elegans* dauer development has clearly been a productive one. However, an equally successful approach has been to harness the ever increasing power of expressed sequence tag (EST) libraries, microarrays and now deep sequencing to perform unbiased searches for parasite-specific genes. Two *Strongyloides* spp., *S. ratti* and *S. stercoralis*, are especially well suited to this approach due to their free-living and parasitic generations, which enables the comparison of transcriptional profiles from iL3 versus free-living L3 (Mitreva et al., 2004; Thompson et al., 2006; Ramanathan et al., 2011), parasitic versus free-living adults (Thompson et al., 2005) and even parasitic adults from naïve versus immune hosts (Thompson et al., 2008; O'Meara et al., 2010). The stage-specific expression of candidate parasite-specific genes from *S. ratti* was further characterized by real-time PCR and western blotting, with one protein being detected in the excretory/secretory products of the parasitic adult, demonstrating the power of this unbiased approach (Spinner et al., 2012).

In addition, deep sequencing has allowed a large-scale analysis of dauer developmental control homologues in *S. stercoralis* (Stoltzfus et al., 2012b). Of particular interest, this study discovered a paucity of insulin-like ligands and an expansion of DAF-7-like TGF-β ligands in *S. stercoralis*, with distinct expression patterns, which suggest a far more nuanced role for insulin and *daf-7* signaling in the control of *S. stercoralis* infective larval formation and activation (Stoltzfus et al., 2012b). Clearly the increasing availability of parasitic nematode whole genome sequences and the decreasing cost of next generation sequencing technologies will lead to a greater understanding of what it means to be a parasite.

7. Conclusion

The original dauer hypothesis stated that the life-history and physiological traits of dauer larvae of free-living nematodes confer features that are ideally suited to infectivity (Rogers and Sommerville, 1963; Hawdon and Schad, 1991; Hotez et al., 1993) and, by extension, these features may constitute a pre-adaptation to the evolution of parasitism. Therefore the simplest hypothesis describing how this evolutionary event may have occurred would be that the mechanisms which control dauer entry and exit are used to control entry into and exit from infective larval development plus, where this life-style option exists, between infective larval development and free-living development.

Despite the simplicity of this hypothesis, the picture that has emerged over the last three decades is far more nuanced than anyone would have expected. It is clear that the sensory neuroanatomy used to control entry into and exit from arrested larval development is conserved between free-living and parasitic nematodes. It is also clear that the four molecular pathways used to control entry into dauer development are present in a wide range of parasitic nematodes. However, what was a surprise is that the role of these pathways appears both conserved (insulin signaling, *daf-12* and dafachronic acid) and divergent (*daf-7* signaling). It is especially interesting to note that even the divergence of *daf-7* signaling is not conserved across all parasitic nematodes. In addition, work with the facultative parasitic nematode *P. trichosuri* and multiple isogenic *C. elegans* lines suggests that phenotypic plasticity in dauer formation may be one trait upon which selective pressure has acted in the evolution of parasitism (Harvey et al., 2008; Stasiuk et al., 2012).

The work over the last 20 years has painted a compelling picture not only of how parasitic nematodes control their entry into and exit from developmental arrest, but also how the conservation and divergence of those control mechanisms may have facilitated the colonization of a new environment and the evolution of parasitism. However, many questions still remain: what is the role of *daf-7* signaling in parasitic nematodes, how are the multiple signals (host entry, temperature, pH) collated into a developmental output, what pathways lie downstream of the *daf-7* and insulin pathways, what role do these pathways have in parasitic nematodes with multiple hosts or facultative free-living generations? One hopes that new tools currently being developed make the next 20 years as fruitful as the last.

Acknowledgments

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Highlights

- Parasitism has evolved multiple times across the phylum Nematoda.

- Similarities between dauer larvae and infective larvae suggest that dauer larvae are a pre-adaptation for parasitism.

- The dauer hypothesis: "the mechanisms used to control dauer development are conserved in parasitic nematodes".

- The neurobiology of the control of larval arrest and exit is conserved between free-living and parasitic nematodes.

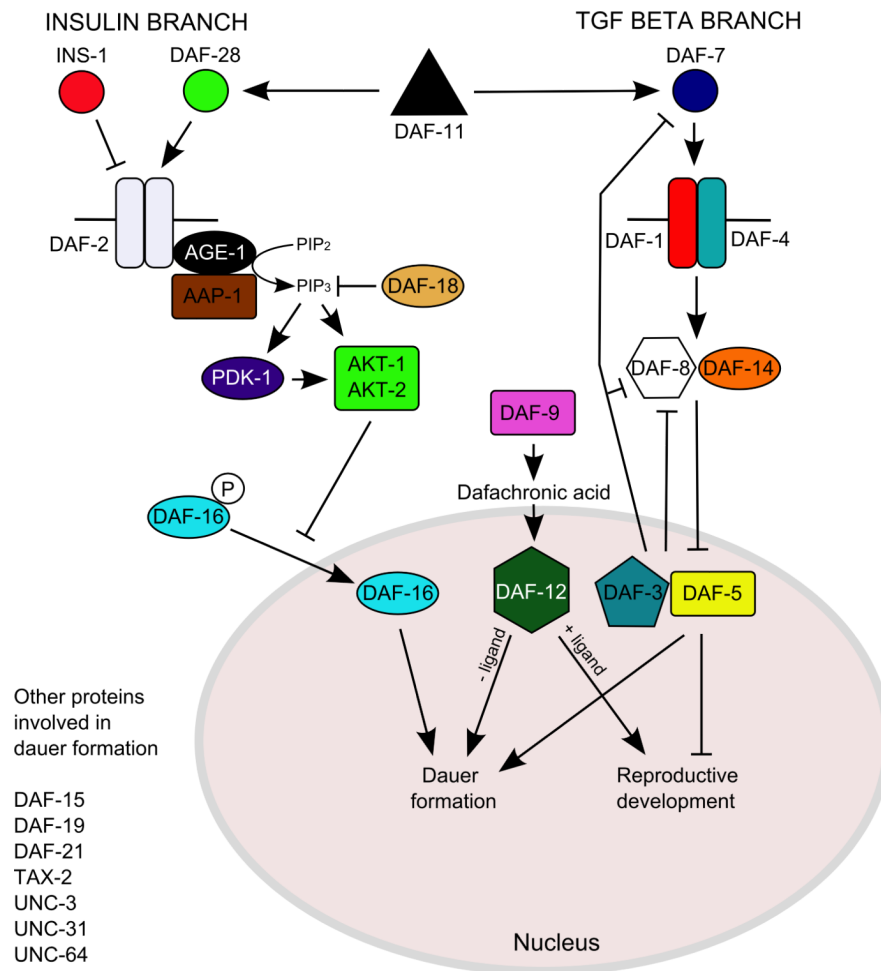- However, these pathways in infective larvae are both conserved (insulin signaling) and divergent (TGF-β signaling).

Fig. 1.
The molecular pathways that control entry into and exit from dauer development. Adapted from Von Stetina et al. (2007) and modified using information from Park et al. (2010). For a more in-depth graphical representation of these molecular pathways, readers are referred to Stoltzfus et al. (2012b).
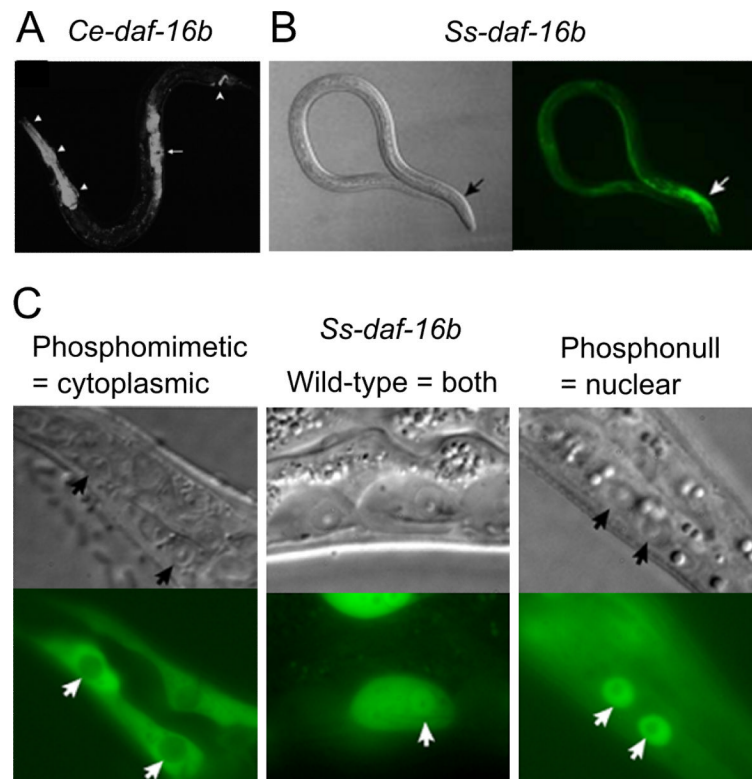
Fig. 2.
Conservation of sensory neuroanatomy between (A) *Caenorhabditis elegans* and (B)
Stronglyoides stercoralis. Pale pink circles labeled Axx represent amphidial neurons in *S. stercoralis* that have been identified by positional homology. Adapted from
www.wormatlas.org (Altun and Hall, 2008) and Ashton et al. (1998).

Fig. 3.
Conservation of expression patterns for *daf-16b* from *Caenorhabditis elegans* (*Ce*) and *Strongyloides stercoralis* (*Ss*). (A) *Ce-daf-16b* expression in the pharynx, somatic gonad and tail neurons. (B) *Ss-daf-16b* expression, predominantly in the pharynx. (C) The localisation of phosphomimetic (i.e. constitutive insulin signaling) and phosphonull (no insulin signaling) forms of Ss-DAF-16b mimic that of Ce-DAF-16b in the presence and absence of insulin signaling. Adapted from Lee et al. (2001) (A) and Castelletto et al. (2009) (B and C).
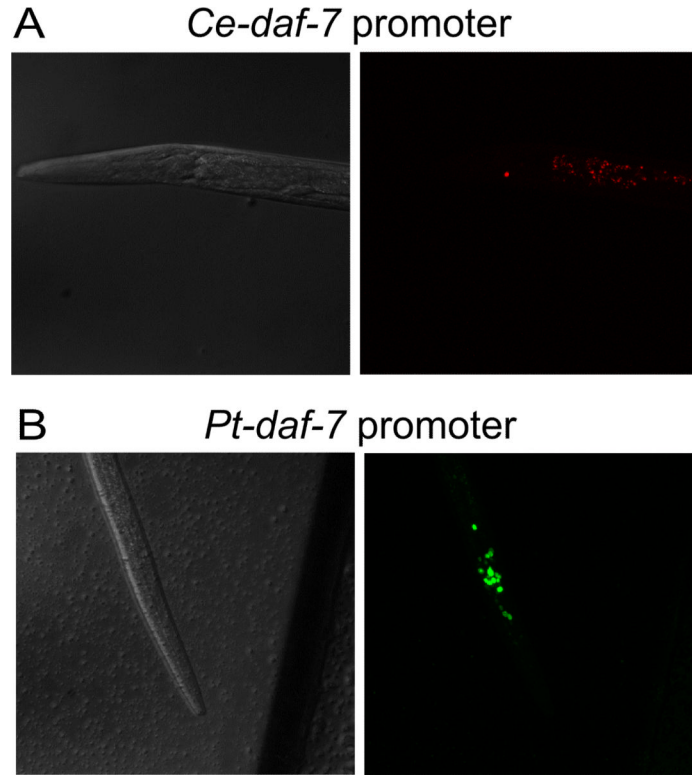
Fig. 4.
Divergent control of spatial and temporal expression by (A) *Caenorhabditis elegans* and (B) *Pt-daf-7* promoters in *C. elegans*. DsRed under the control of the *Ce-daf-7* promoter is restricted to the ASI neuron pair only and is visible only in L1s and L2s (L2 pictured). In contrast, GFP under the control of the *Pt-daf-7* promoter is expressed in a large number of cells in the head, including amphidial neurons, predominantly in dauer larvae (pictured). This expression pattern from the *Pt-daf-7* promoter correlates with its peak expression in *Parastrongyloides trichosuri* infective larvae (Crook et al., 2005). Adapted from Crook et al. (2010).