Parasitic worms infect billions of people worldwide. Current treatments rely on a small group of drugs that have been used for decades. A shortcoming of these drugs is their inability to target the intractable infectious stage of the parasite. As well-known therapeutic targets in mammals, nuclear receptors have begun to be studied in parasitic worms, where they are widely distributed and play key roles in governing metabolic and developmental transcriptional networks. One such nuclear receptor is DAF-12, which is required for normal nematode development, including the all-important infectious stage. Here we review the emerging literature that implicates DAF-12 and potentially other nuclear receptors as novel anthelmintic targets.
Nuclear receptors: emerging drug targets for parasitic diseases

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Introduction

One of the most prevalent classes of parasitic organisms is the helminth (parasitic worms), which collectively infect more than 1 billion people worldwide and cause a range of maladies including malnutrition, growth and mental retardation, disfigurement and physical disabilities, and death (1). Helminths that infect humans are generally considered to comprise two major phyla: Nematoda (round worms), both soil-transmitted and filarial; and Platyhelminthes (flatworms), both flukes and tapeworms. Parasitic worms represent one of the most successful and diverse extant forms of life. For example, of the 40,000 or more presumed nematode species, it is estimated that over half are parasitic (2). There also are believed to be more than 15,000 species of flatworm parasites. Mammalian parasites that are transmitted orally or through the skin from food or soil contaminated with eggs or young larvae usually colonize host intestines. Filarial parasites that are transmitted from biting insect vectors such as black flies or mosquitos enter the definitive host when the vector takes a blood meal. Filarial parasites infect a variety of different human tissues, with tissue specificity determined by the individual species (1). Unfortunately, few therapies exist to treat these parasites, and the long history of extensive use of anthelmintic drugs has resulted in a growing rate of resistance, which is now pervasive in nematode parasites infecting livestock (3). In the absence of alternative therapeutic strategies (e.g., vaccines), novel drugs to treat these infections are urgently needed.

Nuclear receptors are ligand-gated transcription factors that regulate diverse biological processes, including metabolism, development, and reproduction (4). Because of the lipophilic nature of their ligands and their ability to modulate the expression of multiple genes within the same pathway, nuclear receptors have become attractive targets for the development of orally available small-molecule drugs (4). Importantly, nuclear receptors have been identified in all categories of helminths (see ref. 5 for a systematic review of nuclear receptors found in helminths). We propose that targeting nuclear receptors would provide a novel therapeutic strategy that is distinct from current anthelmintic drugs, which target tubulins (benzimidazoles), ion channels (ivermectin and diethylcarbamazine), or symbiotic bacteria of parasites (doxycycline). Here we review the current data suggesting nuclear receptors may be effective drug targets for treating helminthic diseases.

Nuclear receptors in parasitic nematodes

Although nuclear receptors are found in all worm phyla, the most studied have been in nematodes. It is of particular interest that the free-living nematode Caenorhabditis elegans has 284 nuclear receptors (6), the most of any known species (n.b., humans are a distant second with 48). The expansion of nuclear receptors in nematodes suggests they subserve an important biological advantage. Indeed, in addition to their roles in development and reproduction, several of these receptors have been shown to play key roles in survival by adapting the worm to various environmental stresses. One of the most studied of these receptors is DAF-12.

The nuclear receptor DAF-12. DAF-12 was initially described in C. elegans (7, 8), which is closely related to several species of human parasitic nematodes and has been used for anthelmintic drug discovery (9). C. elegans is able to adjust its developmental fate based on its environment. Under favorable conditions, i.e., low population density, proper temperature, and abundant food, C. elegans undergoes a rapid development from egg to reproductive adult through four larval stages, called L1 to L4. Under unfavorable conditions, however, C. elegans arrests its normal growth and enters a metabolic and developmental diapause at the third larval stage, called L3 dauer (L3d). The dauer diapause permits the worm to survive, often for long periods of time, until favorable conditions return (10). The primary function of DAF-12 is to regulate the nematode’s environment-directed larval development as a transcriptional regulator through four larval stages, called L1 to L4. Under unfavorable conditions, however, C. elegans arrests its normal growth and enters a metabolic and developmental diapause at the third larval stage, called L3 dauer (L3d). The dauer diapause permits the worm to survive, often for long periods of time, until favorable conditions return (10). The primary function of DAF-12 is to regulate the nematode’s environment-directed larval development as a transcriptional regulator through four larval stages, called L1 to L4. Under unfavorable conditions, however, C. elegans arrests its normal growth and enters a metabolic and developmental diapause at the third larval stage, called L3 dauer (L3d). The dauer diapause permits the worm to survive, often for long periods of time, until favorable conditions return (10). The primary function of DAF-12 is to regulate the nematode’s environment-directed larval development as a transcriptional regulator through four larval stages, called L1 to L4. Under unfavorable conditions, however, C. elegans arrests its normal growth and enters a metabolic and developmental diapause at the third larval stage, called L3 dauer (L3d). The dauer diapause permits the worm to survive, often for long periods of time, until favorable conditions return (10). The primary function of DAF-12 is to regulate the nematode’s environment-directed larval development as a transcriptional regulator through four larval stages, called L1 to L4. Under unfavorable conditions, however, C. elegans arrests its normal growth and enters a metabolic and developmental diapause at the third larval stage, called L3 dauer (L3d). The dauer diapause permits the worm to survive, often for long periods of time, until favorable conditions return (10). The primary function of DAF-12 is to regulate the nematode’s environment-directed larval development as a transcriptional regulator through four larval stages, called L1 to L4. Under unfavorable conditions, however, C. elegans arrests its normal growth and enters a metabolic and developmental diapause at the third larval stage, called L3 dauer (L3d). The dauer diapause permits the worm to survive, often for long periods of time, until favorable conditions return (10). The primary function of DAF-12 is to regulate the nematode’s environment-directed larval development as a transcriptional regulator.
The rule of the infective third stage. An orthologous stage to dauer, called the infective third stage, exists in most parasitic nematodes (2). These parasites infect humans or other animals through a specialized infective L3 (iL3) larval stage, which in monoxenous species (species that have a single host) is often transmitted through contact with contaminated soil (e.g., hookworms) and in heteroxenous species (species that have an intermediate host) is often transmitted by blood-sucking insect vectors (e.g., hookworms). Homologs of DAF-12 have been isolated and characterized in several soil-borne parasites, including hookworms (Necator americanus, Ancylostoma ceylanicum, Ancylostoma caninum) and Strongyloides stercoralis, an incurable and often fatal parasite (17-19). Genome analysis also has revealed DAF-12 homologs in major species of filarial parasitic nematodes such as Brugia malayi (elephantiasis), Onchocerca volvulus (river blindness), and Loa loa (eye worm), and biochemical characterization of these DAF-12 homologs is underway.

Similar to their free-living counterparts, the iL3 larvae are developmentally arrested but resume their growth to reproductive adulthood after entering their definitive host via skin penetration. This so-called “rule of the infective third stage” (2) is analogous to the arrested L3d diapause stage of C. elegans (Figure 2). In the case of the parasite, the exit from iL3 occurs in the favorable conditions provided by the host, and similarly DAF-12 has been shown to regulate this process (15, 17). In the absence of ligand, DAF-12 permits the formation of iL3 larvae, whereas upon entering the host (which presumably stimulates ligand synthesis) DAF-12 activation induces reproductive development. In S. stercoralis, treatment of infectious iL3 worms outside of their hosts with DAF-12 agonists initiates the resumption of feeding (19). Moreover, continual activation of DAF-12 by pharmacologically administering DA to pre-infective larvae prevents the infective stage by inducing reproductive development of young larvae that would otherwise arrest their development as iL3s (19). Thus, the ligand status of DAF-12 appears to be essential for both initiating and establishing infection. The ability to interrupt the life cycle of the parasite at the infectious third stage by targeting DAF-12 activity is unprecedented and suggests an exciting potential anthelmintic therapeutic strategy.

DAF-12 mechanism of action. In C. elegans the mechanisms by which DAF-12 regulates larval development consist of transcriptional activation of gene programs controlling two basic processes. The first determines cell fate during larval development. This is exemplified by the development of seam cell lineages, which is regulated by the DAF-12 target genes mir-84 and mir-241, two let-7 family miRNAs (13, 20). During reproductive development, liganded DAF-12 induces mir-84 and mir-241 to inhibit the expression of a transcription factor, hbl-1, thereby directing the cells to an L3-specific fate (13, 20). During dauer formation
unliganded DAF-12 retains the seam cells in an L2-specific fate, which delays the switch to the L3-specific cell fate until reproductive development is resumed. The second process regulated by DAF-12 is a coordinated shift in aerobic metabolism to meet the distinct energy requirements during larval development (15). In C. elegans, liganded DAF-12 upregulates a gene network that promotes aerobic metabolism of fat, allowing efficient production of sufficient energy for rapid reproductive growth. Unliganded DAF-12, however, limits fat consumption to a lower rate of anaerobic metabolism. This prevents the dauer larvae from prematurely exhausting energy stores and permits survival in unfavorable conditions.

The mechanistic details of DAF-12 regulation of larval development in parasites remain incompletely elucidated, but basic elements of this pathway appear to be conserved from C. elegans. In the soil-transmitted species S. stercoralis, liganded DAF-12 induces a variety of genes that are involved in fat utilization, and as in C. elegans, inhibition of aerobic fat utilization can inhibit the parasite’s reproductive development (15). Although no let-7 family miRNAs exist in Strongyloides spp. (21), hbl-1 homologs have been identified (WormBase ID: SRAE_1000178600). Therefore, hbl-1 may also mediate DAF-12-regulated larval development in parasitic nematodes through a distinct mechanism from the let-7 family of miRNAs. In contrast, both let-7 family miRNAs and hbl-1 are conserved in filarial nematodes. Moreover, in Brugia pahangi, which causes a zoonotic filariasis, the increased expression of several let-7 homologs correlates well with IL3 exit from dauer-like diapause upon invasion of the host (22). Although the hypothesis remains to be tested, these let-7 homologs potentially mediate the control of developmental progression by DAF-12.

Besides impacting the development of young nematode larvae, DAF-12 can also affect longevity in adult nematodes. In the germline longevity pathway, DAs are synthesized to activate DAF-12 to treat parasitic diseases relies on using exogenous ligands to interfere with the physiologic functions of the receptor. The ideal drugs should be DAF-12 modulators that target entry into and exit from the IL3 stage. One potential drug is Δ7-dafachronic acid (Δ7-DA), which is the endogenous DAF-12 ligand in C. elegans and also activates some parasite DAF-12s, albeit with less potency and efficacy (19).

S. stercoralis is a parasite that has been used to test the therapeutic potential of DA-like drugs. This parasite has evolved two developmental strategies for establishing infections. In one pathway, newly hatched L1 larvae in the host intestine are excreted in the feces, and at ambient environmental temperatures these L1s develop into free-living adults (17). After one free-living life cycle, the second generation of these offspring develops into IL3 larvae that infect new hosts. In the second pathway, the newly hatched L1 larvae stay within the host and at 37°C establish a perennial autoinfection by developing directly into IL3 larvae that continually re-infect the host. The inability of current anthelmintic drugs (e.g., ivermectin, benzimidazoles) to target the latent autoinfective larvae is a major reason why these drugs are often ineffective at curing strongyloidiasis. In contrast, the use of DAF-12 ligands may be a more efficacious strategy for preventing autoinfection and eliminating the parasites. In proof-of-concept experiments in which young, post-free-living S. stercoralis larvae were treated with...
Δ7-DA, the majority of larvae completely bypassed the iL3 stage (17) and instead were driven toward precocious, incomplete reproductive development and death. In addition, when post-parasitic L1 larvae freshly isolated from the host were cultured in host-like conditions (i.e., 37°C) that promote development into autoinfective L3 larvae, treatment with Δ7-DA stimulated development into free-living adults and completely prevented the occurrence of any iL3 larvae (17). The efficacy of Δ7-DA under these conditions likely resulted from Δ7-DA acting as a partial agonist that prevents unliganded DAF-12 from causing iL3 developmental arrest. In contrast, administration of Δ7-DA to hosts infected with *S. stercoralis* might be expected to prevent autoinfection and interrupt normal reproductive development. Given the association between DAF-12 signaling and longevity, disrupting DAF-12 signaling with selective DAF-12 modulators like Δ7-DA would be expected to have two beneficial therapeutic attributes: they would prevent immature worms from entering the infective stage (by preventing the “dauer” pathway) and shorten the lifespan of the adult parasites (by inhibiting the germline pathway). This latter effect might be of particular importance in eliminating the long-lived parasitic nematodes such as the filarial nematodes and hookworms.

Taken together, current research suggests that DAF-12 is a promising therapeutic target. DAF-12 controls important biological processes that are essential for parasite transmission, being required for both entry into and exit from the dauer-like iL3 stages. This dual functionality of DAF-12 provides two potential modes of therapeutic effect. Furthermore, DAF-12 homologs thus far have been identified in all parasitic nematodes that undergo an L3 developmental arrest, which indicates that a drug targeting the receptor would be broadly applicable. It is also noteworthy that the specificity of DA for DAF-12 binding and activation is sufficiently high to prevent cross-over activity with host nuclear receptors, which are only distantly related (11). Finally, the crystal structure of the DAF-12 ligand-binding domain has been solved for two par...
Nuclear receptors in parasitic flatworms

Flukes cause one of the most devastating parasitic diseases, schistosomiasis, and infect over 200 million people worldwide. In humans, schistosomiasis is primarily caused by *Schistosoma mansoni* and *Schistosoma japonicum* (58). So far, 21 nuclear receptors have been found in *S. mansoni* and *S. japonicum*, but their functions are unknown (59). Homologs of several genes that mediate ecdysone responses in insects, including the ecdysone response genes E78 and FTZ-F1 and the heterodimeric partner of EcR, RXR, are also present in *S. mansoni* (59). Furthermore, *S. mansoni* synthesizes two ecdysteroids, ecdysone and 20-hydroxyecdysone, with peak production occurring during the liver stage, in which the parasite larvae develop into mature adults (60). However, to date no EcR homolog has been identified. These findings suggest that an ecdysteroid–nuclear receptor system may control the reproductive development of the parasites, although the identity of the nuclear receptor remains unknown.

*S. mansoni* E78 is a nuclear receptor homologous to the *Drosophila* ecdysone response gene 78 (59, 61). In *Drosophila*, E78 is essential for establishing germline stem cells and thus is crucial for fertility (62). In *S. mansoni*, the E78 homolog is highly expressed in the egg, miracidiae, and daughter sporocyst stages, which all occur in the intermediate host (usually snails) (61). This expression pattern indicates that E78 may play an important role in amplifying the parasite before it infects humans, the definitive host.

One unusual class of nuclear receptors found in *S. mansoni* is composed of three proteins called Sm2DBD-NRα, Sm2DBD-NRβ, and Sm2DBD-NRγ, which atypically contain two DNA-binding domains linked to a single ligand-binding domain (59, 63, 64). NHR-1, a nuclear receptor that is homologous to Sm2DBD-NRγ, has been identified in planarians, which are free-living relatives of *S. mansoni*. NHR-1 and Sm2DBD-NRγ share 74% and 83% sequence identity in the two DNA-binding domains and 44% identity in the ligand-binding domain (65). In planarians, NHR-1 is required for the development of accessory reproductive organs and is important for the differentiation and maturation of germ cells (65). The functions of the Sm2DBD-NRs in *S. mansoni* remain to be determined, but they are differentially expressed in various developmental stages (64), which suggests that they may regulate parasite development and thus be therapeutic targets.

The study of nuclear receptors in tapeworms began only recently in *Echinococcus multilocularis* (66). The life cycle of *E. multilocularis* involves an intermediate host (a rodent) and a definitive host (a fox or dog). Three successive stages of larvae, named onchosphere, metacestode, and protoscolex, live in the intermediate host, and upon entering the definitive host, the protoscolex larvae develop into reproductive adults. Genome analyses have revealed 17 putative nuclear receptors in *E. multilocularis*, most of which are homologous to the nuclear receptors in *S. mansoni* (66), including one named EmNHR-1. Although this nuclear receptor is expressed in all larval stages, it is upregulated in metacestode and dormant protoscolex larvae, which live and arrest in the intermediate host. Thus, EmNHR-1 may be required for parasite development in the intermediate host. The tapeworm species that infect humans (e.g., pork and beef tapeworms) have a similar life cycle to *E. multilocularis*, and it will be interesting to explore the existence and function of EmNHR-1 homologs in these human parasites.
Summary

Nuclear receptors are promising drug targets for treating parasitic diseases. They have been found in all parasitic helminths studied and, like their counterparts in higher animals, they control essential biological processes that govern metabolism, development, and reproduction. Therefore, modulating nuclear receptor signaling may provide exciting and novel therapeutic opportunities. Although nuclear receptors have begun to be studied in depth in their free-living relatives, there has been a paucity of research in helminths. Major limitations to advancing nuclear receptor biology in parasites include the obvious technical difficulties associated with working with parasites and their hosts and the absence of traditional genetic tools for parasitic worms. However, recent advances in technologies such as clustered regularly interspaced short palindromic repeats (CRISPR) may afford a significant opportunity to expand this line of research. Future work will also need to focus on characterizing the biosynthetic pathways that generate ligands of parasite nuclear receptors. Thus far, no endogenous nuclear receptor ligands have been identified in parasitic worms; however, the characterization of the DAF-12 ligands for the free-living nematode *C. elegans* provides a useful precedent and strategy for doing so. Finally, it will be crucial to develop synthetic ligands for parasite nuclear receptors that might be used both as tool compounds to interrogate their biological function and as candidate therapeutics.

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