

# Mode of action of levamisole and pyrantel, anthelmintic resistance, E153 and Q57

R. J. MARTIN *and* A. P. ROBERTSON

*Department of Biomedical Sciences, Iowa State University, Ames, IA, 50011, USA.*

## SUMMARY

Here we review molecular information related to resistance to the cholinergic anthelmintics in nematodes. The amount of molecular information available varies between the nematode species, with the best understood so far being *C. elegans*. More information is becoming available for some other parasitic species. The cholinergic anthelmintics act on nematode nicotinic acetylcholine receptors located on somatic muscle cells. Recent findings demonstrate the presence of multiple types of the nicotinic receptors in several nematodes and the numerous genes required to form these multimeric proteins. Not only are the receptors the product of several genes but they are subject to modulation by several other proteins. Mutations altering these modulatory proteins could alter sensitivity to the cholinergic anthelmintics and thus lead to resistance. We also discuss the possibility that resistance to the cholinergic anthelmintics is not necessarily the result of a single mutation but may well be polygenic in nature. Additionally, the mutations resulting in resistance may vary between different species or between resistant isolates of the same species. A list of candidate genes to examine for SNPs is presented.

Key words: Levamisole, pyrantel, mode of action, anthelmintic resistance, E153 and Q57.

## INTRODUCTION

CARS, the Consortium for Anthelmintic Resistance SNPs, has the aim of identifying single nucleotide changes in parasitic nematodes that are associated with anthelmintic resistance. The molecular detection of resistance to benzimidazoles has been possible in some isolates of parasitic nematodes by looking for single nucleotide polymorphisms (SNPs) in  $\beta$ -tubulin genes (von Samson-Himmelstjerna *et al.* 2003). A phenylalanine-to-tyrosine change at position 200 (F200Y) in the isotype 1  $\beta$ -tubulin gene (TTC to TAC) has been associated with benzimidazole resistance in some isolates of *Haemonchus contortus*. We know, however, that not all benzimidazole resistance is associated with this specific change and other identified and unidentified SNPs are associated with this phenotype. The CARS aim, then, is to identify all SNPs associated with resistance to all types of anthelmintic in all significant parasitic nematodes. The major classes of anthelmintic which CARS is interested in are the benzimidazoles, the nicotinic anthelmintics and the avermectins.

In our review, we focus on cholinergic anthelmintics and review the mode of action of levamisole and pyrantel and use the *C. elegans* model to comment on possible mechanisms of resistance. We suggest that some forms of resistance are polygenic and resistance SNPs will be difficult to find; however, other forms of resistance might be related to

E153G mutations in UNC-38 and Q57G mutations in UNC-63, possibilities which should be tested (Rayes *et al.* 2004; Bartos, Rayes and Bouzat, 2006).

We start with a car metaphor (Fig. 1); consider a Dodge Neon and its engine and *Ascaris suum*. The response of the engine to the ignition of fuel is affected by many factors, including the setting of the carburetor, the timing of ignition, and the timing of the opening of the fuel inlet and exhaust valves. In the same way, the contractile response of *Ascaris* muscle to levamisole or pyrantel is affected by the molecular settings of components of the muscle receptor signalling-contraction machinery. The point of the metaphor is that the response of a nematode to a cholinergic anthelmintic like levamisole or pyrantel depends on the settings of a large number of interconnecting components, as does the performance of the internal combustion engine. The response of the parasite will depend on the sensitivity of nicotinic acetylcholine (ACh) receptors (nAChRs) on muscle, and on how much depolarization and entry of  $\text{Ca}^{2+}$  levamisole/pyrantel produces, together with the sensitivity of the contractile proteins to  $\text{Ca}^{2+}$  (Fig. 2). If the muscle contraction response to the cholinergic anthelmintic is sufficiently reduced by any of a number of different components in the receptor signal-transduction pathway, the parasite will show reduced sensitivity (resistance) to the drug.

## USE OF LEVAMISOLE, PYRANTEL AND RELATED COMPOUNDS AGAINST PARASITIC NEMATODES

Pyrantel and levamisole are used for the control of hookworms and roundworms of humans, livestock

Corresponding author: Martin, R. J. Email: rjmartin@iastate.edu

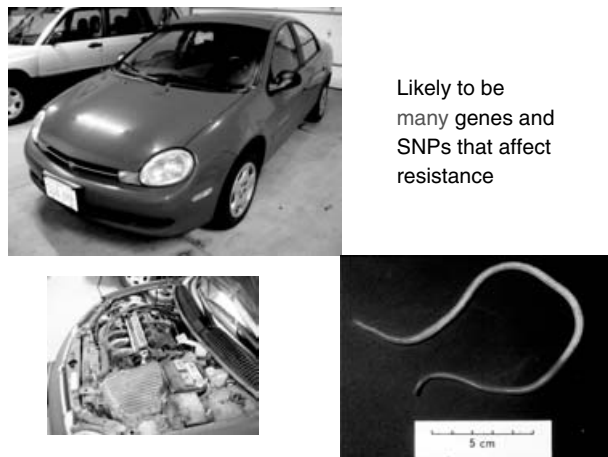


Fig. 1. CARs and a car metaphor for resistance. CARs, the Consortium seeking Anthelmintic Resistance Single nucleotide polymorphisms, and a car (Dodge Neon and engine). The engine of a car is a complex machine with many parts that can be adjusted to affect the performance of the engine in response to combustion of the fuel. Likewise, a nematode parasite like *Ascaris suum* has many component proteins that are responsible for the response to the cholinergic anthelmintics, levamisole and pyrantel.

and companion animals. They are used for treatment of animal and human faecally-transmitted parasitic nematodes, but they are used less often than the benzimidazoles or the avermectins. These drugs are referred to by the World Health Organization, WHO (2000), as essential drugs for treatment of intestinal nematodes. Levamisole and pyrantel are typically effective against roundworms (ascarids) in companion animals and humans, as well as hookworms (*Necator americanus* and *Ancylostoma duodenale*), but are not very effective against whipworms (*Trichuris trichiura*) (Albonico, Crompton and Savioli, 1999). The reduced efficacy of pyrantel against whipworms gave rise to the development of oxantel because of the need for a drug with a higher efficacy against *Trichuris* species. A combination of oxantel and pyrantel can be used and has the advantage of a broader spectrum of action (Albonico *et al.* 2002).

Regrettably, resistance has developed following use of these compounds (Sangster and Dobson, 2002) and is now recognized in parasites of both humans and domesticated animals. Resistance to cholinergic agonists may be produced by four general mechanisms: (1) changes in drug translocation (e.g. increased metabolism or excretion of the drug); (2) changes in receptor numbers (e.g. reduced number of receptors); (3) receptor modification (e.g. change in receptor binding sites due to amino acid substitution); or (4) post-receptor modification (e.g. change in the downstream pathway to contraction). All of these mechanisms could play a role in resistance to levamisole and pyrantel and so it is anticipated that

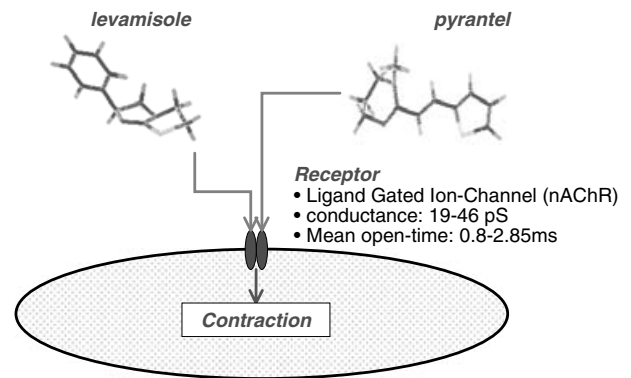


Fig. 2. A simplified but now out-dated model of the mode of action of levamisole and pyrantel. These two compounds act on nicotinic acetylcholine receptors of muscle to gate these channels open, to allow entry of calcium and to produce contraction and spastic paralysis.

resistance to these drugs is polygenic (Sangster and Dobson, 2002).

#### GENERAL MODE OF ACTION OF CHOLINERGIC ANTHELMINTICS

Fig. 2 shows the chemical structures of the cholinergic anthelmintics levamisole and pyrantel, together with a model for their mode of action. In 1970, papers introducing the pharmacology of levamisole and pyrantel described effects of these compounds on the body muscle of *A. suum* (Aceves, Erlij and Martinez-Maranon, 1970; Aubry *et al.* 1970). These authors showed that these compounds were cholinergic agonists that selectively produced depolarization and spastic paralysis of body muscle cells in parasitic nematodes. Electrophysiological responses to these anthelmintics can be observed in muscle cells of the nematode parasites *A. suum* and *Oesophagostomum dentatum* (Martin, 1982; Pennington and Martin, 1990; Robertson and Martin, 1993; Evans and Martin, 1996; Martin *et al.* 1998; Robertson, Bjorn and Martin, 1999; Robertson *et al.* 2002; Trailovic *et al.* 2002, 2005; Levandoski *et al.* 2005). These studies identified nAChRs over the nematode muscle cell surface that respond to these anthelmintics and have described, down to the single-channel level, the agonist action and channel-blocking effect of the anthelmintics.

Levamisole opens ACh-gated cation-selective ion-channels, which constitute the nAChRs, on nematode muscle membranes, producing depolarization and muscle contraction (Fig. 2). The ion-channels have conductances of 19–46 pS and mean open-times of 0.5–2.0 ms. Nematode muscle nAChRs have pharmacological and biophysical properties similar to those of vertebrate neuronal receptor channels, but there are also significant pharmacological differences. The pharmacological differences allow levamisole and pyrantel to have a selective therapeutic

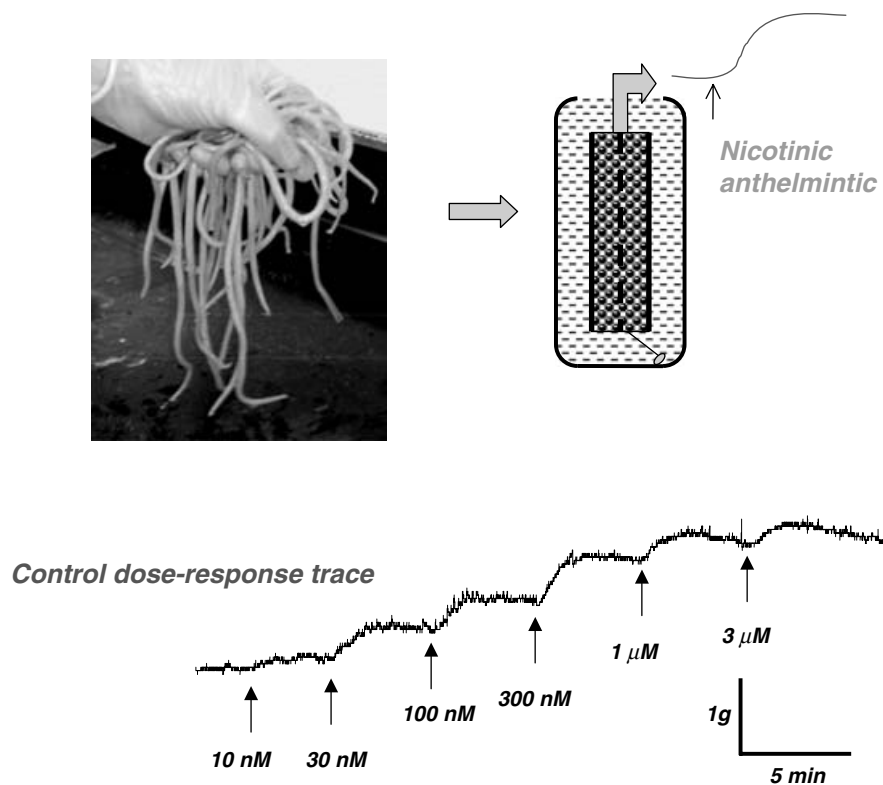


Fig. 3. *Ascaris suum* muscle strip preparation cumulative-dose response relationship to a cholinergic anthelmintic. *Ascaris suum* are collected from the packing plant and a 2 cm half-body flap (muscle strip) is prepared from behind the head region and placed in a tissue bath with an isometric transducer. The nicotinic anthelmintic is added cumulatively, without washout, to produce the concentration-contraction-response.

effect on the nematode muscle receptors, minimizing toxic effects on mammalian (host) muscle.

#### LEVAMISOLE AND PYRANTEL ACTIVATE *N*-, *L*- AND *B*-nAChR SUBTYPES

We have used muscle strips from the parasitic nematode *A. suum* (Fig. 3) for pharmacological experiments. We have obtained cumulative dose-response plots for a number of cholinergic agonists (Fig. 4) in the presence of competitive antagonists like paraherquamide, 2-desoxoparaherquamide and methyllycaconitine (Zinser *et al.* 2002; Robertson *et al.* 2002). We have been able to identify three pharmacologically distinct nAChRs (Fig. 5) that are preferentially activated by the different cholinergic anthelmintics. These include: (1) the *N*-subtype, preferentially activated by methyridine and nicotine; (2) the *L*-subtype, preferentially activated by levamisole and pyrantel and antagonized by paraherquamide; and (3) the *B*-subtype, preferentially activated by buphenium and antagonized by paraherquamide and 2-desoxoparaherquamide.

We have also been able to distinguish the three subtypes at the single-channel level (Qian, Martin and Robertson, 2006) using cell-attached patch recordings from somatic muscle vesicles (Fig. 6). These recordings have allowed a comparison of the

effects of levamisole and buphenium at the single-channel level and the examination of the effects of the novel anthelmintics paraherquamide and 2-desoxoparaherquamide (Zinser *et al.* 2002) that act as competitive antagonists (Robertson *et al.* 2002). These observations emphasize the different selectivities of the cholinergic anthelmintics among the subtypes of cholinergic receptor. In levamisole-resistant isolates of *O. dentatum* (Fig. 7), we have found a reduction in the sensitivity to levamisole in larval migration assays (Fig. 8). This reduction in sensitivity is also observed with pyrantel but not with nicotine and methyridine (Martin *et al.* 2004). When we examined the single-channel properties of levamisole-sensitive and -resistant isolates, we found that the G35 pS channel of *O. dentatum* (corresponding to the *L*-subtype of *A. suum*) was absent from the levamisole-resistant isolates (Robertson *et al.* 1999). These observations confirm that there are differences in the nAChR subtype selectivities among the cholinergic anthelmintics and that, at least in some types of levamisole-resistance, there is a loss of the *L*-subtype (Fig. 9). The experiments also suggest that in some cases, it may be possible to overcome levamisole resistance by using cholinergic agonists (methyridine: *N*-subtype) with different subtype selectivities or an antagonist (2-desoxoparaherquamide, *B*-subtype) with selectivity for other nAChR subtypes.

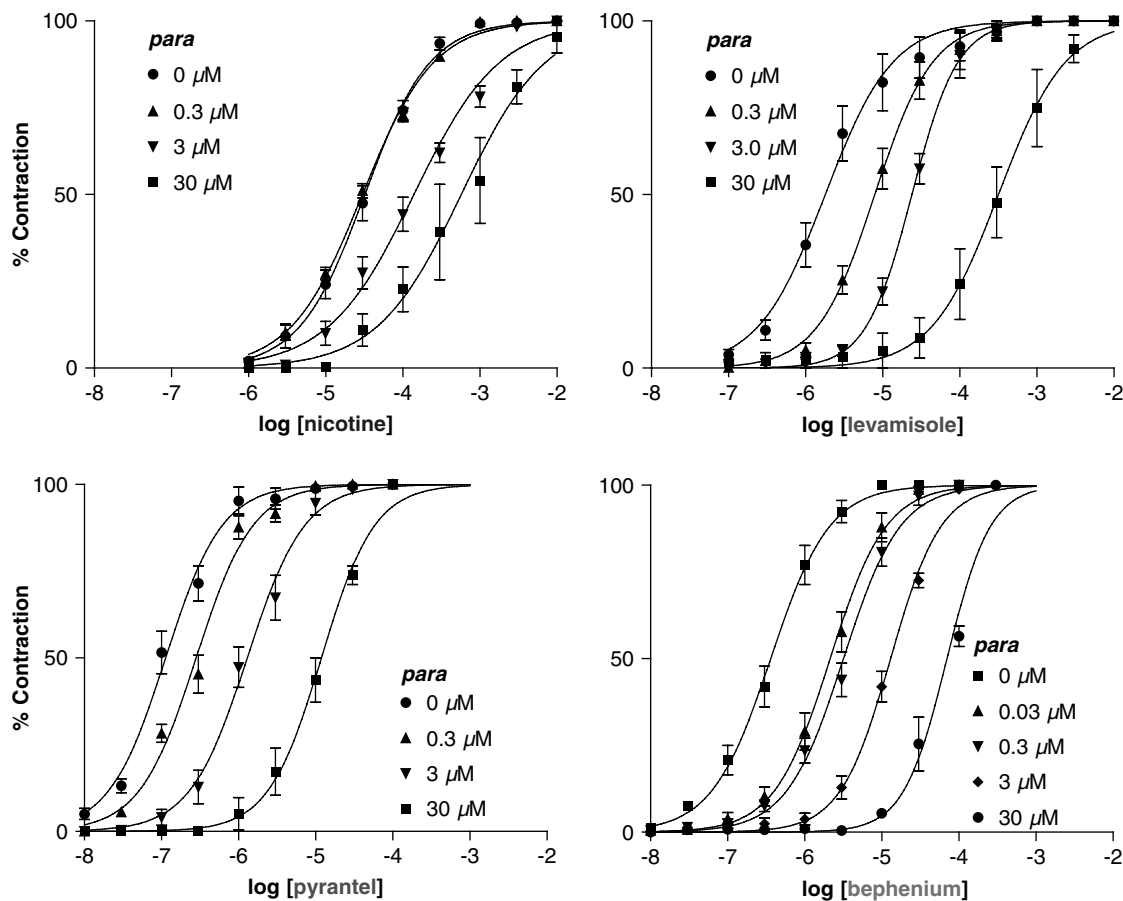


Fig. 4. Paraherquamide-antagonism of cumulative-contraction response anthelmintic concentration plots for nicotine, levamisole, pyrantel and bephenium. The concentration-response plots were produced in the absence of paraherquamide and in the presence of increasing concentrations of paraherquamide. Note that paraherquamide acts as a competitive antagonist but that nicotine is not well antagonised, while the other anthelmintics are. The difference in the dose-ratios produced by the antagonist shows that the receptors activated by nicotine are not the same as those activated by levamisole and pyrantel.

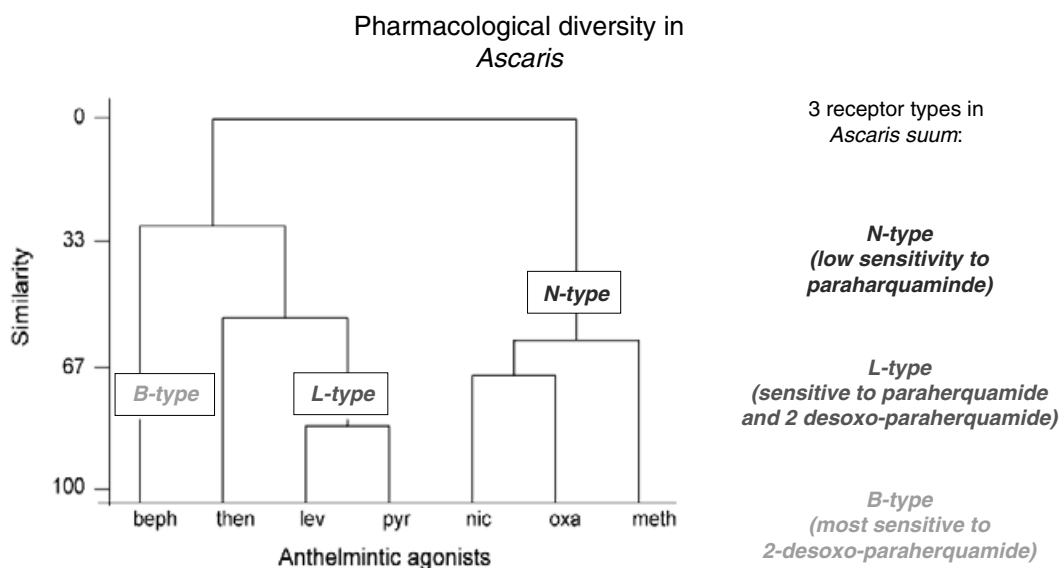


Fig. 5. Dendrogram showing the similarity for different cholinergic anthelmintics when antagonised by paraherquamide, 2-desoxyparaherquamide and methyllycaconitine. pA 2 values for 3 antagonists are compared using cluster analysis and this separates 3 receptor subtypes in *Ascaris* muscle. The three types of receptor are: N-, L- and B-types. The N-type is preferentially activated by oxantel, methyridine and nicotine. The L-type is preferentially activated by levamisole and pyrantel. The B-type is preferentially activated by bephenium.

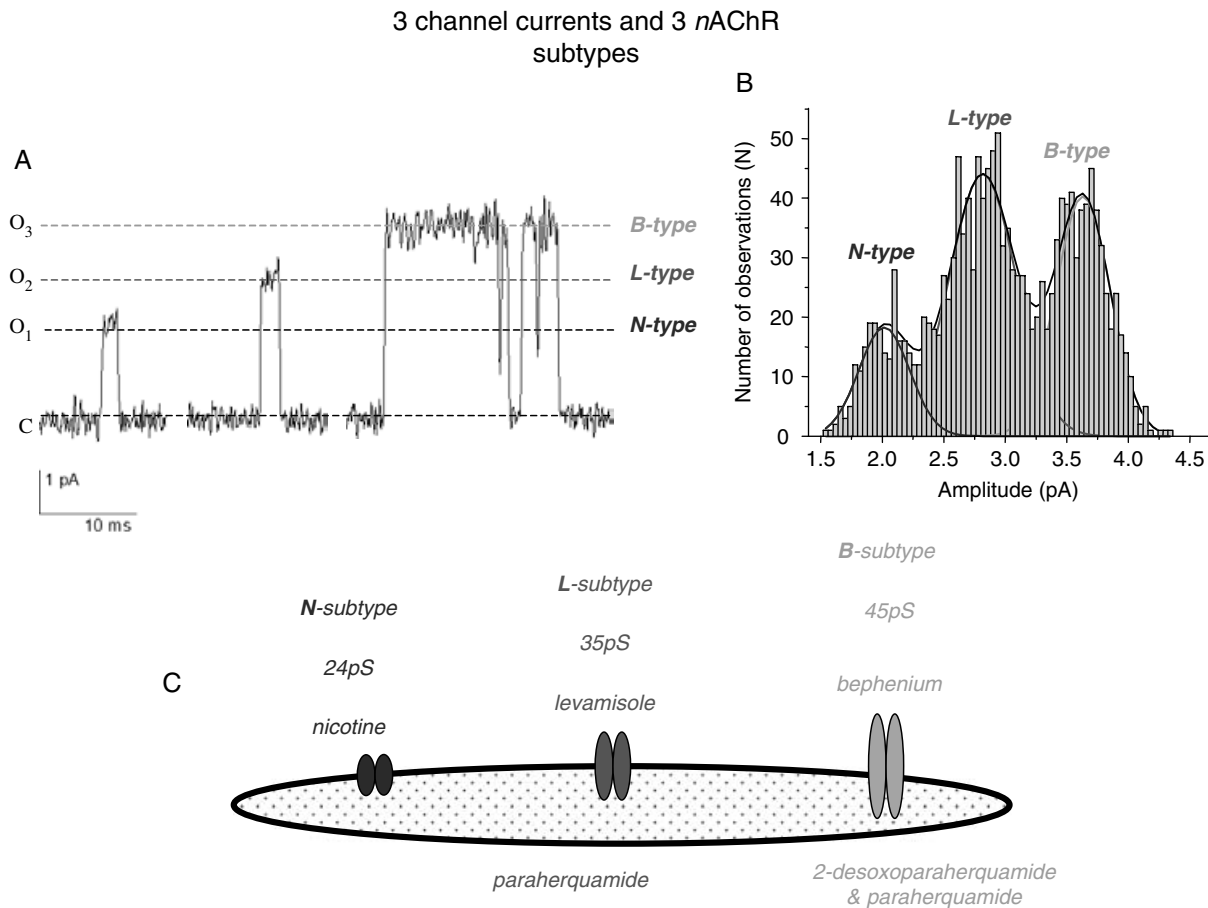


Fig. 6. Single-channel properties of *N*-, *L*- and *B*-types of channel. A: The conductance of the *N*-subtype channel is ~25 pS, the *L*-type is ~35 pS, and the *B*-type is ~45 pS. Representative openings from the same patch at +75 mV. B: Current amplitude histogram of the same patch recording with the 3 separate *N*-, *L*- and *B*-subtypes. C: Summary diagram of the channel properties and agonist and antagonist selectivities of the *N*-, *L*- and *B*-subtypes.

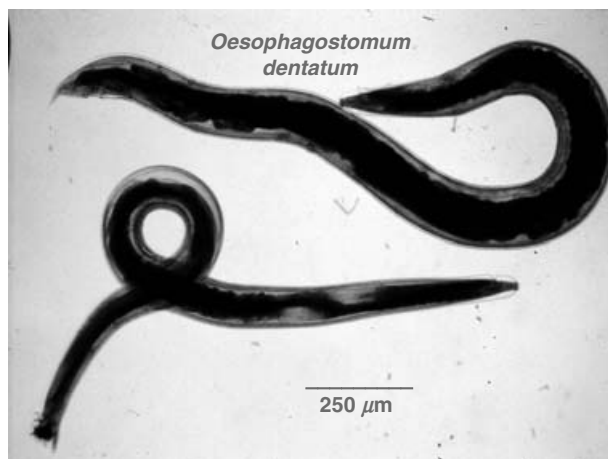


Fig. 7. Unstained photograph of an adult male and female *Oesophagostomum dentatum*.

MUSCLE CONTRACTION RESPONSES TO CHOLINERGIC AGENTS CAN BE MODIFIED

Larval migration assays, muscle contraction assays and electrophysiological experiments have revealed considerable variation in responses in individual animals, even when using the same preparation and the same species of parasite. This variation was

initially frustrating until we developed the view that the variation could be physiologically important. If it was better understood, it might be possible to exploit it to increase the size of responses to cholinergic anthelmintics. Neuropeptides are, at least in part, responsible for this variation. Fig. 10 illustrates effects of the neuropeptide AF2 (KHEYLRF-NH<sub>2</sub>) on *Ascaris* muscle electrophysiological responses and muscle-contraction responses to ACh (Trailovic *et al.* 2005). Brief exposure to AF2 produces long-lasting potentiation of ACh responses, which are likely to extend to all cholinergic anthelmintics. Thus, other receptors, for example receptors for the neuropeptides, may be responsible for and influence the sensitivity and resistance to cholinergic anthelmintic agents. Changes in expression or properties of an AF2 receptor might be associated with some types of levamisole resistance.

THE *C. ELEGANS* MODEL OF THE LEVAMISOLE RECEPTOR, E153 AND Q57

Understanding of the structure and function of nematode AChRs has been advanced by the study of *C. elegans*, the model soil nematode that has



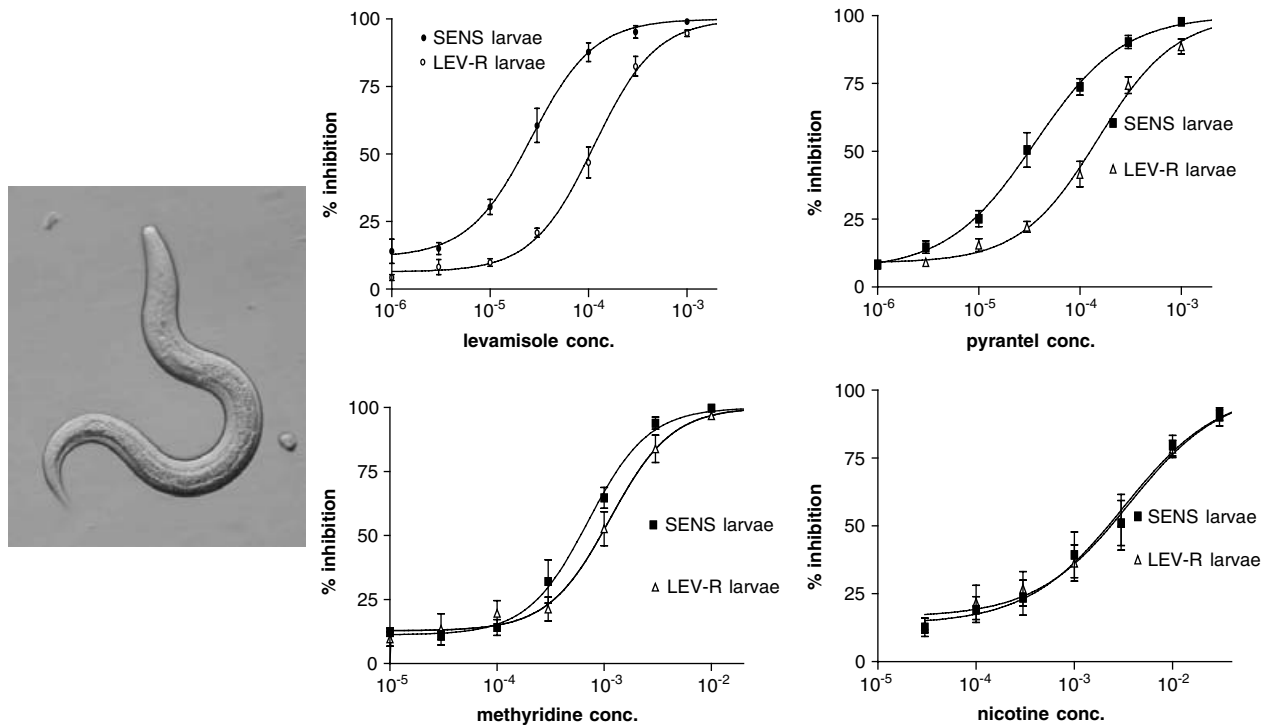


Fig. 8. Photograph *O. dentatum* larvae used in migration assays. Plots: Percent inhibition larval migration assays by levamisole, pyrantel, methyridine and nicotine for levamisole sensitive (SENS) and levamisole resistant (LEV-R) L3 of *O. dentatum*. There is a significant change in the inhibition curves of the two strains for levamisole and pyrantel, but not nicotine and methyridine.

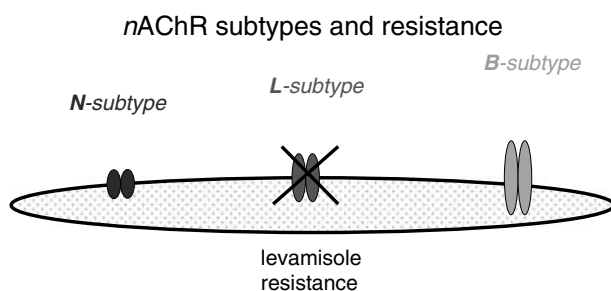


Fig. 9. Summary diagram showing the loss of the L-subtype of receptors associated with levamisole resistance in *Oesophagostomum dentatum*.

undergone detailed genetic and genomic analysis. Three genes encoding nAChR subunits in *C. elegans*, associated with strong levamisole resistance, were recognized initially (Fleming *et al.* 1997): *unc-38*, *unc-29* and *lev-1*. *Unc-38* encodes  $\alpha$ -like subunits, while *unc-29* and *lev-1* encode non- $\alpha$  subunits. The levamisole-responsive UNC-38 subunit homologues in the parasitic nematodes *A. suum* (ASAR-1: Access no. AJ011382), *Trichostrongylus colubriformis* (Wiley *et al.* 1996) and *Haemonchus contortus* (Hoekstra *et al.* 1997) are about 90% identical to *C. elegans*, implying that information derived from the model nematode will be a useful guide for parasitic nematodes. We caution that there are expected to be significant differences between *C. elegans* and other parasitic

nematodes because of the enormous evolutionary distance between some of the species (Blaxter *et al.* 1998). With the completion of the *C. elegans* genome, the size of the nematode nAChR gene family has expanded significantly. Analysis by a consortium found that more than 50 predicted open reading frames had substantial similarity to nAChR subunits of other organisms. Transcription of 17 of the predicted nAChR subunit genes has been confirmed and the genes have been designated using *acr* (acetylcholine receptor) numbers. There are 27 AChR subunit genes (Jones and Sattelle, 2004), the largest number of AChR subunit genes in a single species. Homology has allowed these 27 subunits to be divided into five groups named: DEG-3-like; ACR-16-like; ACR-8-like; UNC-38-like; and UNC-29-like. The UNC-29-like subunits are non- $\alpha$  subunits, while the others are mostly  $\alpha$  subunits. The possible combinations of  $\alpha$  and non- $\alpha$  subunits remains to be determined, but the theoretical possibilities are enormous.

For a variety of developmental and physiological reasons, expression of particular subunits and combinations is restricted to particular nerve cells or muscle cells. UNC-38, UNC-29, UNC-63, LEV-1, ACR-8, ACR 16 and ACR-13 (=LEV-8) GFP-tagged protein subunits have been found in somatic muscle cells of *C. elegans* (Fleming *et al.* 1997; Richmond and Jorgensen, 1999; Culetto *et al.* 2004; Gottschalk *et al.* 2005; Towers *et al.* 2005). Multiple

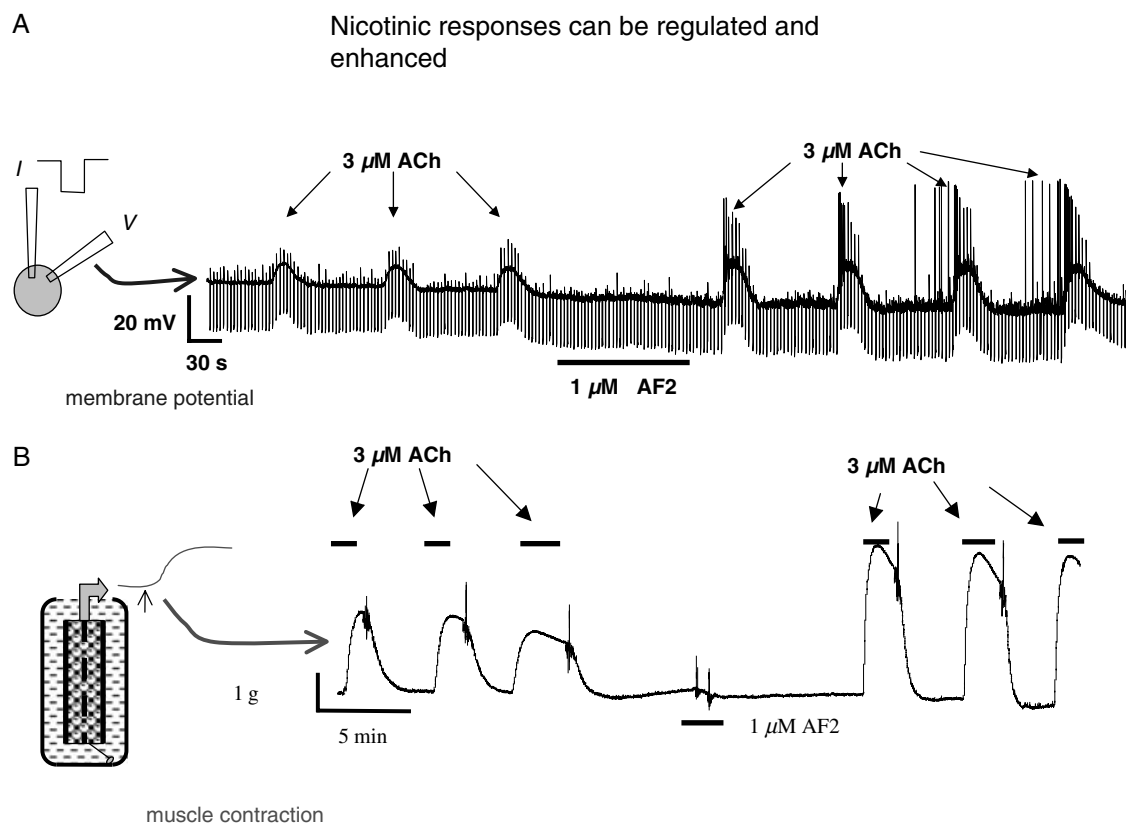


Fig. 10. AF2 potentiation of responses to electrophysiological responses acetylcholine of *Ascaris* muscle. A: Current-clamp recording of membrane potential and conductance during short pulses of acetylcholine before and after a 2-minute application of AF2. The post-AF2 responses are increased in size. B: *Ascaris* muscle strip contraction responses to acetylcholine before and after AF2 application.

pharmacological AChR subtypes present on somatic muscle of *C. elegans* are revealed by null mutation experiments and contraction assays. Null mutants of *unc-38*, *unc-29*, *unc-63* produce very high level resistance to levamisole; a null mutant of *acr-13* (*lev-8*) reduces sensitivity to levamisole; a null mutant of *acr-16* confers nicotine resistance with little effect on sensitivity to levamisole; and electrophysiological responses to levamisole but not nicotine are abolished in *unc-38*, *unc-29*, *unc-63* null mutants and reduced (but not abolished) in *lev-1* and *acr-13* (*lev-8*) null mutants (Richmond and Jorgensen, 1999; Culetto *et al.* 2004; Towers *et al.* 2005); responses to ACh but not levamisole were abolished by *acr-16* null mutants, and a phenotype was not observed with *acr-8* null mutants (Touroutine *et al.* 2005).

As a minimal model, there are at least two types of nAChR present on the somatic muscle cell of *C. elegans*: (1) the *N*-type receptor composed of 5 ACR-16 subunits and most sensitive to nicotine; and (2) the *L*-type, most sensitive to levamisole (Richmond and Jorgensen, 1999) and composed of UNC-38, UNC-29, UNC-63, LEV-1 and LEV-8. We have pointed earlier to the presence of *N*-, *L*- and *B*-subtypes of nAChR in *A. suum*; at this stage, we do not know if there are more than two subtypes of

muscle nAChR in *C. elegans* or if their single-channel properties match those of the parasitic nematodes. We anticipate that there will be similarities as well as significant differences between the *C. elegans* receptors and those of parasitic nematodes.

Fig. 11 shows a putative structure for the *L*-type receptor that contains UNC-38, UNC-63 and LEV-8 (ACR-13)  $\alpha$ -subunits and UNC-29 and LEV-1 non- $\alpha$  subunits. Information on the ordering of the subunits is unknown. The putative structure is based on the comprehensively studied *Torpedo californica* nAChR (Changeux *et al.* 1990). It is assumed (but not tested) that nematode nAChRs are very similar in structure. The *Torpedo* nAChR is composed of two  $\alpha$ - and three non- $\alpha$  subunits (one  $\beta$ -, one  $\gamma$ - and one  $\delta$ -subunit) arranged in a 5-membered ring around the central pore that forms the transmembrane ion-channel. The pore of the ion-channel is lined by the second transmembrane region (TM2) of each of the 5 subunits. The binding sites of the agonists are formed by 6 loops of amino acids: 3 loops (A, B and C) from the  $\alpha$ -subunit and 3 loops (D, E and F) from the adjacent non- $\alpha$  subunit (Corringer *et al.* 1998; Brejc *et al.* 2001). The C loop contains two adjacent cysteines that define a subunit as an  $\alpha$ -subunit. Since the putative structure of the *C. elegans* levamisole receptor has 3  $\alpha$  subunits, there are 3 or more

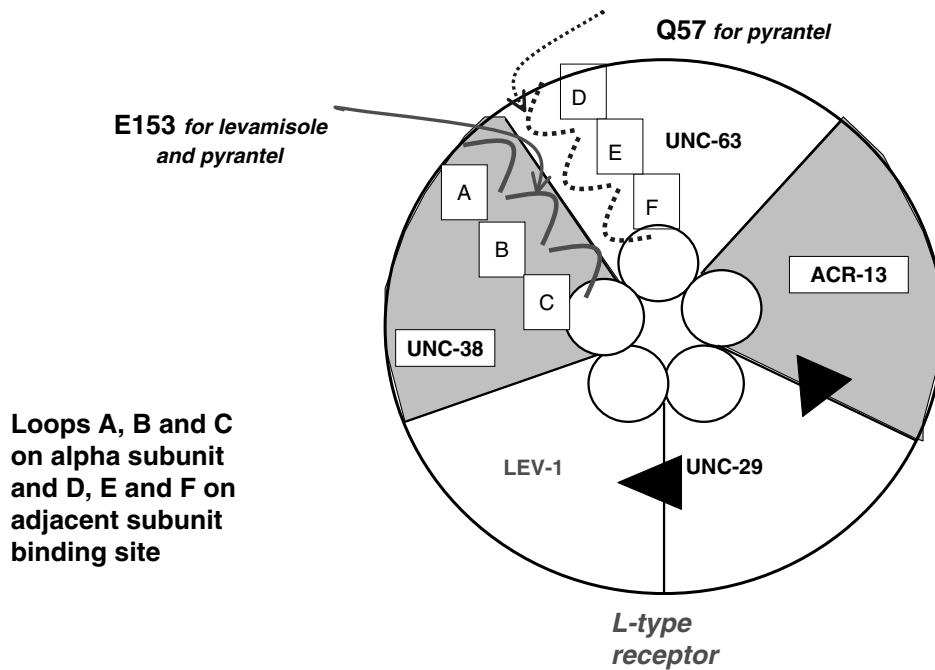


Fig. 11. Agonist binding sites of the putative levamisole receptor from *C. elegans*. The subunits are composed of 5 subunits UNC-38, UNC-63, ACR-13 (LEV-8), UNC-29, and LEV-1. ► represents the agonist binding sites formed at the junction between an alpha subunit and an adjacent unit. The agonist binding site between UNC-38 and UNC-63 shows the A, B, C loops of UNC-38 and D, E and F loops of UNC-63. Two amino acids, E153 (Rayes *et al.* 2004) and Q57 (Brown *et al.* 2006) are predicted to be required for selectivity of levamisole (E153) and pyrantel (E153 and Q57).

non-identical ligand binding sites on each receptor channel: (1) between UNC-38 and an adjacent subunit; (2) between ACR-13 and an adjacent subunit; and (3) between UNC-63 and an adjacent subunit. These non-equivalent sites may bind levamisole with differing affinities. A significant role for UNC-38 is likely, with E153 on loop B being necessary for a selective effect of levamisole (Rayes *et al.* 2004); point mutation of glutamate at position 153 reduces the potency of levamisole. Many point mutations in UNC-38, including mutations in the pore-forming M2 region, may alter the opening of the receptor channel in the presence of levamisole. If equivalent changes were to occur in parasitic nematodes, there would be a loss of sensitivity to levamisole and perhaps pyrantel. This loss in sensitivity to levamisole may be associated with a loss of fitness if there is a concurrent loss of the response to the normal neurotransmitter, ACh.

Pyrantel has additional structural requirements (Bartos *et al.* 2006). Pyrantel requires the presence of a glutamine in position 57 on the complimentary subunit binding site (Fig. 11, loop D, UNC 63): pyrantel requires E153 and Q53, but levamisole requires only E153. The different amino-acid requirements for levamisole and pyrantel could explain why *O. dentatum* and *O. quadrispinulatum* can acquire resistance to pyrantel but not to Levamisole (Bjorn *et al.* 1990). The lowest sensitivity to levamisole and pyrantel would occur in a parasite nAChR that

contained glycine at positions 153 and 57, similar to the mammalian muscle nAChR. Bartos *et al.* (2006) have suggested that Glu153 may be provided by UNC-38 and Gln57 provided by UNC-63; this combination of subunits would thus lead to a receptor very sensitive to pyrantel.

#### OTHER *C. ELEGANS* GENES ARE INVOLVED IN RESISTANCE TO LEVAMISOLE

Forward and reverse mutation experiments in *C. elegans* starting with Lewis *et al.* (1980) have identified 16 genes (Table 1, Fig. 12) that are associated with resistance to levamisole (Lewis *et al.* 1980, 1987; Benian, L'Hernault and Morris, 1993; Kagawa, Takuwa and Sakube, 1997; Fleming *et al.* 1997; Culetto *et al.* 2004; Gally *et al.* 2004; Jones and Sattelle, 2004; Touroutine *et al.* 2005; Towers *et al.* 2005; Jones, Buckingham and Sattelle, 2005; Wormbase, www.wormbase.org). These genes encode nAChR subunits (UNC-38, UNC-29, UNC63, LEV-1 and LEV-8) and interacting proteins, kinases and phosphatases (NRA-1, SOC-1, TAX-6 and TPA-1), the ryanodine receptor (UNC-68), contractile elements (UNC-22 and LEV-11) and levamisole receptor subunit processing proteins (UNC-50, RIC-3 and UNC-74). It seems very likely that other genes will be identified that encode proteins that can be associated with resistance to levamisole in *C. elegans*.



Table 1. *C. elegans* genes associated with levamisole resistance.

Gene name: www.wormbase.org	Function	Wormbase Gene ID and reference source
<i>unc-38</i>	Encodes $\alpha$ -nicotinic channel subunit	WBGene00006774
<i>unc-29</i>	Encodes a non- $\alpha$ nicotinic channel subunit	WBGene00006765
<i>unc-63</i>	$\alpha$ -nicotinic channel subunit	WBGene00006797
<i>lev-1</i>	Non- $\alpha$ nicotinic channel subunit	WBGene00002974
<i>lev-8</i>	$\alpha$ -nicotinic channel subunit	WBGene00002975
<i>lev-10</i>	Cub protein localizing levamisole receptors to neuromuscular synapse	WBGene00002977
<i>nra-1</i> or <i>t28f3.1</i>	Encodes a copine – a novel nicotinic receptor associated protein	WBGene00012128: Gottschalk <i>et al.</i> 2005, EMBO J. 24, 2566–2578
<i>soc-1</i>	Encodes a receptor tyrosine kinase signalling protein	WBGene00004928
<i>tax-6</i>	Encodes an atypical serine-threonine phosphatase 2B orthologue	WBGene00006527
<i>tpa-1</i>	Encodes an atypical serine-threonine protein kinase C1	WBGene00006599
<i>unc-68</i>	Encodes a ryanodine receptor orthologue in body muscle	WBGene00006801
<i>unc-22</i>	Encodes twitchin, a giant intracellular protein with kinase activity and required for muscle contraction and relaxation cycle	WBGene00006759
<i>lev-11</i>	Encodes tropomyosin	WBGene00002978
<i>unc-50</i>	Encodes an integral membrane protein required for nicotinic receptor trafficking: mutant reduces receptor numbers.	WBGene00006785
<i>ric-3</i>	Encodes a novel charged protein that is required for at least 4 types of nicotinic receptor	WBGene00004363
<i>unc-74</i>	Encodes a protein disulphide isomerase necessary for levamisole receptor function	WBGene00006806

Wormbase 1D and function of proteins encoded by *C. elegans* genes associated with levamisole resistance.

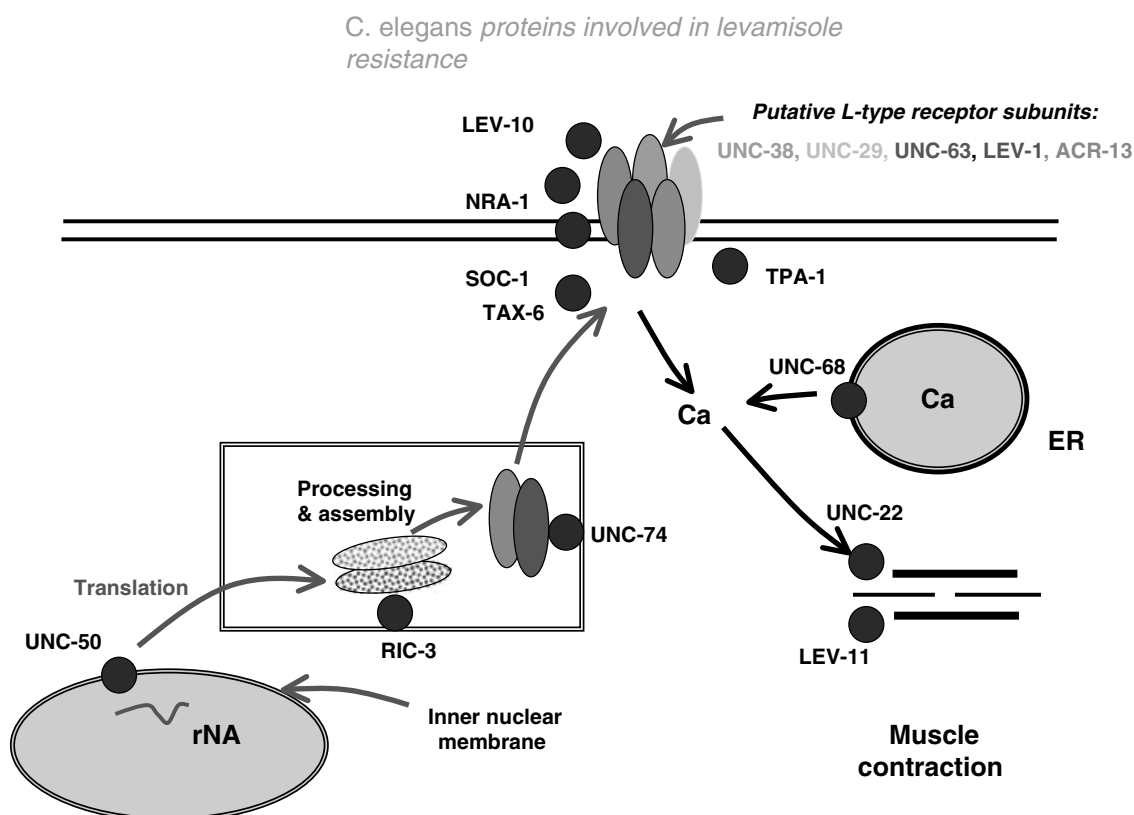


Fig. 12. Summary diagram of the proteins and their functions known to be associated with levamisole resistance in *C. elegans*. The proteins are involved in the signal-transduction process and expression of levamisole receptors on the muscle membrane surface.

LIMITATIONS OF THE *C. ELEGANS* MODEL FOR PARASITIC NEMATODES

The *C. elegans* model is expected to be a useful, but not complete model for a range of nematode parasites, just as mice are for humans. There are at least two limits to the value of the *C. elegans* model for parasitic nematodes. (1) Parasitic nematodes have to be able to complete their life-cycle and maintain adequate movement to reproduce and survive in hostile environments like the intestine, and must elude or defend against attack by the host immune system. The repertoire of genetic mutations that may be available to parasitic nematodes for resistance to levamisole and pyrantel is expected to be more limited if the parasite has to retain sufficient fitness to survive in these hostile conditions. Paralysed *C. elegans* can survive on agar plates seeded with *E. coli* for food, but parasitic nematodes with compromised motility cannot complete their life-cycles. (2) There is also a difference in the concentration of levamisole used experimentally for *C. elegans* resistance experiments and therapeutically for parasitic nematodes. Very high concentrations of levamisole,  $>10^{-4}$  M, will produce toxic effects in the mammalian host animal and so cannot be used therapeutically, but concentrations as high as 1 mM are used on agar plates in *C. elegans* levamisole resistance experiments. Thus, there may be a 10-fold difference in the level of levamisole resistance when considering observations from *C. elegans* and from parasitic nematodes.

Thus, genes that can produce lower levels of resistance in parasitic nematodes but do not compromise the physiology and movement of the parasitic nematode may be significant clinically but have not yet been revealed by *C. elegans* experiments. These two different perspectives should be recognized.

Sequencing of parasite genomes has progressed enormously since the lead that was set by *C. elegans*. The *Brugia malayi* genome is complete (Ghedini *et al.* 2004) and additional nematode projects are underway at the Genome Sequencing Center, St Louis, USA; the Sanger Institute Hixton, UK; Edinburgh University, Scotland, UK and other sites (see [www.nematode.net](http://www.nematode.net)). As of this writing, some genomic information is available for 43 species of nematodes, including animal, plant and human parasites, and 2,353,560, ESTs from 28 animal parasites. Among these nematodes, 30–70% of the ESTs are nematode specific (Mitreva *et al.* 2005), suggesting the possibility that these specific genes could be useful drug targets. Note, however, that levamisole and pyrantel act on drug receptors that are not unique to the nematodes but are present broadly in invertebrates and vertebrates. The small but significant differences in the amino acid sequences of the anthelmintic targets give rise to the unique pharmacology of the drug targets in parasites that have been exploited (benzimidazole anthelmintics have a selective effect

on nematode  $\beta$ -tubulins and levamisole has a selective effect on the nAChRs of nematodes). It is also important to point out that 23% of the ESTs observed so far are unique to the nematode species from which they are derived (Mitreva *et al.* 2005). This observation, along with the two limitations (life-cycle requirement for parasitic nematodes and limiting concentrations of levamisole), suggests that some of the genes associated with resistance in parasitic nematodes will not be the same as those genes associated with levamisole resistance in *C. elegans* (Fig. 12, Table 1).

Despite these comments, we remain positive about the value of the *C. elegans* model for understanding levamisole and pyrantel resistance in parasitic nematodes, particularly as each of the nAChR receptor subunits for the nematode parasite sequences have close homology to one of the *C. elegans* nAChR subunit families (Brown *et al.* 2006). We should expect to find similar principles but different details. The identification of SNPs associated with levamisole resistance is expected to involve a number of genes and a number of alleles of those genes, some of which will be species-specific.

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