

## What Can *Caenorhabditis elegans* Tell Us About Nematocides and Parasites?

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**Abstract** Nematode infections compromise human health and reduce agricultural productivity. Experiments that exploit the powerful molecular genetics of the free-living nematode *Caenorhabditis elegans* have contributed to our understanding of how the major classes of anthelmintic nematocides kill worms and how worms might evolve resistance to these drugs. In *C. elegans*, as in parasites, benzimidazoles interfere with microtubule polymerization, the imidazothiazoles/tetrahydropyrimidines activate nicotinic acetylcholine receptors, and the macrocyclic lactones activate glutamate-gated chloride channels. Mutant alleles of genes that encode drug targets often confer resistance in *C. elegans*. Preliminary evidence suggests that alleles of homologous genes in parasites will, in many cases, also play a role in resistance. Thus, information acquired from *C. elegans* can be usefully applied to understand the mechanisms of drug sensitivity and the genetics of resistance in parasites.

**Keywords:** nematocides, anthelmintics, nematodes, *C. elegans*, drug resistance

### INTRODUCTION

Approximately 1 person in 5 is infected with a parasitic nematode. The relatively benign nematode infections, such as ascariasis, are a health burden for the poor, undernourished populations that are at highest risk. Whereas the filariases can be devastating. Onchocerciasis, for instance, causes blindness in about 30% of affected, untreated individuals [1]. In addition to the human health burden, virtually every major livestock animal is susceptible to infection by parasitic nematodes that reduce agricultural productivity. The nematode parasites cost farmers and ranchers billions of dollars [2]. Drugs that kill nematodes, known as nematocides or, when specifically used to kill parasites, anthelmintics, are the primary means of alleviating the human suffering and preventing the agricultural loss resulting from parasitic nematodes.

Currently used nematocidal compounds were discovered in screens that required no knowledge of the compound's mechanism of action. While that knowledge is not necessary for antiparasitic drugs to be effective, it has become increasingly important for two reasons. First, the evolution of drug resistance in treated parasites can rapidly compromise the efficacy of nematocides. A better understanding of the action of the drug and the specific genetic changes that can make parasites resistant may help us to manage resistance and maintain the effectiveness of nematocides. Second, as our ability to design new nematocides becomes more so-

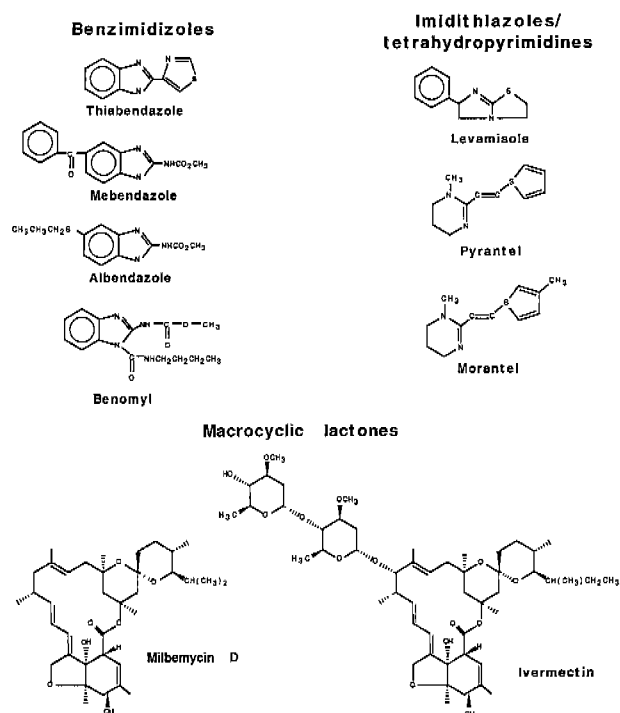
phisticated, an understanding of the mechanism of action of existing, successful compounds will, we hope, help us to identify optimal targets for new drug screens.

That parasites often make poor experimental subjects is an obstacle to their use for studies of nematocides. Parasites usually cannot complete their life cycle in culture and therefore must be bred in and extracted from their hosts. Obtaining sufficient experimental material can be difficult. The technical problems associated with the need to cross parasites in the host, together with the need to use strains that are not genetically homogeneous, limit the genetic analysis of resistance. The ability to transform parasites with genes suspected to play a role in resistance would be useful but is not generally feasible.

*Caenorhabditis elegans* is a useful model organism from which to learn about nematocides. Although *C. elegans* is a free-living soil nematode, not a parasite, it offers many compensating experimental advantages. Thousands of *C. elegans* can be easily grown in a small Petri dish, the life cycle is only 3 days and hermaphrodites can self-fertilize. These qualities of *C. elegans* simplify screens for rare, recessive genetic mutations that affect drug sensitivity. In addition, the complete cell lineage, which is invariant, is known, the adult anatomy has been reconstructed from serial electron micrographs, and the entire genome has been sequenced. The existence of a complete physical map, consisting of overlapping cosmids and YACs, in combination with the ability to transform worms, makes cloning relatively easy. Finally, electrophysiological techniques that can be applied to *C. elegans* are useful for studying drugs that target the nervous system. In this review, I will assess the utility of *C. elegans* for studies of nematocides

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**Fig. 1.** The structures of the major anthelmintic drugs grouped according class. Each class has a different protein target as described in the text.

I will summarize what has been learned in *C. elegans* about sensitivity and resistance to a variety of nematocides and I will compare the results to studies of parasites.

## BENZIMIDIZOLES

### Introduction

The benzimidazoles (BZs) are effective against fungi and nematodes (reviewed in [3,4]). Some of the most common anthelmintic BZs are thiabendazole, mebendazole, albendazole and benomyl (Fig. 1). Benzimidazoles are used to treat a wide variety of human and animal parasites [5]. The toxicity of BZs derives from their ability to disrupt microtubules (MTs).

### Effects on *C. elegans*

When exposed to benomyl from hatching, *C. elegans* becomes severely paralyzed in the L2-L3 larval stages. Paralysis occurs at concentrations as low as 2.5  $\mu\text{g}/\text{mL}$  for benomyl and at concentrations 2- and 4-fold higher for mebendazole and thiabendazole respectively [6]. The therapeutic dose of thiabendazole (25mg/kg) is similar to the dose that affects *C. elegans* [5]. In spite of the paralysis, worms treated with BZs complete larval development and are fertile, although they produce

fewer eggs [6-8]. Worms are more sensitive to BZs at lower temperatures [6]. Treating *C. elegans* with BZs depletes MTs, as determined by ultrastructure [8,9]

### Genetics of Resistance

*C. elegans* strains resistant to mebendazole [10], albendazole [9] and benomyl [6] have been isolated by chemical mutagenesis and selection in the presence of the drug. In a saturated screen, alleles of only one resistance gene, *ben-1*, were isolated (Table 1)[6,10]. *ben-1* is cross-resistant to all the BZs [6,9,10]. The degree of resistance can be greater than 30-fold, depending on the assay and the BZ derivative [6,9]. Null alleles of *ben-1* (i.e. alleles that completely eliminate protein expression) are recessive at 15°C and semidominant or dominant at 25°C.

### Molecular Biology

*ben-1* encodes a  $\beta$  tubulin subunit, one of at least 6  $\beta$  tubulin genes found in *C. elegans* [6,11]. It belongs to the family of nematode tubulins that form 11 proto-filament microtubules (11 PF MTs). This is in contrast to the vertebrate tubulins, which form 13 PF MTs, and to the *mec-7*-encoded tubulin, which forms the 15 PF MTs found in mechanosensory neurons of *C. elegans* [8]. At concentrations of BZs that depolymerize MTs in wild-type worms, 11 PF MTs are stable in the *ben-1* mutant. Interestingly, 15 PF MTs are not susceptible to BZs. However, a mutation in the *mec-7* gene, which eliminates 15 PF MTs, results in their replacement by 11PF MTs that are susceptible [8]. BEN-1\* has a phenylalanine at amino acid 200 (F200), which is thought to be necessary for BZ binding [12,13]. In contrast, TUB-1, a BZ insensitive tubulin from *C. elegans*, has Y200 (the MEC-7 tubulin has F200 but may lack other residues necessary for BZ binding) [6]. Binding of mebendazole to *C. elegans* MTs *in vitro* is temperature dependent, which explains the temperature dependence of the drug's effect on worms. In binding studies that used tubulin purified from *C. elegans*, mebendazole ( $B_{\text{max}}$ ) specifically bound to MTs was reduced in the *ben-1(u107)* mutant relative to wild-type [14]. Strikingly, even in worms homozygous for null alleles of *ben-1*, no other phenotype is apparent, indicating that the BEN-1 tubulin is largely redundant with other tubulins.

**Relevance to Parasites.** In parasites (and fungi), MTs play the same central role in BZ sensitivity and resistance as in *C. elegans* [3,12,15,16]. BZ treatment disrupts MTs in parasites [17,18]. A decrease in the  $B_{\text{max}}$  of BZ binding to MTs correlates with resistance in *Haemonchus contortus* [4,15,19] and in *Trichostrongylus colubriformis* [14,20]. Moreover, specific genetic polymorphisms

\* The protein product of a gene is signified by the gene name in capital letters. Thus, BEN-1 is the protein encoded by the *ben-1* gene. The mutant allele of a gene is indicated in parenthesis after the gene name.

**Table 1.** Nematocide resistance genes in *C. elegans*

Drug	Gene	Inheritance	Gene product
Benzimidazoles	<i>ben-1</i>	Recessive at 15°C Semi/dominant at 25°C	β-tubulin
Imidazothiazoles/ Tetrahydro-pyrimidines	<i>lev-1, unc-29, unc-38</i> <i>unc-63, unc-74, unc-50, lev-8,</i> <i>lev-9, lev-10</i> <i>lev-11, unc-22, unc-68</i>	Recessive or semidominant  Recessive	Subunits of a nicotinic acetylcholine receptor unknown  Tropomyosin, twitchin, ryanodyne receptor
Macrocyclic lactones	<i>avr-14, avr-15, glc-1</i>  <i>unc-7, unc-9</i>  Dyf genes	Recessive, Synthetic phenotype   Recessive, single-gene	α subunits of Glutamate-gated Chloride channels Subunits of innexin gap-junctional channels Proteins involved in generation of amphid sensory structures
Bt toxin	<i>bre-1-5</i>	Recessive	unknown

in MT genes correlate with BZ resistance in *H. contortus* [21-23]. *In vitro* selection for BZ resistance in *H. contortus* resulted in homozygosity of a particular allele of the isotype 1 tubulin gene [24]. The resistant allele contains three mutations including a change from phenylalanine to tyrosine at position 200 (F200Y)[25]. To demonstrate the necessity of the F200Y mutation for resistance, susceptible and resistant *H. contortus* tubulin alleles were used to transform *C. elegans ben-1* mutants. A transgene encoding the sensitive allele, but not one containing the resistant allele, conferred thiabendazole sensitivity on *C. elegans* (though not sensitivity to benomyl)[26]. The F200Y variant also seems to be important for resistance in natural populations of *Teladorsagia circumcincta* and *T. colubriformis* [27,28].

## LEVAMISOLE

### Introduction

In 1966, researchers reported the discovery of two families of structurally related nematocidal compounds: the imidazothiazoles, including tetramisole and levamisole (the l isomer of d,l-tetramisole), and the tetrahydropyrimidines, including pyrantel and morantel (Fig. 1) [29,30]. These compounds are effective against a wide variety of nematodes and are used in both human and animal medicine. These compounds all activate nicotinic acetylcholine receptors (nAChRs).

### Effects on *C. elegans*

In *C. elegans*, 1 mM levamisole causes hypercontraction of the body wall muscles used in locomotion [31]. After about 30 minutes, adults relax and the worms eventually die. However, worms can recover if removed from levamisole before relaxation occurs. The high concentration of levamisole needed to get the full toxic effect of the drug appears to be the result of the

low permeability of the thick nematode cuticle. By cutting worms to allow access of drug to the pseudocoelom, the internal cavity of the worm, one can observe the same muscle contractions at a concentration about twenty-fold lower.

### Genetics of Resistance

One of the first genetic screens performed in *C. elegans* identified mutants that were resistant to tetramisole [32]. Subsequent screens on 1 mM levamisole identified hundreds of strains that were levamisole resistant. The resistant strains either do not hyper-contract in response to levamisole or recover rapidly from hypercontraction [33]. The resistant alleles represented in these mutant strains define 12 genes (Table 1). The mutants can be broken down into several classes based on their phenotypes. The largest class consists of mutants that have a modest degree of uncoordinated locomotion, that is, the sinusoidal crawling of the worms is not as smooth and fluid as in wild-type worms both in the presence and in the absence of the drug. This class of mutants includes recessive alleles of the genes *unc-29, unc-63, unc-38, unc-74, unc-50* and semi-dominant alleles of *lev-1*.

A second class is comprised of mutants, called pseudo-wild-types, whose locomotion is nearly wild-type. The mutations in these strains occurred mostly in the same set of genes as in the first class but generally at a much lower frequency. For example, locomotion was pseudo-wild-type for only 2 of 76 alleles of *unc-29* isolated. One interpretation of these results is that, in the pseudo-wild-type mutants, the target of levamisole retains some of its normal function but binds levamisole less avidly. Alleles of the *lev-1* gene represent the exception to the rule that the pseudo-wild type alleles are more rare. Thirteen *lev-1* alleles were pseudo-wild-type but only two were uncoordinated. Interestingly, the two uncoordinated alleles of *lev-1* are also semi-dominant. Possibly the null phenotype of *lev-1* is

pseudo-wild-type, but the proteins encoded by the dominant gain-of-function alleles interfere with the products of the other resistance genes.

A third set of genes consists of those for which only pseudo-wild type alleles were identified. These relatively rare mutations occur in the genes *lev-8*, *lev-9* and *lev-10*. Null alleles of these genes may be lethal but the levamisole resistance screen makes it possible to isolate the unusual mutation that will alter the response to levamisole but retain protein function. Finally, there are three genes that affect the contractile process. Mutations in these genes presumably do not affect the ability of levamisole to interact with its targets, but rather they mitigate the ill-effects of levamisole by preventing the worms from undergoing hyper-contraction. The genes encode twitchin (*unc-22*) and tropomyosin (*lev-11*) and the ryanodine receptor (*unc-68*).

Pharmacological studies indicate that resistant alleles affect neurotransmission by altering nAChRs. Nicotine, like levamisole, causes muscle hyper-contraction and blockers of nAChRs, such as mecamylamine, also block the effects of levamisole [31]. The levamisole-resistant mutants are cross-resistant to nicotine. Furthermore, the mutants are just as resistant in the cut-worm assay as in the whole worm assay, indicating that the source of the resistance is not a decrease in the permeability of the cuticle to levamisole. *unc-29* mutants are cross-resistant to morantel and pyrantel, as expected if these compounds also act on nAChRs.

Assays that measure binding of radiolabelled meta-aminolevamisole (MAL) to *C. elegans* extracts confirm that the nAChRs are targets of levamisole [34]. MAL binds both saturable and non-saturable sites. Cholinergic agonists like morantel and nicotine compete for MAL binding sites. Cholinergic antagonists such as mecamylamine activate binding, apparently by an allosteric mechanism. Neither cholinesterase inhibitors nor muscarinic antagonists affect MAL binding.

The results of MAL binding assays on levamisole-resistant strains indicate that some resistant alleles affect the nAChRs targeted by levamisole [35]. Based on their MAL binding activity, the resistant mutants were grouped into roughly three classes. *unc-29*, *unc-50* and *unc-74* mutants have severely reduced high-affinity, saturable MAL binding activity, presumably reflecting the absence of the target receptor in these strains. *unc-63*, and *unc-38* mutants have somewhat reduced saturable MAL binding. Interestingly, MAL binds *unc-63* and *unc-38* mutants with higher affinity than it binds wild-type. The affinity of binding is more typical of wild-type treated with mecamylamine and the affinity is not increased by the addition of mecamylamine. Thus, mutating *unc-63* and *unc-38* changes the properties of the levamisole-binding complex without destroying it. Alleles of *lev-1* comprise the third class and exhibit similarities to both of the above classes. The semidominant alleles of *lev-1* confer roughly normal saturable binding but the affinity is higher than wild-type and is not increased by mecamylamine. The recessive *lev-1* alleles have lower levels of saturable binding and a lower bind-

ing affinity that was not increased by addition of mecamylamine.

## Molecular Biology

As predicted, several levamisole resistance genes encode subunits of a levamisole-sensitive nAChRs. nAChRs are ligand-gated ion channels consisting of five subunits that assemble to form a pore in the membrane. The subunits can be encoded by one gene (a homomeric channel) or encoded by several genes (heteromeric channels). Based on sequence, the subunits can generally be grouped into  $\alpha$  or non- $\alpha$  subunits and heteromeric channels contain at least one of each. *unc-29*, *unc-38* and *lev-1* all encode subunits of a heteromeric levamisole-sensitive nAChR [36]. UNC-29 and LEV-1 are non- $\alpha$  subunits whereas UNC-38 is an  $\alpha$  subunit. The *lev-1(x548)* allele, a deletion of the coding region, has the partially resistant pseudo-wild-type phenotype, which is therefore the null phenotype. *lev-1* semidominant alleles, *x61* and *x21*, contain, respectively, an amino acid insertion and a missense mutation in or near the second transmembrane domain. This transmembrane domain is thought to line the channel pore. Incorporation of the semi-dominant LEV-1 mutant subunits into the channel likely interferes with the ability of the channel to conduct ions. Strong levamisole resistance and uncoordinated locomotion are the phenotypes of *unc-38* null alleles. Probably the same is true for *unc-29*.

The absence of the UNC-29/UNC-38/LEV-1 acetylcholine receptor from the body (somatic) muscle accounts for the phenotypes of the subunit mutants. A reporter construct that fused green fluorescent protein (GFP) to the carboxy terminus of *unc-29* expresses in body muscle and rescues the uncoordinated locomotion of several *unc-29* alleles [36]. The reporter construct may also express in some neuronal cells. However, a second construct in which the UNC-29::GFP fusion protein was driven by the promoter of a muscle-specific myosin, also restored normal locomotion. Thus, the effects of levamisole are direct effects on the muscle.

The electrophysiological properties of the UNC-29/UNC-38/LEV-1 channels have been characterized in *Xenopus* oocytes and in *C. elegans* muscle preparations. Levamisole-responsive channels were only seen when more than one subunit was co-expressed in oocytes and robust currents were seen consistently only when all three were co-expressed [36]. However, the currents generated when these channels were activated with levamisole were of relatively small amplitude, indicating that other factors/subunits may be necessary to form a channel *in vivo*. The other levamisole resistance genes may encode these other factors. Nevertheless, the channels expressed in oocytes reflected many of the properties expected of *in vivo* channels. They responded to acetylcholine (ACh), and were blocked by mecamylamine. Whole cell patch clamp recordings from a *C. elegans* body muscle preparation demonstrated the presence of a levamisole-sensitive but nicotine-insensitive

channel that is absent in *unc-29* and *unc-38* mutants [37]. Because they were unable to record endogenous post-synaptic potentials in the *unc-29* and *unc-38* mutants, the receptors must be synaptic.

The nAChR genes that have been identified so far in the levamisole resistance screens are only a small fraction of the ~40-odd nAChR-like genes found in the *C. elegans* genome [38,39]. Some of the remaining subunits can form levamisole-sensitive channels. *acr-2*, which encodes a non- $\alpha$  type nAChR closely related in sequence to *lev-1* and *unc-29*, does not form homomeric channels when expressed in *Xenopus* oocytes but forms channels in association with the UNC-38  $\alpha$  subunit. Levamisole activates this channel and the response to levamisole is blocked by mecamylamine [40]. *acr-3* maps close to *acr-2* and also encodes a non- $\alpha$  type subunit. When co-expressed with *unc-38*, it too forms a channel that responds to levamisole, a response that is inhibited by mecamylamine and *d*-tubocurarine [41]. Whether the ACR-2 and ACR-3 subunits co-assemble with UNC-38 *in vivo* and what relevance those channels might have to levamisole sensitivity and resistance is unclear.

Not all *C. elegans* nAChRs are activated by levamisole. When recording from body muscle, Richmond and Jorgensen also found a synaptic levamisole-insensitive but nicotine-sensitive nAChR that required neither *unc-29* nor *unc-38* encoded subunits [37]. *acr-16* (p.k.a. Ce21) encodes an nAChR subunit that forms a homomeric channel in oocytes. The channel is not activated but partially blocked by levamisole [39,42]. There also appears to be a nAChR in the pharyngeal muscle that mediates neurotransmission by the MC motor neuron but is not sensitive to levamisole [43,44]. Finally, *deg-3* and *des-2* (p.k.a. *acr-4*) encode subunits of a nAChR expressed in the mechanosensory neurons. When expressed in oocytes, the *deg-3* and *des-2* gene products formed an acetylcholine-sensitive channel although levamisole sensitivity was not examined [45]. A dominant gain-of-function mutation in *deg-3* results in neuronal degeneration although it is not clear how the mutation produces this effect [46].

### Relevance to Parasites

As in *C. elegans*, levamisole activates nicotinic-type acetylcholine receptors in muscles of parasitic nematodes [47]; reviewed in [48]. Treating parasites with levamisole or pyrantel causes tonic contraction of the somatic muscles. Furthermore, several cDNAs that have been cloned from parasites may encode orthologs of *unc-38* [39,49-51].

Studies of levamisole resistant parasites are still in early stages. So far, the data is consistent with the hypothesis that alleles that alter levamisole-sensitive nAChRs confer resistance. Resistant isolates of *H. contortus* have altered low-affinity levamisole-binding sites [52]. In levamisole-resistant isolates of *Oesophagostomum dentatum*, the single channel properties of somatic muscle levamisole receptors are altered. Particularly noteworthy is the absence of one of the four conductance

subtypes, a phenotype that might result from the absence of a channel subunit [53]. A similar spectrum of changes in the single-channel properties of nAChRs occur in a pyrantel-resistant *O. dentatum* strain [54]. No alleles of an *unc-38*-like gene cloned from *H. contortus* segregated with resistance in one resistant strain [50]. But more resistant strains and more *H. contortus* orthologs of *C. elegans* resistance genes need to be examined.

## AVERMECTINS

### Introduction

The avermectins were introduced in the 1980s and are now used for many veterinary and human applications [55]. Ivermectin is a semi-synthetic derivative of natural products of the bacterium *Streptomyces avermitilis*. Milbemycin D and moxidectin are derived from products of *Streptomyces hygroscopicus* [56]. All of these compounds have a similar structure and mechanism of action and can be grouped into the larger class of macrocyclic lactones (Fig. 1). The macrocyclic lactones appear to affect all nematodes as well as insects, arachnids and crustacea (*i.e.* arthropods). Ivermectin's low toxicity in humans makes it the drug of choice for treating onchocerciasis [5]. The macrocyclic lactones all activate glutamate-gated chloride channels.

### Effects on *C. elegans*

Ivermectin affects worms at concentrations as low as 2 ng/mL [43,57,58], which is comparable to therapeutic concentrations of ivermectin (100-400  $\mu$ g/kg) [5]. Ivermectin paralyzes *C. elegans* [57,59]. Ivermectin also inhibits pharyngeal pumping, preventing *C. elegans* from eating [43,60].

### Genetics of Resistance

Synthetic resistance is a salient feature of the genetics of ivermectin resistance in *C. elegans* (Table 1). Synthetic resistance is a genetic term that describes the situation where mutation of a single gene has no effect on the worm's sensitivity to a drug but mutations in two or more genes substantially decreases drug sensitivity. In a growth assay that primarily measures the sensitivity of pharyngeal pumping to ivermectin, recessive mutations in the genes *avr-15*, *avr-14*, *unc-7* or *unc-9* have almost no effect on ivermectin sensitivity [58,60]. Worms that carry simultaneous mutations in two of *avr-14*, *unc-7* or *unc-9* are also as sensitive as wild-type. However, worms that carry mutations in both *avr-15* and any one of the other three genes exhibit a ~10-500 fold decrease in ivermectin sensitivity. Thus, there are two independent genetic pathways through which ivermectin can kill worms, one that acts through *avr-15* and another pathway that requires *avr-14*, *unc-7* and *unc-9*. The *glc-1* gene appears to define a third pathway.

Homozygous recessive mutations in *glc-1* increase resistance in worms that already have mutations in both *avr-15* and *avr-14*, but not alone and not in combination with any one other mutant gene. Finally, single gene mutations in members of a large class of genes called the Dyf genes confer a low level (2-5 fold) of ivermectin resistance. The addition of a mutation in a Dyf gene to any of the other genetic backgrounds results in a modest, roughly additive increase in ivermectin resistance.

Interestingly, *avr-14*, *avr-15* and *glc-1* mutants have mild behavioral phenotypes: *avr-15* mutants exhibit a slight reduction in eating efficiency and *avr-14* mutants have an increased frequency of reversal when crawling [58,60]. In contrast, locomotion in *unc-7* and *unc-9* mutants is uncoordinated. It should also be noted that, in spite of its locomotory deficits, single *unc-9* mutants are resistant to paralysis by ivermectin [59]. Thus, the ivermectin resistance genes probably play diverse roles in behavior and one's assessment of the importance of a particular gene in ivermectin sensitivity may depend strongly on the behavior assayed.

Ivermectin binds to a single high-affinity site in membrane preparations from *C. elegans*. The ability of ivermectin analogs to inhibit ivermectin binding correlates well with their ability to paralyze worms [61,62]. An active ivermectin derivative photoaffinity labels proteins of 8, 47 and 53 kDa [63]. The *avr-14*, *avr-15* and *glc-1* gene products each account for a fraction of the binding activity ( $B_{max}$ ) and together they account for all of the measurable high-affinity binding [58]. Thus, these genes are necessary for the formation of ivermectin's targets and may encode some of the proteins found by affinity labeling.

## Molecular Biology

Three of the ivermectin resistance genes, *avr-14*, *avr-15* and *glc-1*, encode  $\alpha$  subunits of glutamate-gated chloride channels (GluCl $\alpha$ s), the targets of ivermectin. The GluCl $\alpha$ s belong to the superfamily of channel subunit genes that includes the nAChRs. Like the nAChRs, the GluCl $\alpha$ s are likely to be pentameric.

*glc-1* cDNAs were isolated by expression cloning in *Xenopus* oocytes. When oocytes express whole *C. elegans* mRNA, ivermectin- and glutamate-activated currents result [64,65]. Ivermectin potentiates the glutamate current, indicating that the two ligands likely interact with the same channel. Two cDNAs were isolated, both encoding subunits of ligand-gated ion channels that are most similar in sequence to the vertebrate GABA $_A$ /glycine channels [66]. When expressed in oocytes, one subunit, GLC-1/GluCl $\alpha$ 1, forms a homomeric ivermectin-activated chloride channel that is insensitive to glutamate. The subunit encoded by the other cDNA, GLC-2/GluCl $\beta$ , forms a homomeric chloride channel that is glutamate-gated but is insensitive to ivermectin.

*avr-14* and *avr-15* were isolated by a combination of positional cloning and the identification of candidate genes. *avr-15* encodes at least two alternatively-spliced  $\alpha$  subunits of GluCl channels [60]. When expressed in

*Xenopus* oocytes, AVR-15/GluCl $\alpha$ 2 forms a homomeric ivermectin-sensitive channel that has a weak response to glutamate. AVR-14/GluCl $\alpha$ 3 (p.k.a. *gbr2/gbr-3/GluClX*) also produces two transcripts encoding  $\alpha$  subunits [58,67]. The subunit encoded by one *avr-14* transcript forms a homomeric ivermectin- and glutamate-sensitive channel when expressed in *Xenopus* oocytes.

The *in vivo* subunit composition of the GluCl $\alpha$ s is an open question. AVR-15/GluCl $\alpha$ 2 and GLC-2/GluCl $\beta$  appear to associate to form the pharyngeal muscle GluCl. This GluCl is the synaptic receptor of the inhibitory glutamatergic motor neuron M3 [60]. Ivermectin activates the pharyngeal GluCl to inhibit pharyngeal pumping. AVR-15/GluCl $\alpha$ 2 must be a subunit of the pharyngeal GluCl since, in an *avr-15* mutant, the pharyngeal muscle is insensitive to glutamate and M3 neurotransmission is absent. GLC-2/GluCl $\beta$  is likely to be a component of the pharyngeal GluCl since GLC-2/GluCl $\beta$  appears to be expressed exclusively in the pharyngeal muscle, it associates with  $\alpha$ 2 in oocytes and it would provide the glutamate sensitivity that  $\alpha$ 2 lacks [60,68,69]. GLC-1/GluCl $\alpha$ 1 also associates with GluCl $\beta$  to form a glutamate-sensitive channel in *Xenopus* oocytes [66,69]. But  $\alpha$ 1 is not necessary for the formation of the pharyngeal GluCl and nothing is known about its pattern of expression (J. Dent, unpublished observation). Based on a GFP reporter construct, AVR-14/GluCl $\alpha$ 3 appears to be expressed in the extra-pharyngeal nervous system. Finally, there are two other GluCl-like channel subunits in the *C. elegans* genome. Based on sequence, GLC-3/ZC317.3 is clearly an  $\alpha$  subunit. C27H5.8, may be a  $\beta$  subunit [70].

*unc-7* and *unc-9* both encode innexins, which are subunits of invertebrate gap junctions [71-73]. They have no sequence similarity to GluCl $\alpha$ s or to vertebrate gap junction subunits (connexins). Unlike the GluCl $\alpha$ s, they apparently do not bind ivermectin strongly [58]. Rather, mutations in these genes probably affect ivermectin sensitivity indirectly by altering the connectivity of the nervous system. One important effect of mutations in *unc-7* and *unc-9* is to prevent changes in membrane potential caused by ivermectin's activation of AVR-14/GluCl $\alpha$ 3 from spreading through the nervous system [58].

The Dyf genes, of which there are about 20, share a phenotype when mutated. Externally applied dyes, which are normally able enter the body of the worm through specialized sensory structures (amphids) in the otherwise impermeable cuticle, are excluded from the body in the Dyf mutants [74,75]. The Dyf genes that have been characterized encode diverse proteins that are likely to affect the formation of these sensory structures [74,76]. Thus, one plausible hypothesis is that mutations in Dyf genes make worms more resistant to ivermectin because they make worms less permeable to the drug.

## Relevance to Parasites

The GluCl $\alpha$ s are the relevant targets of the avermectins.

tins in parasitic nematodes. Ivermectin inhibits feeding in *H. contortus* and *T. colubriformis* [77-80]. Electrophysiological studies have demonstrated the presence of avermectin-gated chloride channels in body muscle and pharyngeal muscle of *Ascaris suum* [81,82]. cDNAs encoding several GluCl-like channel subunits have been cloned from parasites including *H. contortus*, *Ascaris suum*, *Dirofilaria immitis* and *Onchocerca volvulus* (the nematode that causes onchocerciasis) [70,83-85]. These cDNAs include apparent orthologs of the *C. elegans* GluCl $\beta$  and AVR-14/GluCl $\alpha$ 3 genes. A subunit of a GluCl has also been cloned from *Drosophila melanogaster* and accounts for ivermectin sensitivity in arthropods [86,87].

It remains to be seen whether orthologs of the *C. elegans* ivermectin-resistance genes will be a source of resistance in parasites. There are some experiments that point to changes in GluCl $\alpha$ s as a mechanism of resistance. In two different *H. contortus* ivermectin-selected strains, the allele frequencies of an  $\alpha$  GluCl subunit are altered relative to the unselected strains from which the resistant strains were derived [88]. The allele frequencies of a  $\beta$  subunit gene are unaffected. Thus, the  $\alpha$  subunit gene is likely to be linked to a resistance locus and may itself be the locus. The kinetics of glutamate-binding differ in resistant and sensitive strains of *H. contortus*, possibly as a result of changes in a GluCl [89]. Pharyngeal function in particular is less sensitive to the effects of ivermectin and glutamate in resistant strains of *H. contortus* [78,80]. However, there may be resistance mechanisms in parasites that have not been found in *C. elegans*. In one strain of ivermectin-resistant *H. contortus*, the high-affinity ivermectin binding site was unaffected [90]. Resistance in another *H. contortus* strain is dominant, in contrast to the recessive resistance found in *C. elegans* [91,92]. Amplified expression of or mutations in a P-glycoprotein may represent a resistance mechanisms in *H. contortus* [93-96]. Gene amplification could account for the dominant resistance.

## OTHERS

### Amidantel

Amidantel and its deacylated derivative are effective against various nematode parasites [97,98]. In *C. elegans*, amidantel acts on nAChRs. It causes rigid paralysis at concentrations around 180-350  $\mu$ M. The paralysis is blocked by the nicotinic channel blocker d-tubocurarine [99]. Moreover, amidantel-resistant mutants were isolated and found to be cross-resistant to levamisole and ACh [100]. Although the mutant genes were never identified, based on their phenotypes, they could be alleles of levamisole resistance genes.

### *Bacillus thuringiensis* Toxin

Members of the family of *B. thuringiensis* (Bt) toxins are the most widely used natural insecticides due, in

part, to the propagation of transgenic plants expressing Bt toxin genes. However, nematode infestations also cause significant crop damage and Bt toxins kill a variety of nematodes including *C. elegans* [101-106]. When ingested by *C. elegans*, the Cry5B Bt toxin kills worms with an LD<sub>50</sub> of 12.6  $\mu$ g/mL [107]. Death from Bt toxins results from intestinal degeneration in *C. elegans*, which is consistent with the Bt toxin-induced pathology in insects [103,107]. In a genetic screen for Bt resistance, recessive alleles of five genes were identified but the genes have not yet been characterized.

## CONCLUSION

Based on the substantial body of knowledge outlined in this review, we can say with confidence that the mechanisms of drug sensitivity and the genetics of drug resistance in *C. elegans* parallel those characterised in parasites. But can we make precise predictions about parasites based on what we know about *C. elegans*?

### Drug targets

In every case where the targets of antiparasitic drugs have been identified, the targets belong to the same class of proteins in parasitic nematodes as in *C. elegans*. It is not yet clear that the drug targets in parasites will be encoded by orthologs of the *C. elegans* resistance genes. The *H. contortus*  $\beta$  tubulin isotype 1 gene, alleles of which confer BZ resistance, is not obviously orthologous to a particular *C. elegans*  $\beta$  tubulin gene (J. Dent, unpublished observation). There appear to be parasite orthologs of *C. elegans* levamisole- and ivermectin-resistance genes but no alleles of the parasite genes have yet been shown to confer resistance [51,83,86]. However, even in the absence of true orthologs, cloned *C. elegans* genes will be useful for identifying members of homologous gene families in parasites.

### Resistance Mechanisms

The correspondence between resistance mechanisms in *C. elegans* and various parasites is likely to be strong enough to justify using *C. elegans* as a starting point from which to begin looking for candidate genes involved in parasite resistance. The best characterised resistance mechanism in a parasite, BZ resistance in *H. contortus*, is strikingly similar to the mechanism of BZ resistance in *C. elegans*. Alleles that affect some aspect of the formation of a levamisole-sensitive nAChR channel apparently underlie levamisole-resistance in *O. dentatum* just as they do in *C. elegans*.

That said, resistance mechanisms in parasites probably do not mirror the resistance mechanisms in *C. elegans* as closely as the drug targets in parasites correspond to the targets in *C. elegans*. This is because the selective pressures on worms in the laboratory are minimal and permit the isolation of strains that would not be viable in the wild. As an example, the uncoordi-

nated locomotion exhibited by the innexin mutants and many of the levamisole mutants would presumably prevent their survival in the wild. Moreover, even subtle behavioural or metabolic changes that result from a resistance allele may prevent a parasitic worm from performing some vital step of its complicated life cycle. Even in cases where resistance in parasites is mediated by orthologs of *C. elegans* resistance genes, it is unlikely that resistance will result from sequence variations common to *C. elegans* and parasites. Only in cases where resistance were conferred by a missense mutation that reduces drug sensitivity while retaining some critical protein function (e.g. consider [87]) would a specific genetic change be likely to be conserved among resistant alleles. For example, we might expect to find parasite equivalents of the rare pseudo-wild-type alleles of the levamisole-resistance genes in *C. elegans*.

It is interesting to note that resistance in *H. contortus* results from a sequence variation, F200Y, that is present in wild-type alleles of other tubulin genes. Thus, it is likely that the Y200 variant is a functional tubulin. In contrast, the resistant alleles of *ben-1* in *C. elegans* are often null. F200Y may be a rare sequence variant that reduces drug binding but retains protein function. Perhaps, the reason this variant is retained in *H. contortus* is that the more intense selective pressure on parasites requires some function, however subtle, for that tubulin. Whereas in *C. elegans*, the BEN-1 tubulin is not necessary for survival in the laboratory, so null alleles of the tubulin gene are selected because they are more common and/or because they provide stronger resistance. Of course it is also possible that the tubulin gene is largely redundant in *C. elegans* but essential in *H. contortus*. Either way, the lack of strong selective pressures on *C. elegans* may be reflected in the type of BZ resistance alleles one identifies.

### Looking to the Future

The ability of nematodes to rapidly evolve resistance threatens the efficacy of our best nematocidal drugs. Presumably, a better understanding of drug targets and resistance mechanisms will help prevent the erosion of our ability to control parasitic nematodes. Probes that identify resistance alleles in wild parasites before those alleles become widespread can alert us to the need to rotate drugs or to institute measures to prevent a resistant parasite's dissemination [27,28]. Moreover, our knowledge of how the existing drugs work and how they are compromised by resistance will help us design better nematocides. Ivermectin, in particular, has advantageous properties that might guide the selection of new drug targets. Ivermectin's lack of toxicity derives in part from its specificity for a class of channels, the GluCl's, that is apparently absent from vertebrates. Ivermectin also targets several members of a multigene family, which may slow the evolution of resistance [58,60,108]. The knowledge we have gleaned from *C. elegans* in combination with new genomic and drug-screening technologies that are being developed will

enhance the potency and reduce the side effects of the next generation of anti-parasitic compounds.

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