

Part One

Targets

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Ligand-Gated Ion Channels as Targets for Anthelmintic Drugs: Past, Current, and Future Perspectives

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Abstract

Ligand-gated ion channels (LGIC) are targets for anthelmintic drugs used in human health and veterinary applications. Given the diverse physiological roles of LGICs in neuromuscular function, the nervous system, and elsewhere, it is not surprising that random chemical screening programs often identify drug candidates targeting this superfamily of transmembrane proteins. Such leads provide the basis for further chemical optimization, resulting in important commercial products. Currently, members of three LGIC families are known to be targeted by anthelmintics. These include the nicotinic acetylcholine receptors gating cation channels, glutamate-gated chloride channels, and γ -aminobutyric acid-gated chloride channels. The recent impact of genomics on model invertebrates and parasitic species has been far-reaching, leading to the description of new helminth LGIC families. Among the current challenges for anthelmintic drug discovery are the assessment of newly discovered LGICs as viable targets (validation) and circumventing resistance when exploring further the well-established targets. Recombinant expression of helminth LGICs is not always straightforward. However, new developments in the understanding of LGIC chaperones and automated screening technologies may hold promise for target validation and chemical library screening on whole organisms or *ex vivo* preparations. Here, we describe LGIC targets for the current anthelmintics of commercial importance and discuss the potential impact of that knowledge on screening for new compounds. In addition, we discuss some new technologies for anthelmintic drug hunting, aimed at the discovery of novel treatments to control veterinary parasites and some neglected human diseases.

Introduction

Anthelmintic drugs are central to combating many human and veterinary disorders. One in four of the world's population is infected with a parasitic roundworm or nematode, with infestation being particularly severe in tropical and subtropical regions. The consequent debilitating effects on the workforce and the compounding

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risk of other pathogenic infections represents a considerable social and economic burden. If we add to that a very high level of roundworm infestation among the world's farmed animals, and the devastating impact of trematode parasites in man and animals, then the need for adequate helminth control is transparent [1, 2].

The veterinary economic burden is reflected in the scale of the global animal health drug market (approximately US\$11 billion/annum) [3]. The human health antiparasitic drug market is around US\$0.5 billion/annum. However, it costs around US\$40 million to develop a new drug that controls livestock nematodes, whereas it can cost US\$800 million for a new drug for human use. Understandably, the cost barrier has limited progress, but the size of the global markets for antiparasitic drugs and chemicals make their pursuit of commercial interest as well as an important human and animal health priority.

Exciting new developments in research on vaccines targeting helminth parasites are underway and these, undoubtedly, will make important contributions in the future. However, at present, chemical approaches to helminth control predominate. For example, the world's three top-selling veterinary antiparasitic drugs (imidacloprid, fipronil, and ivermectin) and several others such as selamectin, levamisole, pyrantel, morantel, tribendimidine, piperazine, and amino-acetonitrile derivatives (AADs) act on Cys-loop ligand-gated ion channels (LGICs). These transmembrane receptor molecules facilitate the fast actions of neurotransmitter chemicals at nerve-nerve synapses and neuromuscular junctions (NMJs) in invertebrates. Often they offer rapid control of the pathogen. Much of our current knowledge of these important drug targets stems from the genetic model organism and free-living nematode, *Caenorhabditis elegans*, which possesses the most extensive known superfamily of Cys-loop LGICs, consisting of 102 subunit-encoding genes [4]. They include cation-permeable channels gated by acetylcholine (ACh) and γ -aminobutyric acid (GABA) as well as anion-selective channels gated by ACh, GABA, glutamate, 5-hydroxytryptamine (5-HT), dopamine, and tyramine [5–7]. Less than half of the genes in the *C. elegans* Cys-loop LGIC superfamily have been functionally characterized.

Unfortunately, many of the anthelmintic drugs in current use are under threat (Table 1.1). Important compounds such as ivermectin, which have given excellent service, are at the end of their patent life. Repeated use of effective chemicals leads to the development of pathogen resistance. Indeed, multidrug resistance against the three major classes of anthelmintics including macrocyclic lactones, which target glutamate-gated chloride channels (GluCl_s), has become a global problem for the treatment of gastrointestinal nematode parasites of farm animals [8–10]. The increasing development costs and poor return from conventional screening approaches are also problematic. Together, these factors bring a sense of urgency to the development of new, effective anthelmintics.

The life of a patent has always been finite, but as the time from discovery to market becomes protracted and the bar is raised for new, safer molecules with improved specifications on toxicity and environmental residues, the task of discovery becomes more difficult. The introduction of generic forms of a drug has the potential to lower the cost of treatment and make it available more widely, although this positive benefit

Table 1.1 Target sites for currently used anthelmintics.

Chemical group	Compound name	Site of action
Avermectins	ivermectin, moxidectin	GluCl channel
Imidazothiazole	levamisole, tetramisol	ACh cation channel
Tetrahydropyrimidines	pyrantel, morantel	ACh cation channel
Tribendimine	tribendimine	ACh cation channel
Benzimidazole	albendazole, triclabendazole	β -tubulin
Piperazine	piperazine	GABA _A channel
AADs	monepantel	ACh cation channel
Oxindole alkaloids	paraherquamide, derquantel	ACh cation channel
Cyclo-octadepsipeptides	emodepside	voltage-gated potassium channel
Praziquantel	praziquantel	voltage-gated calcium channel ^{a)}
Salicylanilide	niclosamide	ATPase activity (?)
Surmamin	germanin	ryanodine receptors
Diethylcarbamazine	hetrazan, carbilazine	arachidonic acid

a) Still under discussion [95].

may be offset by expediting the onset of resistance. Anthelmintic resistance is affected by the treatment frequency and dose, drug efficiency, the burden of adult worms, and the host immune reaction [6]. Monitoring drug resistance is expensive. The escalating challenges of drug development mean that new anthelmintic compounds have been added to the list of available treatments at the slow rate of just one per decade [11]. Thus, there is an urgent need to develop new, effective anthelmintics.

The Cys-loop LGICs form a receptor superfamily, rich in known targets for anthelmintics. Is there more mileage in these proven targets? Can new drugs be developed that act at a different locus on the same receptor without necessarily leading to cross-resistance? Are there new LGIC targets yet to be discovered and mined? Can the wealth of new data emerging from genomics [12], forward (e.g., chemistry-to-gene screens) and reverse (e.g., RNA interference (RNAi)) genetics, and functional studies on invertebrate model organisms such as *C. elegans* be harnessed to good effect in expediting anthelmintic drug discovery? Can parasite genome studies (completed or underway) also have a major impact? What can we learn from those parasite genomes already sequenced, following on from pioneering work on the first parasite genome, that of *Brugia malayi* [13]. Where there is no genome, valuable expressed sequence tag resources are sometimes available as starting points in the search for new LGIC targets. One important advance, which will undoubtedly accelerate anthelmintic drug discovery, is the recently reported crystal structure of the *C. elegans* GluCl complexed with its orthosteric ligand (glutamate), the allosteric ligand and anthelmintic drug (ivermectin), and a noncompetitive antagonist (picrotoxin) [14]. This is the first eukaryotic LGIC for which a crystal structure is available. Knowing where and how important drugs interact with a proven target from the LGIC superfamily is a major advance, and will assist in the targeting of other family members.

Finding and validating new targets among LGICs is crucial, and they need to be cloned and expressed so that their functions can be assayed (Figure 1.1). This has

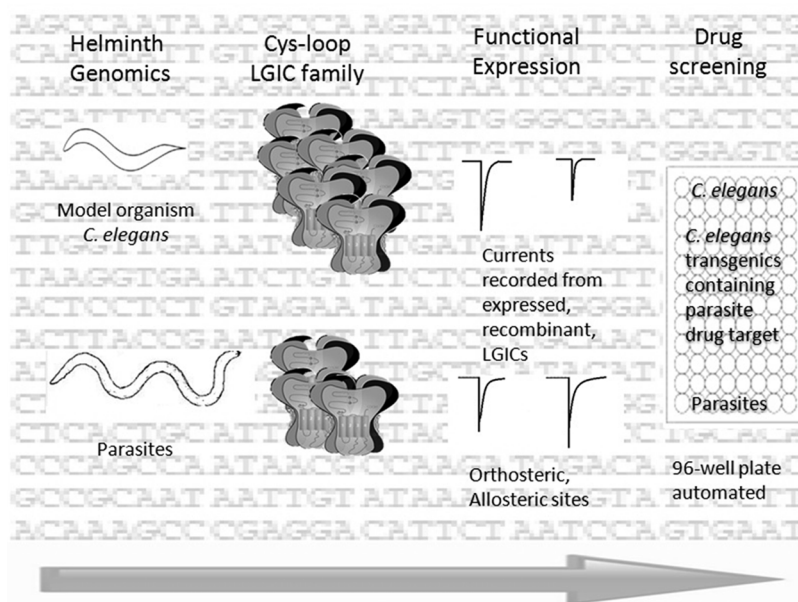


Figure 1.1 Schematic diagram showing some of the current approaches adopted to search for and characterize new anthelmintic drug targets ranging from genomics, via cell-based studies, to automated *in vivo* screening.

proved possible for some model organism LGICs using expression vehicles suited for low/medium-throughput screening; however, developing a platform of cell lines expressing a range of model organism and parasite LGICs is very much a future prospect, and assembling the prerequisite parasite LGIC drug targets remains a challenge. We need to meet this challenge in order to expedite anthelmintic drug discovery using the kinds of high-throughput screening methods deployed in the drug industry for developing new human therapies.

As well as developments in *in vitro* screening methods, developments in *in vivo* testing are urgently needed. Many assays rely on lethal end-points, but it may be that other assays can more sensitively detect important drug modifications of behavior *in vivo* that would be missed in such assays. It may not be necessary to kill worms to dislodge them or by other means prevent or reverse infestation. The difficulty of culturing parasites *ex vivo* remains a problem [15] and new approaches to improve such cultures would be welcomed. It may also be possible to exploit more effectively the recent development of transgenic *C. elegans*, which are easy to culture, in which a *C. elegans* LGIC target is replaced by a parasite ortholog [16]. Novel transgenic lines such as these used in conjunction with newly developed, plate-based, automated behavioral phenotyping may offer important opportunities for improved *in vivo* screening (Figure 1.1; see also Chapter 10 for a discussion on this topic with regard to parasitic helminths). This may even bring *in vivo* screening to bear on parasite species not hitherto considered feasible, where *ex vivo* culture is particularly difficult.

Thus, the emerging crisis of the shrinking pool of anthelmintic drug treatments at a time of growing need for control makes it timely to review the current state of anthelmintics targeting LGICs especially when placed alongside an examination of some emerging new approaches with the potential to expedite the discovery process.

Established LGIC Anthelmintic Drug Targets

Fast chemical neurotransmission at many synapses in humans is mediated by the actions of neurotransmitter molecules such as ACh, GABA, glycine, and 5-HT on specific receptors of the “Cys-loop” LGIC superfamily. These receptors play a crucial role in the function of synaptic signaling in the nervous system and NMJs. The structure of the *Torpedo marmorata* (marbled electric ray) electric organ nicotinic ACh receptor (nicotinic acetylcholine receptor nAChR) has been entirely resolved at 4.6 Å and in part at 4.0 Å [17]. Based on the structure of this prototypical nAChR, all LGICs are thought to be composed of five subunits arranged around a central ion channel. The well-characterized human LGICs are either permeable to cations (e.g., nAChRs and 5-HT₃Rs) or anions (e.g., GABA and glycine receptors) [18]. In nematodes there are, in addition, 5-HT-, glutamate-, ACh-, dopamine- and tyramine-gated anion channels, and GABA-gated cation channels, all of which are part of the Cys-loop LGIC superfamily. Other invertebrates, including *Drosophila melanogaster*, possess histamine-gated anion channels. Ligand-gated anion channels generally lead to rapid inhibitory synaptic responses, whereas ligand-gated cation channels mediate fast excitatory chemical transmission. Each subunit consists of an N-terminal, extracellular domain containing the Cys-loop (which is two disulfide-bond-forming cysteines separated by 13 residues) and six regions (loops A–F) that make up the ligand-binding site, as well as four transmembrane domains. Here, we review recent evidence from the invertebrate genetic model organism *C. elegans* as well as data from parasitic nematodes illustrating the rich diversity of LGICs present. The discovery of new receptor subtypes in helminths, some of which have no counterpart in mammals, may provide opportunities to develop a new generation of more selective, and hence safer, anthelmintics.

GluCl_s – Targets for Ivermectin

Several nematode GluCl subunits have been identified and for some their pharmacology has been characterized by expressing one or more subunits in *Xenopus laevis* oocytes. Cully *et al.* [19] showed that L-glutamate and ivermectin activate GluCl_s generated from heteromers of GluCl α 1 and GluCl β . Since then, other GluCl subunits have been identified in *C. elegans* [20]. These include GluCl α 1–4 and GluCl β . GluCl α 2 (AVR-15) and GluCl β are present in the pharynx of *C. elegans*, whereas GluCl α 3 (AVR-14) is only found in neurons [21]. Both GluCl α 2A and GluCl α 2B function in the inhibition of pharyngeal pumping [21–23], GluCl α 1–4 regulate locomotion [24], and GluCl α 4 functions in olfactory behavior [25].

A chemistry-to-gene screen in *C. elegans* for resistance to ivermectin confirmed the identity of GluCl subunits as drug targets [26]. Mutation of GluCl α 1–3 confers high resistance to ivermectin, whereas mutation of any two channel genes confers low-level or no resistance. Each of the three GluCl genes constitutes a parallel genetic pathway that contributes to ivermectin sensitivity in *C. elegans*. So, mutations affecting only one pathway will not confer resistance.

L-Type nAChRs –Targets for Levamisole, Pyrantel, Morantel, and Tribendimidine

Complete nAChR gene families have been described for both vertebrates and invertebrates. Mammals have 16 genes encoding nAChR subunits, whereas *C. elegans* possesses an extensive and diverse nAChR subunit family [27] with 29 subunits divided into five “core” groups based on sequence homology in addition to four ACh-gated chloride channels (acetylcholine-gated chloride channels AChCls) (Figure 1.2). Each of the core groups have been so named after the first of their number to be discovered. As part of exploring *C. elegans* as a model for genetic analysis, Sydney Brenner mutagenized worms with ethyl methane sulfonate and successfully isolated worm mutants resistant to the paralyzing effects of the anthelmintic, levamisole [28]. This is a classic example of a chemistry-to-gene screen that uncovers molecular components targeted by a drug [29]. Levamisole (a nicotine-like drug) when applied to nematodes triggers enhanced contraction followed by paralysis. Five resistance loci are genes encoding the nAChR subunits making up the levamisole receptor, LEV-1 [30–32], LEV-8 or ACR-13 (acetylcholine receptor) [33], UNC-29 [31], UNC-38 [31], and UNC-63 [32]. Consistent with the paralyzing effects of levamisole, all five subunits are expressed in body wall muscle and electrophysiological studies on neuromuscular preparations from important L-type nAChR mutants confirmed their contribution to levamisole-sensitive nAChRs [32, 33]. Heterologous expression in *X. laevis* oocytes show that UNC-38, UNC-29, UNC-63, LEV-1, and LEV-8 can coassemble in the presence of three other essential proteins, RIC-3 (resistant to inhibitors of cholinesterase), UNC-50, and UNC-74, to form functional recombinant levamisole-sensitive nAChRs [34].

nAChRs (DEG-3 Group) – Targets for AADs

Researchers at Cambria and Novartis identified a novel anthelmintic drug target from the large family of DEG-3-type (degeneration of certain neurons) nAChR subunits in *C. elegans* using a chemistry-to-gene screen. They discovered the AADs, a new chemical class of synthetic anthelmintics effective against various species of nematodes pathogenic in livestock [35]. These compounds have a novel mode of action involving DEG-3-type nAChRs, notably ACR-23. The AADs are well tolerated by host species and exhibit low toxicity to mammals. They also overcome resistance to the currently available anthelmintics. Their excellent host tolerance is such that they are being explored for their potential as possible human anthelmintics.

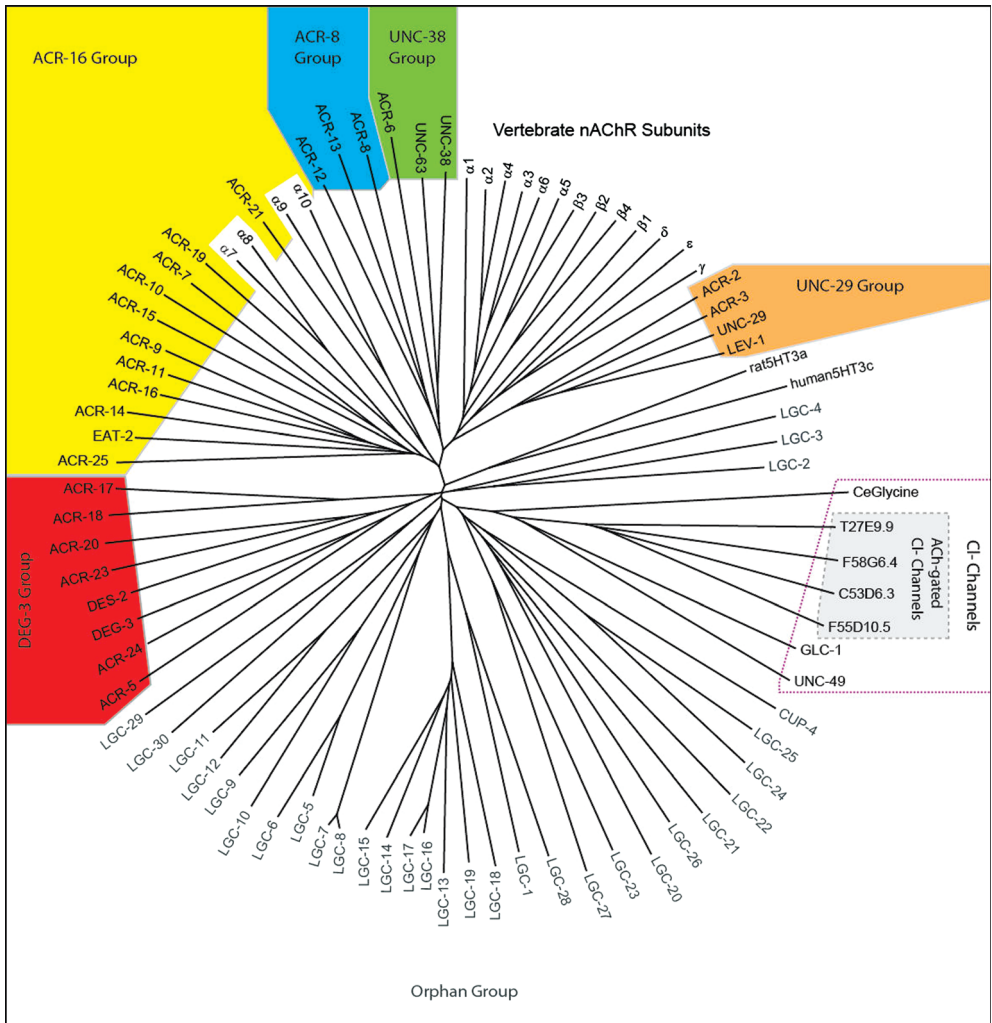


Figure 1.2 Tree showing the nAChR family of *C. elegans*. The worm nAChRs are divided into five core groups, whereas subunits showing substantial homology with other known nAChR

subunits that do not fall within the core groups are designated orphan subunits. (Adapted by permission from Macmillan Publishers Ltd [29] © 2005.)

GABA-Gated Chloride Channels – Targets for Piperazine

GABA-gated chloride channels (γ -aminobutyric acid-gated chloride channel GABA_ACl_s) have been described in *C. elegans*. Products of the *unc-49* gene form a GABA-gated anion channel that mediates body muscle inhibition during locomotion [30]. The *unc-49* gene is alternatively spliced with a single copy of a GABA receptor N-terminus, followed by three tandem copies of GABA receptor C-termini.

At least two of these subunits (UNC-49B and UNC-49C) are colocalized at the NMJ [36]. The UNC-49B subunit is also essential for receptor function and can form both homomeric and heteromeric receptors when co-expressed with UNC-49C in *X. laevis* oocytes [36, 37]. The application of GABA and the GABA receptor agonist muscimol to wild-type and *unc-49* mutant worms confirmed that the *unc-49* gene encodes the GABA receptors that control movement [30]. A separate study indicated that GABA receptors are found in *C. elegans* motor neurons and the ablation of these neurons induced defects in locomotion and defecation [38, 39]. Also, an inhibitory function for GABA receptors in body wall muscles has been shown [30]. There are four other subunits included in this group (Figure 1.3). With regard to other species, members of the UNC-49 group are most closely related to mammalian and insect GABA-gated anion channels [40]. *C. elegans* GAB-1 can contribute to functional GABA receptors when coexpressed in *X. laevis* oocytes with either HG1A or HG1E, which are putative GABA receptor subunits from the parasitic nematode *Haemonchus contortus* [41].

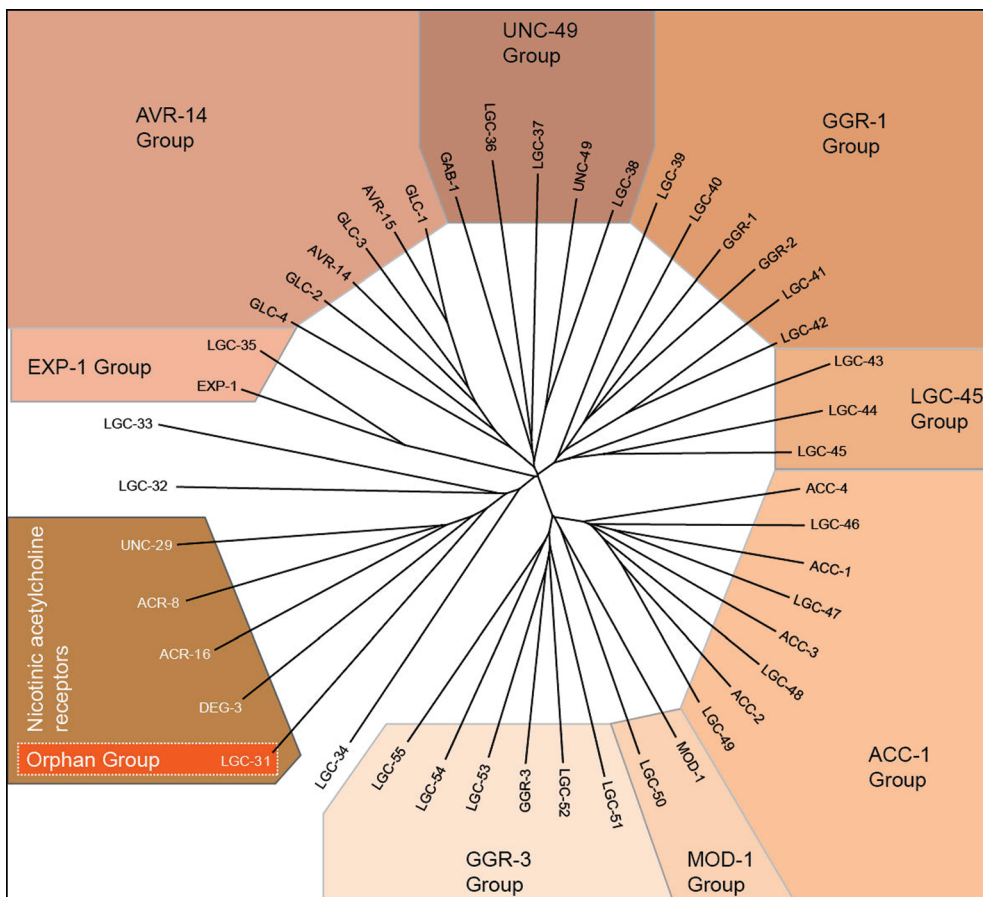


Figure 1.3 Tree showing the Cys-loop LGIC family of *C. elegans*. This figure focuses on Cys-loop LGICs other than nAChRs, although representative members of the nAChRs are shown (Adapted by permission from Springer [4] © 2008.)

Evaluating Possible Anthelmintic Targets for the Future

As our understanding of LGIC subgroups develops, more members come into the frame as candidate drug targets. Assessing the impact of gain- or loss-of-function mutants on nematode viability will be an important indicator of their potential utility as targets.

N-Type nAChRs

The *C. elegans* ACR-16 group, resembling vertebrate $\alpha 7$ -like subunits, contains 11 members [42, 43]. ACR-16 is an essential component of the levamisole-insensitive N-type nAChR [44, 45]. ACR-16 forms functional homomeric channels when expressed in *X. laevis* oocytes [42, 46]. Similar $\alpha 7$ -like subunits have also been found in the trematode *Schistosoma haematobium* [47] and arthropods [48], indicating an ancient lineage for this receptor subtype. They are sensitive to nicotine, not levamisole, and are designated N-type receptors.

Nematode-Specific nAChRs and Orphan nAChR-Like Subunits

The ACR-8 and DEG-3 groups appear to represent nematode-specific receptor subtypes [27]. In addition, there are 26 subunits, denoted orphan subunits (Figure 1.2), which show substantial homology to nAChRs, but do not fall within the five core groups. The orphan subunits CUP-4 and Y58G8A.1 are required for efficient endocytosis of fluids by coelomocytes [49].

GABA Receptor Cation Channels

This group contains EXP-1 (expulsion defective) and LGC-35 (ligand-gated ion channels of the Cys-loop superfamily) (Figure 1.3). EXP-1 is a GABA-gated channel that mediates muscle contraction [50]. When expressed in *Xenopus* oocytes, it forms a GABA receptor that is permeable to cations. EXP-1 lacks the PAR (Pro-Ala-Arg) motif preceding the second transmembrane domain (TM2) which is important for anion selectivity [51]. Instead, it possesses the residues ETE implicated in determining cation selectivity [51, 52]. LGC-35 has not been functionally expressed but also has a glutamate residue preceding TM2 and thus may also be a cation channel.

5-HT Channels

MOD-1 (modulation of locomotion defective) is a 5-HT-gated anion channel [53]. It is expressed in the *C. elegans* nervous system and modulates locomotor behavior [52]. LGC-50 is closely related to MOD-1.

ACh Chloride Channels

This group includes ACC-1 (acetylcholine-gated chloride channel), ACC-2, ACC-3, and ACC-4, which are ACh-gated anion channels [54] (Figure 1.3). ACC-1 and ACC-2

form homomeric channels in *X. laevis* oocytes. ACh and arecoline are agonists, but nicotine is not [54]. The ACh-binding domains of these AChCl subunits diverge substantially from the ACh-binding domain of nAChRs. Unlike ACh-gated anion channels of the snail, *Lymnaea stagnalis*, which appear to have evolved from cation channels through amino acid substitutions in the ion channel pore [55], the ACC-1 group may have arisen from substitutions in the ligand-binding domain of anion channels [54, 55].

GGR-1 Group

Six subunits make up this group, all of which remain to be functionally characterized (Figure 1.3). The neurotransmitters to which these subunits respond cannot easily be envisaged. The subunits most closely resemble glycine α -subunits (25%), whereas LGC-42 shows a 26% identity to insect histamine-gated anion channels [4, 40]. They are likely to be anion-selective as all possess the PAR motif [51]. Recently, LGC-40 was identified as a receptor for 5-HT, and it is also gated by choline and ACh [7].

GGR-3 Group

LGC-53 is a dopamine receptor gating a chloride channel, whereas LGC-55 is a tyramine-gated chloride channel [7, 56]. Both have been functionally expressed in *Xenopus* oocytes [7]. The remaining four subunits making up the GGR-3 group of *C. elegans* have not been characterized.

LGC-45 Group

LGC-43, LGC-44, and LGC-45 (Figure 1.3) remain to be functionally characterized. Like the GGR-1 group, the LGC-45 group members closely resemble human glycine α -subunits and insect histamine-gated anion channels [4]. However, unlike the GGR-1 group, the LGC-45 group subunits are likely to be cation-selective as they lack the PAR motif preceding M2 [51]. LGC-44 and LGC-45 possess a glutamic acid instead of the proline residue in the PAR motif, which is likely to result in a cation-selective channel [52]. LGC-44 also lacks a Cys-loop. The absence of a Cys-loop has also been reported in a bacterial proton-gated ion channel (from *Gloeobacter violaceus*) [57, 58]. Therefore, LGC-44 may represent an ancestral Cys-loop LGIC.

Ungrouped Subunits

The LGC-32, LGC-33, and LGC-34 subunits are highly divergent (Figure 1.3), showing up to 10, 10, and 15% identity, respectively, with other *C. elegans*, human, and *Drosophila melanogaster* Cys-loop LGICs [4]. These subunits have not been functionally characterized. Although these subunits contain some of the features common to Cys-loop LGICs, the Cys-loop is absent [59]. Thus, LGC-34 may also represent an ancestral Cys-loop LGIC.

Cys-Loop LGIC Superfamilies of Other Nematodes

Genome-sequencing projects are enabling the comparison of Cys-loop LGIC superfamilies from different nematode species. For example, *Caenorhabditis briggsae* [48, 60] also has an extensive Cys-loop LGIC gene superfamily, although comparative genomics suggest there appears to be an expansion in *C. elegans* within the nAChR orphan group. For example, there is only one homolog of LGC-28 in *C. briggsae*, whereas LGC-23, LGC-24, and LGC-28 are present in *C. elegans* [61].

The availability of genome sequence information for *B. malayi* and *Trichinella spiralis* (both smaller than the *C. elegans* genome) has facilitated the characterization of their Cys-loop LGICs (30 and 19 subunits, respectively). The Cys-loop LGICs of both parasites are orthologous with those of *C. elegans* with the exception of an nAChR subunit (ACR-26) that, to date, appears specific to *B. malayi*. Williamson *et al.* [62] propose that the difference in the Cys-loop LGIC family sizes may reflect a free-living versus a parasitic lifestyle in that a larger complement of subunits may be required to respond to many environmental cues not encountered by the parasites. Indeed, a study of over 30 nematode genomes (of varying completeness) [63] detected an average of 31 and 57 LGICs in parasitic and nonparasitic species, respectively. Sequencing and annotations are underway for other nematode parasites including *H. contortus*, *Teladorsagia circumcincta*, and the human hookworm, *Necator americanus*. Nomenclature can present problems [64]. Cys-loop LGIC genes have been identified from multiple nematode species, but relatively few have been cloned and expressed [62].

nAChRs in the body wall muscle of *Ascaris suum* have been investigated in detail. Excitatory actions of ACh [65, 66] and anticholinesterases potentiate the actions of ACh [65]. Also, morantel and pyrantel activate nAChRs [66]. Martin *et al.*, using patch-clamp recording from vesicles reconstituted from *Ascaris* muscles, showed the presence of at least two types of ACh-activated channels [67, 68]. Also, they showed that pyrantel is both an agonist and open channel blocker of nicotinic receptors in *A. suum* [69]. The nAChR antagonist, paraherquamide, a novel natural anthelmintic product, was employed to pharmacologically separate populations of *A. suum* muscle N-subtype (nicotine-sensitive) and L-subtype (levamisole-sensitive) nAChRs [70]. Later studies by Martin *et al.* have added a third muscle nAChR subtype, the B-type (bephenium-sensitive) receptor [71]. Williamson *et al.* have found that manipulating concentrations of *Ascaris unc-38* and *unc-29* RNA injected into *Xenopus* oocytes can generate heteromeric receptors that are more sensitive to levamisole or nicotine [72]. As genes encoding the LEV-1 and LEV-8 subunits are not present, this all points to a different composition for muscle nAChRs in *A. suum*. Levamisole-resistance in *Oesophagostomum dentatum* larvae is associated with a loss of L-subtype, but not the N-subtype receptors [73]. As more comparative physiology becomes available it will be of interest to establish whether these receptor subtypes are equivalent in different nematode phyla. For example, are the N-subtypes of *A. suum* and *O. dentatum* composed of homologs of ACR-16? Studies on muscle receptor subtypes of two parasitic species (*O. dentatum* and *A. suum*) confirm the continued utility of *C. elegans* as a model of parasitic nematodes in the search for new drug targets. *C. elegans* and

H. contortus belong to the same phylogenetic group (clade V), and their LGICs are now being extensively studied. There appears to be a large number of *C. elegans* homologs present in *H. contortus*. Neveu *et al.* have identified the L-subtype receptors and successfully functionally expressed these in *X. laevis* oocytes [74, 75]. Other LGICs have been identified, including GABA receptors (Hco-UNC-49B, Hco-UNC-49C) [76], and biogenic amine receptors including Hco-LGC-55 (tyramine) [77] and Hco-GGR-3 (dopamine) [78]. Multiple GluCl α s are present, including the alternatively spliced Hco-GBR/AVR-14 (Hco-GBR-2A and Hco-GBR-2B) [79], HcGluCl α [80, 81], and HcGluCl β [82]. *C. elegans* has shown potential as a suitable system for studying parasitic nematode genes with the successful expression of Hco-AVR-14 in a *C. elegans* mutant line [16]. This method is showing promise as a high-throughput *in vivo* screen for novel anthelmintics. Studies on a *C. elegans* GGR-3 homolog in *H. contortus*, Hco-ggr-3 (84% similarity), showed that this subunit formed a homomeric receptor that responded primarily to dopamine [78]. A homolog of LGC-34 has been described in the parasitic roundworm, *Dirofilaria immitis*, that shows a 52% identity [59].

Genomics

WormBase (www.wormbase.org) is a major publically available dataset, initially created for the storage of *C. elegans* genomic information. It is now the central repository for nematode biology, containing complete genome sequences, gene predictions, and orthology assignments from a range of related nematodes. It relies on a manual curation pipeline ensuring that all data is consistent and of high quality. Genome sequence information and, recently, phylogenomics has been used to predict drug sensitivity in different species [63]. With the significant growth in the amount of sequence data available for nematodes, this has facilitated the prediction of potential drug targets using bioinformatics techniques. Hitherto, all antiparasitic drugs have been discovered by empirical screening in parasites or models such as *C. elegans* [83]. This remains an important and valid approach. Nevertheless, target-based screening has advantages as it generates structure–activity relationships and enables lead optimization at the target itself [84].

Chemistry-to-Gene Screens to Identify New Targets

Chemistry-to-gene screens that involve mutagenesis of *C. elegans* followed by screening for resistance to the chemical under investigation have a proven track record in target identification [29] and are likely to be equally useful in identifying gene targets for new compounds that have been shown to have anthelmintic activity, but for which the site of action is unknown. Double-stranded RNAi [85], a rapid method for individual gene silencing that is now applicable to the entire genome [86, 87], will also complement chemistry-to-gene studies in helping validate future candidate drug targets. The utility of chemistry-to-gene approaches is

illustrated by the discovery that, as well as identifying genes encoding L-type nAChR subunits, the levamisole-resistance loci include genes encoding important proteins acting upstream or downstream of nAChRs (reviewed in [29]). These include: LEV-10, which is required for nAChR aggregation at the NMJ [88]; UNC-50, which is involved in the processing and assembly of receptors [89], for which the mammalian homolog was identified and found to function similarly [90]; LEV-11 and UNC-22, which regulate muscle contraction [90, 91]; and UNC-68 (a ryanodine receptor), which is involved in calcium signaling [92]. Interestingly, ryanodine itself has been used as a pesticide [93] and the receptor has recently been identified as the target of anthranilic diamides – an important new class of chemicals targeting invertebrate pests [94]. Thus, this study also identified a pesticide target that is functionally related to the primary target of levamisole (the L-type nAChR subunits). This suggests that molecular components functionally linked to Cys-loop LGICs and highlighted in chemistry-to-gene screens could also yield new animal health drug targets.

First Crystal Structure of a Cys-Loop LGIC Complexed with a Commercial Animal Health Drug

The first three-dimensional structure of a eukaryotic Cys-loop LGIC was reported in 2011 by Hibbs and Gouaux [14]. The *C. elegans* homomer-forming, anion-selective, GluCl α subunit has been described at 3.3 Å resolution. The structure of the GluCl–Fab complex bound to ivermectin yields important insights into the interactions of a major animal health drug with its allosteric binding site. Ivermectin, which stabilizes the open pore conformation, binds in the transmembrane region, forming a wedge between the M1 and M3 helices, and also interacts with the M2–M3 loop. Other structures were obtained with the neurotransmitter L-glutamate docked in the orthosteric binding site. L-Glutamate binds to the agonist site at the interface between subunits. Arginine residues, in combination with neighboring cationic amino acids, provide the binding pocket with a strongly positive electrostatic potential. The α -amino nitrogen of L-glutamate is stabilized through a 3.8-Å cation– π interaction with Tyr200 on loop C, a hydrogen bond with the backbone carbonyl oxygen of Ser150, and a close interaction with the backbone carbonyl oxygen of Tyr151. Structures obtained with picrotoxin bound show that this open channel blocker binds to the cytoplasmic end of the pore. The tricyclic rings are directed extracellularly and near the 2' Thr, whereas the isoprenyl tail points towards the cytoplasm and is close to the –2' Pro residues. Thus, we can now visualize the binding of important orthosteric, allosteric, and channel blocking ligands and, not least, the important animal health drug, ivermectin, to nematode Cys-loop LGICs.

Conclusions and Future Lines of Research

The adoption of a multigene family in the search for drug targets may be important. The evolution of drug resistance may be slower if several target genes have to be

mutated simultaneously to confer resistance. This may account for the relatively slow onset of resistance to ivermectin. Research on nematode Cys-loop LGIC super-families, accelerated by genomics, is adding to our understanding of their rich diversity. This genomics-led expansion of our knowledge of the physiological spectrum of LGICs also exposes new candidate drug targets. The powerful genetic toolkit available in model organisms such as *C. elegans* is likely to remain useful in helping validate these candidate targets, even though the future holds the exciting prospect of access to many new parasite genomes.

From this brief overview it is evident that both forward and reverse genetic studies on *C. elegans* have contributed significantly to both identifying novel genes involved in nematode synaptic transmission and to determining the receptor subunits that form the molecular targets of antiparasitic drugs. Such approaches will continue to be to the fore when compounds with anthelmintic activity, but for which the target is unknown, are under development. Chemistry-to-gene screens, of the type that have focused attention on a small subset of subunits (from a very large family) as the targets for levamisole, have also proved useful in identifying functionally linked genes that include candidate targets for novel drugs with a quite different mechanism of action. In future, we can envisage generating improved selectivity for parasite over host by exploring further as drug targets those Cys-loop LGICs that are either parasite-specific or where host and parasite orthologs differ considerably in sequence. Studies on nematode parasites have shown that several types of chemistry with antiparasitic activity can act successfully on a single class of receptors such as nAChRs. Studies on laboratory-resistant strains have proved useful in understanding drug targets and synaptic function, and future studies on field-resistant strains will enhance our understanding of resistance mechanisms, which can imperil the longevity of a commercial antiparasitic product.

References

- 1 Garba, A., Toure, S., Dembele, R., Boisier, P., Tohon, Z., Bosque-Oliva, E., Koukounari, A., and Fenwick, A. (2009) Present and future Schistosomiasis control activities with support from the Schistosomiasis control initiative in west Africa. *Parasitology*, **136**, 1731–1737.
- 2 Kaplan, R.M. (2004) Drug resistance in nematodes of veterinary importance: a status report. *Trends Parasitol.*, **20**, 477–481.
- 3 Evans, T. and Chapple, N. (2002) The animal health market. *Nat. Rev. Drug Discov.*, **1**, 937–938.
- 4 Jones, A.K. and Sattelle, D.B. (2008) The cys-loop ligand-gated ion channel gene superfamily of the nematode, *Caenorhabditis elegans*. *Invert. Neurosci.*, **8**, 41–47.
- 5 Raymond, V. and Sattelle, D.B. (2002) Novel animal-health drug targets from ligand-gated chloride channels. *Nat. Rev. Drug Discov.*, **1**, 427–436.
- 6 Barnes, K.C., Grant, A.V., and Gao, P. (2005) A review of the genetic epidemiology of resistance to parasitic disease and atopic asthma: common variants for common phenotypes? *Curr. Opin. Allergy Clin. Immunol.*, **5**, 379–385.
- 7 Ringstad, N., Abe, N., and Horvitz, H.R. (2009) Ligand-gated chloride channels are receptors for biogenic amines in *C. elegans*. *Science*, **325**, 96–100.

- 8 Sutherland, I.A. and Leathwick, D.M. (2011) Anthelmintic resistance in nematode parasites of cattle: a global issue? *Trends Parasitol.*, **27**, 176–181.
- 9 Sargison, N.D., Jackson, F., Wilson, D.J., Bartley, D.J., Penny, C.D., and Gilleard, J.S. (2010) Characterisation of milbemycin-, ivermectin-, imidazothiazole- and benzimidazole-resistant *Teladorsagia circumcincta* from a sheep flock. *Vet. Rec.*, **166**, 681–686.
- 10 VLA (2008) Suspected macrocyclic lactone resistance in common sheep nematodes. *Vet. Rec.*, **163**, 673–676.
- 11 McKellar, Q.A. and Jackson, F. (2004) Veterinary anthelmintics: old and new. *Trends Parasitol.*, **20**, 456–461.
- 12 *C. elegans* Sequencing Consortium (1998) Genome sequence of the nematode, *C. elegans*: a platform for investigating biology. *Science*, **282**, 2012–2018.
- 13 Ghedin, E., Wang, S., Foster, J.M., and Slatko, B.E. (2004) First sequenced genome of a parasitic nematode. *Trends Parasitol.*, **20**, 151–153.
- 14 Hibbs, R.E. and Gouaux, E. (2011) Principles of activation and permeation in an anion-selective cys-loop receptor. *Nature*, **474**, 54–60.
- 15 Brooks, D.R. and Isaac, R.E. (2002) Functional genomics of parasitic worms: the dawn of a new era. *Parasitol. Int.*, **51**, 319–325.
- 16 Glendinning, S.K., Sattelle, D.B., Buckingham, S.D., Wonnacott, S., and Wolstenholme, A.J. (2011) Glutamate-gated chloride channels of *Haemonchus contortus* restore drug sensitivity to ivermectin resistant *Caenorhabditis elegans*. *PLoS One*, **6**, e22390.
- 17 Unwin, N. (2005) Refined structure of the nicotinic acetylcholine receptor at 4Å resolution. *J. Mol. Biol.*, **346**, 967–989.
- 18 Karlin, A. (2002) Emerging structure of the nicotinic acetylcholine receptors. *Nat. Rev. Neurosci.*, **3**, 102–114.
- 19 Cully, D.F., Pareiss, P.S., Liu, K.K., Schaeffer, J.M., and Arena, J.P. (1996) Identification of a *Drosophila melanogaster* glutamate-gated chloride channel sensitive to the antiparasitic agent ivermectin. *J. Biol. Chem.*, **271**, 20187–20191.
- 20 Wolstenholme, A.J. and Rogers, A.T. (2005) Glutamate-gated chloride channels and the mode of action of the ivermectin/milbemycin anthelmintics. *Parasitology*, **131**, S85–S95.
- 21 Dent, J.A., Davis, M.W., and Avery, L. (1997) Avr-15 encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin sensitivity in *Caenorhabditis elegans*. *EMBO J.*, **16**, 5867–5879.
- 22 Pemberton, D.J., Franks, C.J., Walker, R.J., and Holden-Dye, L. (2001) Characterization of glutamate-gated chloride channels in the pharynx of wild-type and mutant *Caenorhabditis elegans* delineates the role of the subunit GluCl-alpha2 in the function of the native receptor. *Mol. Pharmacol.*, **59**, 1037–1043.
- 23 Laughton, D.L., Lunt, G.G., and Wolstenholme, A.J. (1997) Alternative splicing of a *Caenorhabditis elegans* gene produces two novel inhibitory amino acid receptor subunits with identical ligand binding domains but different ion channels. *Gene*, **201**, 119–125.
- 24 Cook, A., Aptel, N., Portillo, V., Siney, E., Sihota, R., Holden-Dye, L., and Wolstenholme, A. (2006) *Caenorhabditis elegans* ivermectin receptors regulate locomotor behaviour and are functional orthologues of *Haemonchus contortus* receptors. *Mol. Biochem. Parasitol.*, **147**, 118–125.
- 25 Chalasani, S.H., Chronis, N., Tsunozaki, M., Gray, J.M., Ramot, D., Goodman, M.B., and Bargmann, C.I. (2007) Dissecting a circuit for olfactory behaviour in *Caenorhabditis elegans*. *Nature*, **450**, 63–70.
- 26 Dent, J.A., Smith, M.M., Vassilatis, D.K., and Avery, L. (2000) The genetics of ivermectin resistance in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA*, **97**, 2674–2679.
- 27 Jones, A.K. and Sattelle, D.B. (2004) Functional genomics of the nicotinic acetylcholine receptor gene family of the nematode, *Caenorhabditis elegans*. *Bioessays*, **26**, 39–49.
- 28 Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. *Genetics*, **77**, 71–94.

- 29 Jones, A.K., Buckingham, S.D., and Sattelle, D.B. (2005) Chemistry-to-gene screens in *Caenorhabditis elegans*. *Nat. Rev. Drug Discov.*, **4**, 321–330.
- 30 Richmond, J.E., and Jorgensen, E.M. (1999) One GABA and two acetylcholine receptors function at the *C. elegans* neuromuscular junction. *Nat. Neurosci.*, **2**, 791–797.
- 31 Fleming, J.T., Baylis, H.A., Sattelle, D.B., and Lewis, J.A. (1996) Molecular cloning and *in vitro* expression of *C. elegans* and parasitic nematode ionotropic receptors. *Parasitology*, (113 Suppl), S175–S190.
- 32 Culetto, E., Baylis, H.A., Richmond, J.E., Jones, A.K., Fleming, J.T., Squire, M.D., Lewis, J.A., and Sattelle, D.B. (2004) The *Caenorhabditis elegans unc-63* gene encodes a levamisole-sensitive nicotinic acetylcholine receptor alpha subunit. *J. Biol. Chem.*, **279**, 42476–42483.
- 33 Towers, P.R., Edwards, B., Richmond, J.E., and Sattelle, D.B. (2005) The *Caenorhabditis elegans lev-8* gene encodes a novel type of nicotinic acetylcholine receptor alpha subunit. *J. Neurochem.*, **93**, 1–9.
- 34 Boulin, T., Gielen, M., Richmond, J.E., Williams, D.C., Paoletti, P., and Bessereau, J.L. (2008) Eight genes are required for functional reconstitution of the *Caenorhabditis elegans* levamisole-sensitive acetylcholine receptor. *Proc. Natl. Acad. Sci. USA*, **105**, 18590–18595.
- 35 Kaminsky, R., Ducray, P., Jung, M., Clover, R., Rufener, L., Bouvier, J., Weber, S.S. *et al.* (2008) A new class of anthelmintics effective against drug-resistant nematodes. *Nature*, **452**, 176–180.
- 36 Bamber, B.A., Beg, A.A., Twyman, R.E., and Jorgensen, E.M. (1999) The *Caenorhabditis elegans unc-49* locus encodes multiple subunits of a heteromultimeric GABA receptor. *J. Neurosci.*, **19**, 5348–5359.
- 37 Bamber, B.A., Tyman, R.E., and Jorgensen, E.M. (2003) Pharmacological characterisation of the homomeric and heteromeric UNC-49 GABA receptors in *C. elegans*. *Br. J. Pharmacol.*, **138**, 883–893.
- 38 McIntire, S.L., Jorgensen, E., and Horvitz, H.R. (1993) Genes required for GABA function in *Caenorhabditis elegans*. *Nature*, **364**, 334–337.
- 39 McIntire, S.L., Jorgensen, E., Kaplan, J., and Horvitz, H.R. (1993) The GABAergic nervous system of *Caenorhabditis elegans*. *Nature*, **364**, 337–341.
- 40 Dent, J.A. (2006) Evidence for a diverse cys-loop ligand-gated ion channel superfamily in early bilateria. *J. Mol. Evol.*, **62**, 523–535.
- 41 Feng, X.P., Hayashi, J., Beech, R.N., and Prichard, R.K. (2002) Study of the nematode putative GABA type-A receptor subunits: evidence for modulation by ivermectin. *J. Neurochem.*, **83**, 870–878.
- 42 Ballivet, M., Alliod, C., Bertrand, S., and Bertrand, D. (1996) Nicotinic acetylcholine receptors in the nematode *Caenorhabditis elegans*. *J. Mol. Biol.*, **258**, 261–269.
- 43 Mongan, N.P., Jones, A.K., Smith, G.R., Sansom, M.S., and Sattelle, D.B. (2002) Novel alpha7-like nicotinic acetylcholine receptor subunits in the nematode *Caenorhabditis elegans*. *Protein Sci.*, **11**, 1162–1171.
- 44 Francis, M.M., Evans, S.P., Jensen, M., Madsen, D.M., Mancuso, J., Norman, K.R., and Maricq, A.V. (2005) The Ror receptor tyrosine kinase CAM-1 is required for ACR-16-mediated synaptic transmission at the *C. elegans* neuromuscular junction. *Neuron*, **46**, 581–594.
- 45 Touroutine, D., Fox, R.M., Von Stetina, S.E., Burdina, A., Miller, D.M. 3rd, and Richmond, J.E. (2005) Acr-16 encodes an essential subunit of the levamisole-resistant nicotinic receptor at the *Caenorhabditis elegans* neuromuscular junction. *J. Biol. Chem.*, **280**, 27013–27021.
- 46 Raymond, V., Mongan, N.P., and Sattelle, D.B. (2000) Anthelmintic actions on homomer-forming nicotinic acetylcholine receptor subunits: chicken alpha7 and ACR-16 from the nematode *Caenorhabditis elegans*. *Neuroscience*, **101**, 785–791.
- 47 Bentley, G.N., Jones, A.K., Parra, W.G.O., and Agnew, A. (2004) ShAR1 alpha and ShAR1 beta: novel putative nicotinic acetylcholine receptor subunits from the

- platyhelminth blood fluke *Schistosoma*. *Gene*, **329**, 27–38.
- 48 Grauso, M., Reenan, R.A., Culetto, E., and Sattelle, D.B. (2002) Novel putative nicotinic acetylcholine receptor subunit genes, D alpha 5, D alpha 6 and D alpha 7 in *Drosophila melanogaster* identify a new and highly conserved target of adenosine deaminase acting on RNA-mediated A-to-I pre-mRNA editing. *Genetics*, **160**, 1519–1533.
- 49 Patton, A., Knuth, S., Schaheen, B., Dang, H., Greenwald, I., and Fares, H. (2005) Endocytosis function of a ligand-gated ion channel homolog in *Caenorhabditis elegans*. *Curr. Biol.*, **15**, 1045–1050.
- 50 Beg, A.A. and Jorgensen, E.M. (2003) EXP-1 is an excitatory GABA-gated cation channel. *Nat. Neurosci.*, **6**, 1145–1152.
- 51 Jensen, M.L., Schousboe, A., and Ahring, P.K. (2005) Charge selectivity of the cys-loop family of ligand-gated ion channels. *J. Neurochem.*, **92**, 217–225.
- 52 Wotring, V.E. and Weiss, D.S. (2008) Charge scan reveals an extended region at the intracellular end of the GABA receptor pore that can influence ion selectivity. *J. Gen. Physiol.*, **131**, 87–97.
- 53 Ranganathan, R., Cannon, S.C., and Horvitz, H.R. (2000) MOD-1 is a serotonin-gated chloride channel that modulates locomotory behaviour in *C. elegans*. *Nature*, **408**, 470–475.
- 54 Putrenko, I., Zakikhani, M., and Dent, J.A. (2005) A family of acetylcholine-gated chloride channel subunits in *Caenorhabditis elegans*. *J. Biol. Chem.*, **280**, 6392–6398.
- 55 van Nierop, P., Keramidias, A., Bertrand, S., van Minnen, J., Gouwenberg, Y., Bertrand, D., and Smit, A.B. (2005) Identification of molluscan nicotinic acetylcholine receptor (nAChR) subunits involved in formation of cation- and anion-selective nAChRs. *J. Neurosci.*, **25**, 10617–10626.
- 56 Pirri, J.K., McPherson, A.D., Donnelly, J.L., Francis, M.M., and Alkema, M.J. (2009) A tyramine-gated chloride channel coordinates distinct motor programs of a *Caenorhabditis elegans* escape response. *Neuron*, **62**, 526–538.
- 57 Bocquet, N., Prado de Carvalho, L., Cartaud, J., Neyton, J., Le Poupon, C., Taly, A., Grutter, T. *et al.* (2007) A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family. *Nature*, **445**, 116–119.
- 58 Tasneem, A., Iyer, L.M., Jakobsson, E., and Aravind, L. (2005) Identification of the prokaryotic ligand-gated ion channels and their implications for the mechanisms and origins of animal cys-loop ion channels. *Genome Biol.*, **6**, R4.
- 59 Yates, D.M. and Wolstenholme, A.J. (2004) *Dirofilaria immitis*: Identification of a novel ligand-gated ion channel-related polypeptide. *Exp. Parasitol.*, **108**, 182–185.
- 60 Stein, L.D., Bao, Z., Blasiar, D., Blumenthal, T., Brent, M.R., Chen, N., Chinwalla, A. *et al.* (2003) The genome sequence of *Caenorhabditis briggsae*: a platform for comparative genomics. *PLoS Biol.*, **1**, E45.
- 61 Jones, A.K., Davis, P., Hodgkin, J., and Sattelle, D.B. (2007) The nicotinic acetylcholine receptor gene family of the nematode *Caenorhabditis elegans*: an update on nomenclature. *Invert. Neurosci.*, **7**, 129–131.
- 62 Williamson, S.M., Walsh, T.K., and Wolstenholme, A.J. (2007) The cys-loop ligand-gated ion channel gene family of *Brugia malayi* and *Trichinella spiralis*: a comparison with *Caenorhabditis elegans*. *Invert. Neurosci.*, **7**, 219–226.
- 63 Rufener, L., Keiser, J., Kaminsky, R., Maser, P., and Nilsson, D. (2010) Phylogenomics of ligand-gated ion channels predicts monepantel effect. *PLoS Pathog.*, **6**, e1001091.
- 64 Beech, R.N., Wolstenholme, A.J., Neveu, C., and Dent, J.A. (2010) Nematode parasite genes: what's in a name? *Trends Parasitol.*, **26**, 334–340.
- 65 Rozhkova, E.K., Malyutina, T.A., and Shishov, B.A. (1980) Pharmacological characteristics of cholinergic receptors in somatic muscles of the nematode, *Ascaris suum*. *Gen. Pharmacol.*, **11**, 141–146.

- 66 Harrow, I.D. and Gration, K.A.F. (1985) Mode of action of the anthelmintics morantel, pyrantel and levamisole on muscle cell membrane of the nematode *Ascaris suum*. *Pestic. Sci.*, **16**, 662–672.
- 67 Pennington, A.J. and Martin, R.J. (1990) A patch-clamp study of acetylcholine-activated ion channels in *Ascaris suum* muscle. *J. Exp. Biol.*, **154**, 201–221.
- 68 Robertson, S.J. and Martin, R.J. (1993) Levamisole-activated single-channel currents from muscle of the nematode parasite *Ascaris suum*. *Br. J. Pharmacol.*, **108**, 170–178.
- 69 Robertson, S.J., Pennington, A.J., Evans, A.M., and Martin, R.J. (1994) The action of pyrantel as an agonist and an open channel blocker at acetylcholine receptors in isolated *Ascaris suum* muscle vesicles. *Eur. J. Pharmacol.*, **271**, 273–282.
- 70 Robertson, A.P., Clark, C.L., Burns, T.A., Thompson, D.P., Geary, T.G., Trailovic, S.M., and Martin, R.J. (2002) Paraherquamide and 2-deoxy-paraherquamide distinguish cholinergic receptor subtypes in *Ascaris* muscle. *J. Pharmacol. Exp. Ther.*, **302**, 853–860.
- 71 Trailovic, S.M., Clark, C.L., Robertson, A.P., and Martin, R.J. (2005) Brief application of AF2 produces long lasting potentiation of nAChR responses in *Ascaris suum*. *Mol. Biochem. Parasitol.*, **139**, 51–64.
- 72 Williamson, S.M., Robertson, A.P., Brown, L., Williams, T., Woods, D.J., Martin, R.J., Sattelle, D.B., and Wolstenholme, A.J. (2009) The nicotinic acetylcholine receptors of the parasitic nematode *Ascaris suum*: formation of two distinct drug targets by varying the relative expression levels of two subunits. *PLoS Pathog.*, **5**, e1000517.
- 73 Martin, R.J., Bai, G., Clark, C.L., and Robertson, A.P. (2003) Methyridine (2-[2-methoxyethyl]-pyridine) and levamisole activate different ACh receptor subtypes in nematode parasites: a new lead for levamisole-resistance. *Br. J. Pharmacol.*, **140**, 1068–1076.
- 74 Boulin, T., Fauvin, A., Charvet, C., Cortet, J., Cabaret, J., Bessereau, J.L., and Neveu, C. (2011) Functional reconstitution of *Haemonchus contortus* acetylcholine receptors in *Xenopus* oocytes provides mechanistic insights into levamisole resistance. *Br. J. Pharmacol.*, **164**, 1421–1432.
- 75 Neveu, C., Charvet, C.L., Fauvin, A., Cortet, J., Beech, R.N., and Cabaret, J. (2010) Genetic diversity of levamisole receptor subunits in parasitic nematode species and abbreviated transcripts associated with resistance. *Pharmacogenet. Genomics*, **20**, 414–425.
- 76 Siddiqui, S.Z., Brown, D.D., Rao, V.T., and Forrester, S.G. (2010) An UNC-49 GABA receptor subunit from the parasitic nematode *Haemonchus contortus* is associated with enhanced GABA sensitivity in nematode heteromeric channels. *J. Neurochem.*, **113**, 1113–1122.
- 77 Rao, V.T., Accardi, M.V., Siddiqui, S.Z., Beech, R.N., Prichard, R.K., and Forrester, S.G. (2010) Characterization of a novel tyramine-gated chloride channel from *Haemonchus contortus*. *Mol. Biochem. Parasitol.*, **173**, 64–68.
- 78 Rao, V.T., Siddiqui, S.Z., Prichard, R.K., and Forrester, S.G. (2009) A dopamine-gated ion channel (HcGGR3^{*}) from *Haemonchus contortus* is expressed in the cervical papillae and is associated with macrocyclic lactone resistance. *Mol. Biochem. Parasitol.*, **166**, 54–61.
- 79 Jagannathan, S., Loughton, D.L., Critten, C.L., Skinner, T.M., Horoszok, L., and Wolstenholme, A.J. (1999) Ligand-gated chloride channel subunits encoded by the *Haemonchus contortus* and *Ascaris suum* orthologues of the *Caenorhabditis elegans* *gbr-2 (avr-14)* gene. *Mol. Biochem. Parasitol.*, **103**, 129–140.
- 80 Cheeseman, C.L., Delany, N.S., Woods, D.J., and Wolstenholme, A.J. (2001) High-affinity ivermectin binding to recombinant subunits of the *Haemonchus contortus* glutamate-gated chloride channel. *Mol. Biochem. Parasitol.*, **114**, 161–168.
- 81 Forrester, S.G., Hamdan, F.F., Prichard, R.K., and Beech, R.N. (1999) Cloning, sequencing, and developmental expression levels of a novel glutamate-gated chloride channel homologue in the parasitic nematode *Haemonchus contortus*.

- Biochem. Biophys. Res. Commun.*, **254**, 529–534.
- 82 Delany, N.S., Laughton, D.L., and Wolstenholme, A.J. (1998) Cloning and localisation of an avermectin receptor-related subunit from *Haemonchus contortus*. *Mol. Biochem. Parasitol.*, **97**, 177–187.
- 83 Woods, D.J. and Williams, T.M. (2007) The challenges of developing novel antiparasitic drugs. *Invert. Neurosci.*, **7**, 245–250.
- 84 Woods, D.J. and Knauer, C.S. (2010) Discovery of veterinary antiparasitic agents in the 21st century: a view from industry. *Int. J. Parasitol.*, **40**, 1177–1181.
- 85 Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*, **391**, 806–811.
- 86 Fraser, A.G., Kamath, R.S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., and Ahringer, J. (2000) Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. *Nature*, **408**, 325–330.
- 87 Gonczy, P., Echeverri, C., Oegema, K., Coulson, A., Jones, S.J., Copley, R.R., Duperon, J. *et al.* (2000) Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. *Nature*, **408**, 331–336.
- 88 Gally, C., Eimer, S., Richmond, J.E., and Bessereau, J.L. (2004) A transmembrane protein required for acetylcholine receptor clustering in *Caenorhabditis elegans*. *Nature*, **431**, 578–582.
- 89 Lewis, J.A., Elmer, J.S., Skimming, J., McLafferty, S., Fleming, J., and McGee, T. (1987) Cholinergic receptor mutants of the nematode *Caenorhabditis elegans*. *J. Neurosci.*, **7**, 3059–3071.
- 90 Fitzgerald, J., Kennedy, D., Viseshakul, N., Cohen, B.N., Mattick, J., Bateman, J.F., and Forsayeth, J.R. (2000) UNCL, the mammalian homologue of UNC-50, is an inner nuclear membrane RNA-binding protein. *Brain Res.*, **877**, 110–123.
- 91 Benian, G.M., L'Hernault, S.W., and Morris, M.E. (1993) Additional sequence complexity in the muscle gene, *unc-22*, and its encoded protein, twitchin, of *Caenorhabditis elegans*. *Genetics*, **134**, 1097–1104.
- 92 Maryon, E.B., Coronado, R., and Anderson, P. (1996) Unc-68 encodes a ryanodine receptor involved in regulating *C. elegans* body-wall muscle contraction. *J. Cell Biol.*, **134**, 885–893.
- 93 Jenden, D.J. and Fairhurst, A.S. (1969) The pharmacology of ryanodine. *Pharmacol. Rev.*, **21**, 1–25.
- 94 Lahm, G.P., Selby, T.P., Freudenberger, J.H., Stevenson, T.M., Myers, B.J., Seburyamo, G., Smith, B.K. *et al.* (2005) Insecticidal anthranilic diamides: a new class of potent ryanodine receptor activators. *Bioorg. Med. Chem. Lett.*, **15**, 4898–4906.
- 95 Pica-Mattoccia, L., Orsini, T., Basso, A., Festucci, A., Liberti, P., Guidi, A., Marcatto-Maggi, A.L. *et al.* (2008) *Schistosoma mansoni*: lack of correlation between praziquantel-induced intra-worm calcium influx and parasite death. *Exp. Parasitol.*, **119**, 332–335.

