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Advances in our understanding of nematode ion channels as potential anthelmintic targets

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ABSTRACT

Ion channels are specialized multimeric proteins that underlie cell excitability. These channels integrate with a variety of neuromuscular and biological functions. In nematodes, the physiological behaviors including locomotion, navigation, feeding and reproduction, are regulated by these protein entities. Majority of the anti-nematodal chemotherapeutics target the ion channels to disrupt essential biological functions. Here, we have summarized current advances in our understanding of nematode ion channel pharmacology. We review cys-loop ligand gated ion channels (LGICs), including nicotinic acetylcholine receptors (nAChRs), acetylcholine-chloride gated ion channels (ACCs), glutamate-gated chloride channels (GluCls), and GABA (γ-aminobutyric acid) receptors, and other ionotropic receptors (transient receptor potential (TRP) channels and potassium ion channels). We have provided an update on the pharmacological properties of these channels from various nematodes. This article catalogs the differences in ion channel composition and resulting pharmacology in the phylum Nematoda. This diversity in ion channel subunit repertoire and pharmacology emphasizes the importance of pursuing species-specific drug target research. In this review, we have provided an overview of recent advances in techniques and functional assays available for screening ion channel properties and their application.

1. Introduction

All living cells are encased in a hydrophobic, semi-permeable cell membrane that restricts large polar molecules and ions. The movement of ions across the cell membrane is governed by specific ion channels formed by intrinsic membrane proteins. These channels are incredibly diverse selective aqueous pores located in virtually all cell and tissue types. They undergo a conformational change to control ionic flux, providing the foundation for membrane excitability, neurotransmission, and cellular communications (Fig. 1). These biophysical entities are essential for a wide variety of physiological and homeostatic functions, thus playing a fundamental role in all organisms' viability. Nematodes are no exception as these dynamic proteins are intimately involved in critical physiological processes such as locomotion, feeding, sensory, and reproductive behaviors (Perry and Maule, 2004). These processes are interrupted through chemotherapeutics that target the neuromuscular system of these organisms. Parasite ion channels have been the subject of in-depth research as targets for anthelmintic drugs. Some of the most successful anthelmintic agents act on these channels and help alleviate the parasitic burden, which is a significant public health issue and threatens global food-security (Wolstenholme, 2012; Greenberg, 2014; Martin et al., 2015; Abongwa et al., 2017).

Many of the current anthelmintics target nematode cys-loop ligand-gated ion channels; macrocyclic lactones act on glutamate-gated chloride channels (GluCls) while the cholinomimetics (such as pyrantel, levamisole, tribendimidine) act on nicotinic acetylcholine receptors (nAChRs) (Wolstenholme, 2012; Holden-Dye et al., 2013; Greenberg, 2014; Martin et al., 2015; Abongwa et al., 2017). These membrane-bound channels face an extracellular matrix, making them easily accessible to drug molecules and are thus 'druggable' targets. Furthermore, ion channels that are either invertebrate-specific or show differences in pharmacology between parasite and host homologs are suitable targets for chemotherapeutic intervention (Jones and Sattelle, 2004; Robertson and Martin, 2007; Wolstenholme, 2011; Holden-Dye et al., 2013). These proteins' heterogenic nature gives rise to many different subtypes of nematode receptor that often have specific tissue expression and are integrated with vital biological functions (Wolstenholme and Rogers, 2005; Wolstenholme, 2011; Holden-Dye et al., 2013). In this review we discuss the progress made in understanding function, subtype diversity, and pharmacology of several, but not all, ion

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channel types from parasitic nematodes that have been explored as drug targets. We provide an in-depth analysis of nAChRs, acetylcholine-chloride gated ion channels (ACCs), GluCls, and GABA (γ-aminobutyric acid) receptors, and other ionotropic receptors (transient receptor potential (TRP) channels and potassium ion channels). Ion channels sensitive to biogenic amines (such as serotonin, dopamine and tyramine), glutamate-gated cation channels (NMDA and AMPA receptor-type channel), DEG/ENac (DEGenerins/Epithelial amiloride-sensitive Na^+ Channel) family of sodium channels, calcium channels, and cyclic nucleotide gated (CNG) ion channels have not been discussed in this review.

2. Ligand-gated ion channels

Ligand-gated ion channels (LGICs) constitute an integral class of membrane proteins that are fundamental for nervous system signaling, and intracellular communication. These ionotropic channels are oligomeric receptor proteins that conduct ion flux across the membrane in response to the binding of a ligand, such as a neurotransmitter. The binding of a ligand, extracellularly or intracellularly, to the ion channel triggers a conformational change that results in the conducting state and forms the basis of membrane excitability and inhibition. Most LGICs in the nematode genome fall into the cys-loop LGICs superfamily, which are characterized by the presence of cys-loop motif in the extracellular domain of a subunit (Fig. 2) (Jones and Sattelle, 2008). The conserved cys-loop motif consists of a disulfide bond between two invariant cysteine resides separated by 13 amino acid residues. All the cys-loop receptors are composed of five, pseudo-symmetrically arranged, subunits that surround a central ion-conducting pore (Fig. 2). Each receptor subunit has an N-terminal extracellular domain that contains the orthosteric ligand-binding sites, a transmembrane domain (TMD) formed by four helical segments, and an intracellular domain (Thompson et al., 2010). Some of the cys-loop LGICS are cation selective such as nicotinic acetylcholine receptors (nAChRs) and some are anion selective such as glutamate-gated chloride channels (GluCls).

2.1. Nicotinic acetylcholine receptors

nAChRs are pentameric cys-loop LGICs that are involved in physiological responses to acetylcholine and mediate fast excitatory neurotransmission (Thompson et al., 2010). nAChRs have played a prominent role in the history of pharmacology and were the first neurotransmitter ion channel receptors to be isolated (Changeux, 2012). Central to the concept of a pharmacological receptor were the investigations to localize curare's physiological action by Claude Bernard in 1857. He determined the junction between motor nerve and muscle to be the site of action (Bennett, 2000; Cousin, 2013; Martindale and Lester, 2014). In 1885 Paul Ehlrich introduced the concept of "chemoreceptors" suggesting that chemical groups in toxins interacted with the specific side-chains on the cell surface (Bosch and Rosich, 2008). John Newport Langley proposed the most integral theory regarding receptors and their interactions in 1905. His research involved the study of the effect of alkaloids on nerves and muscles. He concluded that a contractile molecule has a "receptive substance" (currently designated as receptors) that is combined with nicotine and curare to mediate contractions, and these receptors could receive stimulus from the nerve and transmit it to the effector cell (Langley, 1901, 1905; Bennett, 2000). It was Bernard Katz who used the term "channels" for receptors, and in 1955 the receptors were recognized as proteins by David Nachmansohn (Nachmansohn, 1959; Colquhoun, 2006). Katz and Miledi (1970) estimated the properties of single-channel nAChRs using the noise analysis technique, and Neher and Sakmann (1976) confirmed these results by using the new patch-clamp method. The first nAChR (from electric ray, *Torpedo californica*) was identified, purified, and biochemically characterized in 1970 (Changeux et al., 1970; Miledi and Potter, 1971). The first nAChR subunit was cloned in 1982 (Noda et al., 1982), followed by the pioneering work of Nigel Unwin, who unraveled the three-dimensional structure of the nicotinic receptors over the years (Unwin et al., 1988; Miyazawa et al., 1999; Unwin, 2005). Thus, nAChRs have been paramount in the discovery of neurotransmitter receptors and their pharmacology. These are perhaps the most thoroughly characterized of all the ligand-gated ion channels, and their studies have helped in the evolution of various subfields of science.

The nAChRs are cell-surface transmembrane complexes composed of five subunits that assemble to form a functional protein. They are arranged to form a pseudo-symmetrical rosette with a central ion-conducting pore that runs through the channel's entire length (Changeux, 2012). nAChRs are primarily cation conducting channels, but invertebrates also express anion-selective acetylcholine gated channels (van Nierop et al., 2005; MacDonald et al., 2014; Wever et al., 2015). Each receptor subunit consists of a signal peptide region, an extracellular hydrophilic ligand-binding domain, four transmembrane (TM1–TM4) segments, and an intracellular cytoplasmic domain. The extracellular domain contains a canonical cys-loop motif and six loops (loop A-F) that make up the ligand-binding site. The subunit genes are categorized into two classes: α-subunits and non-α subunits. The presence of "vicinal" (adjacent) cysteine residues on the extracellular loop-C in α subunits distinguishes them from the non-α subunits. These cysteines are essential in ligand binding (Kao et al., 1984; Arias, 1997). The ligands' binding to the extracellular domain between the principal face contributed by an α subunit and complementary face of an adjacent subunit induces channel opening (Sine and Engel, 2006). The nAChRs are either composed of five identical (homopentamers) or different (heteropentamers) polypeptide chains (Corringer et al., 2000; Karlin, 2002). These receptors assemble from a diverse collection of homologous subunits encoded by a large multigene family (Corringer et al.,

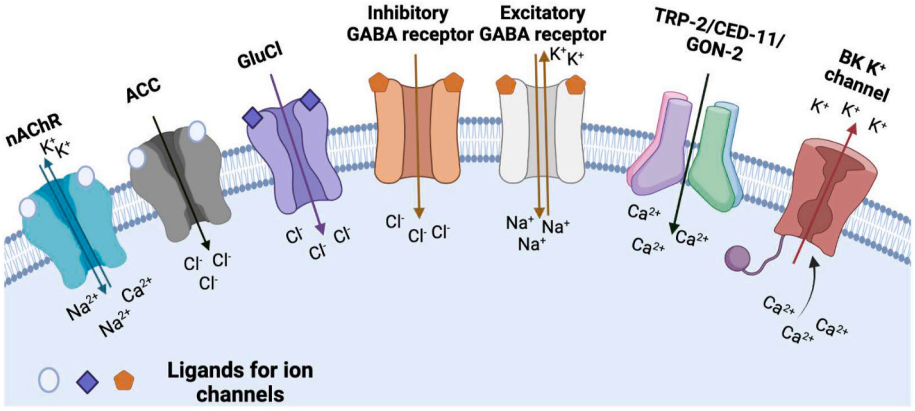

Fig. 1. Different types of nematode ligand- and voltage-gated ion channels in plasma membrane along with their ion selectivity.

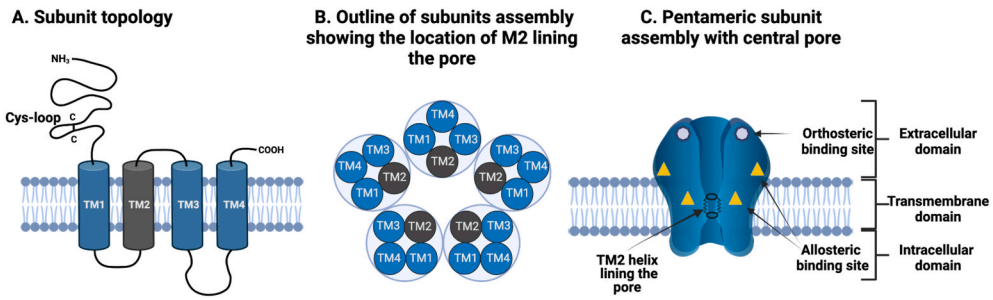

Fig. 2. Structure and topology of cys-loop ligand-gated ion channels. **A.** The schematic representation of topography of the receptor showing N-terminal extracellular domain, intracellular domain, four membrane spanning transmembrane cylindrical domains, cys-loop having cysteine residues participating in disulfide formation. **B.** Top view of a pentameric ion-channel showing transmembrane helical arrangement of each subunit showing the location of TM2 lining the pore. **C.** Schematic of a generic cys-loop LGIC showing the arrangement of subunits to form a central ion pore. The notional orthosteric- and allosteric-ligand binding site are indicated in the extracellular and transmembrane domains.

2000; Sine and Engel, 2006). A rich repertoire of nAChRs is generated by assembling different combinations of subunits that confer distinct features, specific physiology, and pharmacology (Le Novère et al., 2002). The nAChRs are widely expressed in metazoans, including mammals, nematodes, and insects (Le Novère and Changeux, 1995).

2.1.1. *Caenorhabditis elegans* nAChRs

A large number of nAChR subunits have been identified in nematodes which, in addition to the primordial role of neurotransmission, also regulate a variety of physiological behaviors, including feeding, locomotion, sensory functions, reproduction, and development depending on their tissue expression (Table 1; Fig. 3) (Treinin et al., 1998; Yassin et al., 2001; McKay et al., 2004; Rand, 2007; Holden-Dye et al., 2013; Abongwa et al., 2017; Choudhary et al., 2020). These receptors have long been realized as valuable anthelmintic drug targets to control helminth infections in animals and humans (Martin et al., 1991; Abongwa et al., 2017). The cholinomimetic anthelmintics, including levamisole, monepantel, and pyrantel, target nematode somatic muscle nAChRs resulting in spastic paralysis and rapid expulsion of worms from the host (Martin et al., 1991; Wolstenholme, 2011). The free-living nematode, *Caenorhabditis elegans* (Clade V), is a popular model organism and has played a vital role in the deduction of function, genetics, and pharmacology of nematode nAChRs (Jones et al., 2005). *C. elegans* is one of the simplest organisms to employ acetylcholine as a neurotransmitter; yet *its* genome possesses the largest and most diverse nAChR gene collection described to date with 29 nAChR subunits divided into five "core" groups by homology (Mongan et al., 1998; Jones and Sattelle, 2004; Sattelle, 2009). Additionally, there are 32 "orphan" non-alpha subunits which show homology to nAChRs outside of the core groups (Jones et al., 2007; Holden-Dye et al., 2013). Multiple nAChR subunit genes generate a greater diversity of receptor types by varying the subunits' combination, and stoichiometry (Table 1). The core groups of nAChRs are named after the first subunit of the group characterized: DEG-3 group (8 members; DEGeneration of certain neurons), ACR-16 group (11 members; ACetylcholine Receptor), UNC-29 group (3 members; UNCoordinated), UNC-38 group (4 members), and ACR-8 group (3 members) (Mongan et al., 1998; Jones et al., 2007). The DEG-3, ACR-8, and UNC-38 groups contain α subunit members exclusively, while all the UNC-29 group members are non-α subunits. ACR-16 group members share high homology to vertebrate neuronal α7–10 nAChR subunits (Ballivet et al., 1996; Mongan et al., 1998). UNC-38 group members are most homologous to insect nAChR subunits, while UNC-29-like subunits most closely resemble AChR-like Drosophila protein (ARD; non-α subunit) of *Drosophila* and vertebrate muscle non-α subunits (Jones and Sattelle, 2004). The DEG-3 and ACR-8 group members do not have homologs in vertebrates (Mongan et al., 2002). Most of the receptor subunits in the groups show characteristics common to nAChRs, but some subunits possess modifications that alter their pharmacological properties. The YxCC motif (loop-C) is highly conserved in α type nAChR subunits. It is replaced with the FxCC motif in DEG-3 group subunits, ACR-24 and ACR-5, which decreases the affinity for acetylcholine (Galzi et al., 1991). In ACR-10, a member of the ACR-8 group, and UNC-38 subunit, the motif is replaced by YxxCC instead (Mongan et al., 1998). Another modification has been identified in the ACR-8 group subunits in which a basic residue (histidine) flanks the TM2 region instead of the highly conserved acidic residue. All these modifications and diversity in key motifs suggest that the resulting active receptors could have different biophysical and pharmacological properties (Jones and Sattelle, 2004).

Major types of nAChRs identified in *C. elegans* are levamisole-sensitive (L-type) nAChRs, nicotine-sensitive (N-type) nAChRs, DEG-3/DES-2 (DEgeneration Suppressor) nAChRs, pharyngeal nAChRs, and neuronal ACR-2 nAChRs. Each of these receptor types varies in their expression patterns and is essential for a vital physiological behavior. Levamisole-sensitive and nicotine-sensitive receptors are expressed in body wall muscles and regulate locomotory behavior (Richmond and Jorgensen, 1999). DEG-3/DES-2 receptors are expressed in non-synaptic regions and chemosensory neurons' sensory endings (Treinin et al., 1998). EAT-2 nAChRs are expressed in the pharyngeal muscles and control feeding function (McKay et al., 2004).

L-type receptors mediate excitatory neurotransmission at the neuromuscular junctions of nematodes and constitute a major target of anthelmintic drugs, including levamisole, pyrantel, and tribendimidine (Wolstenholme, 2011; Martin et al., 2012). In 1974, Sydney Brenner isolated the first *C. elegans* mutants resistant to levamisole; successively, James Lewis and colleagues showed that these mutants, which exhibit impaired locomotion, lack pharmacological acetylcholine receptors (Brenner, 1974; Lewis et al., 1980b, 1987). Further studies conducted by Fleming et al. (1997) demonstrated three genes associated with levamisole resistance encode for nAChR subunits, LEV-1 (LEVamisole resistant), UNC-29, and UNC-38. These genes were co-expressed in *Xenopus* oocytes to reconstitute a functional receptor activated by levamisole and blocked by nicotinic antagonists. The levamisole current was very small (around 5 nA), suggesting the involvement of additional subunits in the native receptors. Whole-cell voltage-clamp recordings from *C. elegans* body wall muscles provided electrophysiological evidence regarding the requirement of subunits encoded by *unc-29* and *unc-38* for levamisole nAChR activity (Richmond and Jorgensen, 1999). Subsequent studies identified additional nAChR subunit encoding genes, *unc-63* and *lev-8* (also called *acr-13),* associated with levamisole resistance (Culetto et al., 2004; Towers et al., 2005). Thus, five subunits, two non-α-subunits (LEV-1 and UNC-29) and three α-subunits (LEV-8, UNC-38, UNC-63), all of which are expressed in the body wall muscle, have been identified as important for levamisole sensitivity (Fleming et al., 1997; Culetto et al., 2004; Jones and Sattelle, 2004; Towers et al., 2005; Boulin et al., 2008). The expression of the channel is reduced by >97% in the absence of one or more of these subunit genes, while levamisole induced inward currents are absent or substantially decreased in mutants of all five nAChR subunits (Richmond and Jorgensen, 1999; Culetto et al., 2004; Towers et al., 2005; Boulin et al., 2008; Holden-Dye et al., 2013). Three ancillary proteins, RIC-3 (Resistance to Inhibitors of Cholinesterase), UNC-50, and UNC-74, are required for successful

Table 1
A summary of various subtypes of nematode nAChRs.

Organism	Receptor type	nAChR subunit	Tissue distribution	Pharmacology
C. elegans	Levamisole sensitive (L-type) heteromeric nAChRs	UNC-38 α type	Body wall and vulval muscles, nerve ring, dorsal and ventral nerve cord, nervous system, copulatory spicule, enteric muscle[1,2,3,4,5]	• L-AChR (*Cel*-Lev-8, *Cel*-UNC-63, *Cel*-UNC-38, *Cel*-UNC-29, and *Cel*-LEV-1) • Agonist profile: acetylcholine > levamisole > pyrantel >>> nicotine[11] • Acetylcholine $EC_{50} = 26\ \mu M$[11] • Levamisole $EC_{50} = 10.1\ \mu M$[11] • Antagonist profile: d-tubocurarine (100 μM) > methyllycaconitine (10 μM) > hexamethonium (100 μM) > α-bungarotoxin (100 nM) > dihydro-β-erythroidine (10 μM)[11] • L-AChR-2.1: *Cel*-ACR-8, *Cel*-UNC-63, *Cel*-UNC-38, *Cel*-UNC-29, and Cel-LEV-1[13] • Acetylcholine $EC_{50} = 20\ \mu M$[13] • Levamisole $EC_{50} = 24.6\ \mu M$[13] • L-AChR-2.2: *Cel*-ACR-8, *Cel*-UNC-63, *Cel*-UNC-38, and *Cel*-UNC-29 • Acetylcholine $EC_{50} = 18.9\ \mu M$[13] • Levamisole $EC_{50} = 51.7\ \mu M$[13]
		UNC-63 α type	Body wall and vulval muscle, nervous system, motoneurons, head neurons, tail neurons, ventral nerve cord[1,6,7]	
		LEV-8 (ACR-13) α type	Body wall muscles, DD neurons, anal depressor muscles, head neurons, vulval muscles[1,3,9]	
		UNC-29 non-α type	Body wall and head muscles, head neurons, dorsal and ventral nerve cord, nerve ring[1,2,5,8,10]	
		LEV-1 non-α type	Body wall muscles, dorsal and ventral cord, ventral cord neurons[1,3,8]	
		ACR-8 α type	Body wall muscle, head and tail neurons, enteric and vulval muscles, nerve ring, nerve cord, ventral nerve cord motoneurons[1,3,13]	
	Nicotine-sensitive (N-type) homomeric nAChRs	ACR-16 α type	Body wall musculature, muscle cell, head muscle, anal depressor muscle, some head and tail neurons, linker cell[1,5,14,15,16]	• Acetylcholine $EC_{50} = 55\ \mu M$[17] • Nicotine $EC_{50} = 12\ \mu M$[17] • Levamisole behaves as an antagonist of acetylcholine-mediated response $IC_{50} = 36\ \mu M$[17] • Antagonist profile: d-tubocurarine (10 μM) ≅ hexamethonium (100 μM) > dihydro-β-erythroidine (10 μM) > methyllycaconitine (10 μM) > α-bungarotoxin (100 nM)[17]
	Choline-sensitive heteromeric nAChRs	DEG-3 α type	Body wall muscle, head and tail neurons, cell bodies of touch receptor neurons, two PVD neurons, IL1 neurons, two PVC neurons, single AVG, and mechanosensory neurons[1,18,19]	• DEG-3/DES-2 heteromeric channel • Acetylcholine behaved as a partial agonist with an $EC_{50} = 2.9\ mM$[19] • Choline produced ~13-fold higher current amplitude than acetylcholine and has slightly higher sensitivity with an $EC_{50} = 1.8\ mM$[19] • d-tubocurare $IC_{50} = 3.5\ \mu M$[19] • Strychnine $IC_{50} = 114\ \mu M$[19]
		DES-2 α type	PVC neurons, PVD neurons, sensory endings of IL2, FLP neurons, ALM neurons, m1 head muscles[1,19]	
	Choline-monepantel sensitive nAChRs	ACR-23 α type	Head muscles, multiple interneurons, six mechanosensory neurons (ALM, PLM, AVM and PVM)[21,22]	• Choline and betaine are potent agonists of ACR-23 homomeric channel ($EC_{50} = 1.4\ mM$) [21,22] • Monepantel produced direct activation as well as potentiated choline- and betaine-mediated responses[21,22] • Acetylcholine and nicotine behaved as weaker agonists[21,22]
	ACR-20 nAChRs	ACR-20 α type	Body wall muscle and nervous systems[1]	• Betaine $EC_{50} = 25\ \mu M$[23] • Choline $EC_{50} = 1.2\ mM$[23] • Monepantel produced direct activation and positive allosteric modulation of betaine-mediated responses[23]
	EAT-2 pharyngeal nAChRs	EAT-2 non-α type	pharyngeal muscle (pm4 and 5)[24]	• expresses as homomeric channel only when co-expressed with EAT-1825

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Table 1 (*continued*)

Organism	Receptor type	nAChR subunit	Tissue distribution	Pharmacology
		EAT-18	pharyngeal muscle and pharyngeal neuron M5 and some neurons in extrapharyngeal nervous system[24]	• Agonist profile: Acetylcholine > methacholine > nicotine > carbachol > butyrylcholine > epibatidine > oxantel ≫>>> DMPP = tribendimidine = bephenium = cytisine = lobeline = levamisole = SIB 1508Y = α-cotinine = nornicotine = anabasine = pyrantel[25] • Acetylcholine EC_{50} = 15.5 μM[25] • Nicotine EC_{50} = 64 μM[25] • Antagonist profile: d-tubocurarine (30 μM) > paraherquamide (30 μM) > derquantel (10 μM) ≫>>> α-bungarotoxin (10 μM) ≈ DhβE (30 μM)[25]
	ACR-2, ACR-3 and ACR-12 nAChRs	ACR-2 non-α type	Ventral cord neurons: VA, VB, DA, DB cholinergic motoneurons, PVQ and DVC tail neurons, IL-1, RMD tail neurons, vulval muscles[26,27]	• ACR-2R (ACR-2, ACR-3, ACR-12, UNC-38 and UNC-63) nAChR • Agonist profile: Acetylcholine > nicotine > DMPP >>> choline ≈ levamisole[28] • Acetylcholine EC_{50} = 14.1 μM[28] • Mecamylamine produced potent blocking of acetylcholine-mediated currents[28] • ACR-2/UNC-38 nAChR[28] • Levamisole-sensitive channel • Mecamylamine blocked levamisole-sensitive currents • ACR-3/UNC-38 nAChR[30] • Levamisole-sensitive channel • Mecamylamine and d-tubocuraraine blocked levamisole-sensitive currents
		ACR-3 non-α type	Subset of neurons[1]	
		ACR-12 α type	Ventral cord motoneurons, cholinergic motoneurons (DA, VA, DB, VB) and GABA motoneurons (DD and VD), tail and head neurons[3,28]	
A. suum	Levamisole-sensitive nAChRs	UNC-29 non-α type	Muscle cells and intestinal cells[31,32]	• UNC-29:UNC-38 1:1[31] • Agonist profile: levamisole > acetylcholine > nicotine • Acetylcholine pEC_{50} = 4.97 ± 0.16 • Levamisole pEC_{50} = 4.78 ± 0.25 • Nicotine pEC_{50} = 4.79 ± 0.22 • UNC-29:UNC-38 1:5[31] • Agonist profile: Levamisole > pyrantel > acetylcholine > nicotine>>> oxantel • Acetylcholine pEC_{50} = 5.95 ± 0.12 • Levamisole pEC_{50} = 5.53 ± 0.37 • Nicotine pEC_{50} = 5.88 ± 0.95 • Pyrantel pEC_{50} = 6.4 ± 0.18 • UNC-29:UNC-38 5:1[31] • Agonist profile: oxantel > nicotine > acetylcholine > levamisole>>> pyrantel • Acetylcholine pEC_{50} = 6.14 ± 0.12 • Levamisole pEC_{50} = 5.62 ± 0.49 • Nicotine pEC_{50} = 5.57 ± 1.56 • Oxantel pEC_{50} = 5.39 ± 1.06
		UNC-38 α type	Muscle cells and intestinal cells[31,32]	
	Nicotine-sensitive nAChRs	ACR-16 α type	somatic muscle, pharynx, ovijector, and head[33]	• Agonist profile: nicotine ≈ cytisine ≈ 3-bromocytisine ≈ epibatidine > DMPP > oxantel >>> choline = betaine = lobeline = A*844606* = morantel = levamisole = methyridine = thenium = bephenium = tribendimidine = pyrantel[33] • Acetylcholine EC_{50} = 5.9 μM[33] • Nicotine EC_{50} = 4.5 μM[33] • Antagonist profile (10 μM): mecamylamine = MLA = *d*TC > paraherquamide ~ derquantel ≈ hexamethonium ≈ DHβE > α-BTX[33]
	ACR-26 nAChRs	ACR-26 α type	Head muscles[34]	• sensitive to acetylcholine (EC_{50} between 10 and 100 nM) and nicotine (EC_{50} = 25 μM)[34]
	Pharyngeal nAChRs	EAT-2 non-α type	Pharynx, reproductive tissues, and head region[25]	• expresses as homomeric channel only when co-expressed with EAT-1825 • Agonist profile: nicotine > ACh > cytisine > epibatidine > DMPP > oxantel[25] • Acetylcholine EC_{50} = 1.7 μM[25] • Antagonist profile (30 μM): d-tubocurarine ≈ mecamylamine > hexamethonium > methylly caconitine derquantel > DhβE[25]
		EAT-18 auxiliary protein	Pharynx, reproductive tissues, gut tissues, and head region[25]	
H. contortus	Levamisole-sensitive nAChRs	UNC-29 non-α type (UNC29.1, UNC-29.2, UNC-29.3 and UNC-29.4 variants	Body muscles and uterus muscles[36]	• *Hco*-L-AChR1 (ACR-8, UNC-29.1, UNC-38 and UNC-63a)[35,36] • Agonist profile: levamisole > acetylcholine > DMPP > pyrantel > nicotine • Acetylcholine EC_{50} = 5.8 μM[35]; 2.38 μM[36] • Levamisole EC_{50} = 6.08 μM; 4.91 μM[36] • Antagonist profile: d-tubocurarine (100 μM) ≈ mecamylamine (30 μM) > DhβE (10 μM)[36] • *Hco*-L-AChR2 (UNC-29.1, UNC-38 and UNC-63a)[35] • Agonist profile: pyrantel > DMPP > acetylcholine > nicotine > levamisole • Acetylcholine EC_{50} = 19.2 μM[35] • Levamisole EC_{50} = 48.4 μM[35] • Antagonist profile: Both d-tubocurarine (100 μM) and DhβE (10 μM) produced efficient blocking of acetylcholine-mediated currents[35] • *Hco*-L-AChR1.3 (ACR-8, UNC-29.3, UNC-38 and UNC-63a)[36]
		UNC-38 α type	Not known	
		UNC-63 α type	Not known	
		ACR-8 α type	Not known	

(*continued on next page*)

Table 1 (*continued*)

Organism	Receptor type	nAChR subunit	Tissue distribution	Pharmacology
				• Agonist profile: acetylcholine > levamisole > DMPP >>> bephenium ≈ pyrantel ≈ nicotine • Acetylcholine EC_{50} = 3.19 μM • Levamisole EC_{50} = 1.15 μM • Antagonist profile: d-tubocurarine (100 μM) > mecamylamine (30 μM) > DhβE (10 μM)[36] • *Hco*-L-AChR1.4 (ACR-8, UNC-29.4, UNC-38 and UNC-63a)[36] • Agonist profile: acetylcholine > levamisole > DMPP >>> bephenium ≈ pyrantel ≈ nicotine • Acetylcholine EC_{50} = 3.03 μM[35] • Levamisole EC_{50} = 2.48 μM[35] • Antagonist profile: d-tubocurarine (100 μM) > mecamylamine (30 μM) > DhβE (10 μM)[36]
	DEG-3 family cholinemonepental nAChRs	DEG-3 α type	Not known	• DEG-3/DES-2 heteromeric channel:[37] • Preferentially activated by choline • Choline EC_{50} = 9.9 mM • Monepantel and monepantel sulfone (AAD-4670) potentiated choline-mediated currents • MPTL-1 homomeric channel:[23] • Activated by betaine and choline • Betaine EC_{50} = 41 μM • Choline EC_{50} = 1.3 mM • 3 μM monepantel behaved as superagonist of the channel with currents much larger than those elicited by maximal concentrations of choline and betaine • Monepantel produced potentiation of Choline- and betaine-mediated currents
		DES-2 α type	Not known	
		MPTL-1 α type	Not known	
	Morantel sensitive nAChRs	ACR-26 α type	Body wall muscles and nerve ring[38]	• ACR-26/27 assemble as a heteromeric channel[38] • Agonist profile: morantel > acetylcholine > pyrantel[38] • Acetylcholine EC_{50} = 80.1 μM[38] • Morantel EC_{50} = 29 μM[38] • Pyrantel EC_{50} = 6.8 μM[38]
		ACR-27 non-α type	Body wall muscles and nerve ring[38]	
O. dentatum	Levamisole-/Pyrantel-/Tribendimidine/Acetylcholine - sensitive nAChR	UNC-38 α type	Not known	• Ode-UNC-29/UNC-63 Pyrantel-nAChR:[39] • Agonist profile: pyrantel > tribendimidine > nicotine ≈ levamisole ≈ acetylcholine • Pyrantel EC_{50} = 0.09 μM • Tribendimidine EC_{50} = 3.9 μM • Acetylcholine EC_{50} = 72.4 μM • Ode-UNC-29/UNC-63/UNC-38 Pyrantel/tribendimidine-nAChR:[39] • Agonist profile: pyrantel ≈ tribendimidine > acetylcholine > nicotine ≈ levamisole • Pyrantel EC_{50} = 0.4 μM • Tribendimidine EC_{50} = 2.2 μM • Acetylcholine EC_{50} = 13.2 μM • Ode-UNC-29/UNC-63/ACR-8 Acetylcholine-nAChR:[39] • Agonist profile: acetylcholine > tribendimidine ≈ levamisole ≈ pyrantel > nicotine ≈ thenium ≈ bephenium • Acetylcholine EC_{50} = 3.5 μM • levamisole EC_{50} = 2.2 μM • Tribendimidine EC_{50} = 0.8 μM • Ode-UNC-29/UNC-63/UNC-38/ACR-8 Levamisole-nAChR:[39] • Agonist profile: levamisole > acetylcholine > tribendimidine ≈ levamisole ≈ thenium ≈ bephenium ≈ pyrantel > nicotine • Levamisole EC_{50} = 3.1 μM • Acetylcholine EC_{50} = 4.2 μM • Tribendimidine EC_{50} = 69 μM
		UNC-63 α type	Not known	
		ACR-8 α type	Not known	
		UNC-29 non-α type	Not known	
P. equorum	Morantel sensitive nAChRs	ACR-26 α type	Body wall muscles and nerve ring[38]	• ACR-26/27 assemble as a heteromeric channel[38] • Agonist profile: acetylcholine > morantel > levamisole > pyrantel[38] • Acetylcholine EC_{50} = 34.9 μM[38] • Morantel EC_{50} = 0.98 μM[38] • Pyrantel EC_{50} = 0.32 μM[38]
		ACR-27 non-α type	Body wall muscles and nerve ring[38]	
Hookworms (*A. caninum, A. ceylanicum, N. americanus*)	Nicotine sensitive nAChRs	ACR-16 α type	Not known	• *Aca*-ACR-16 homomeric channel[40] • Agonist profile: 3-bromocytisine > acetylcholine > epibatidine > cytisine > nicotine > DMPP • Acetylcholine EC_{50} = 50 μM • 3-Bromocytisine EC_{50} = 1.5 μM • Antagonist profile (10 μM): d-tubocurarine ≈ mecamylamine ≈ DHβE > derquantel > hexamethonium > α-BTX • *Ace*-ACR-16 homomeric channel[41] • Agonist profile: nicotine > nornicotine > choline > oxantel > pyrantel > morantel > bephenium • Acetylcholine EC_{50} = 20.64 μM • Nicotine EC_{50} = 24.33 μM • Levamisole and mecamylamine produced potent inhibition of acetylcholine-mediated currents • *Nam*-ACR-16 homomeric channel[41] • Agonist profile: nicotine > pyrantel > betaine

(*continued on next page*)

Table 1 (*continued*)

Organism	Receptor type	nAChR subunit	Tissue distribution	Pharmacology
				• Acetylcholine $EC_{50} = 170.1$ μM • Nicotine $EC_{50} = 570.7$ μM • Levamisole and mecamylamine produced potent inhibition of acetylcholine-mediated currents
T. suis	Oxantel-sensitive nAChRs	ACR-16 α type	Not known	• Agonist profile: oxantel ~ ACh >>> pyrantel >>> epibatidine > nicotine ~ 3-bromocytisine ~ DMPP ~ morantel ~ cytisine ~ levamisole[42] • Acetylcholine $EC_{50} = 14.5$ μM[42] • Oxantel $EC_{50} = 9.49$ μM[42] • Pyrantel $EC_{50} = 148.5$ μM[42] • Antagonist profile: α-BTX >>> DHβE > derquantel[42]

[1] www.wormbase.org.
[2] Fleming et al. (1997).
[3] Gottschalk and Schafer (2006).
[4] Rowland et al. (2006).
[5] Liu et al. (2011).
[6] McKay et al. (2003).
[7] Hunt-Newbury et al. (2007).
[8] Culetto et al. (2004).
[9] Towers et al. (2005).
[10] Gally et al. (2004).
[11] Boulin et al. (2008).
[12] Touroutine et al. (2005).
[13] Blanchard et al. (2018).
[14] Fox et al. (2007).
[15] Feng et al. (2006).
[16] Francis et al. (2003).
[17] Ballivet et al. (1996).
[18] Treinin and Chalfie (1995).
[19] Treinin et al. (1998).
[20] Yassin et al. (2001).
[21] Rufener et al.(2013).
[22] Peden et al. (2013).
[23] Baur et al. (2015).
[24] McKay et al. (2004).
[25] Choudhary et al. (2020).
[26] Jospin et al. (2009).
[27] Qi et al. (2013).
[28] Petrash et al. (2013).
[29] Squire et al. (1995).
[30] Baylis et al. (1997).
[31] Williamson et al. (2009).
[32] McHugh et al. (2020).
[33] Abongwa et al. (2017).
[34] Bennett et al. (2012).
[35] Boulin et al. (2011).
[36] Duguet et al. (2016).
[37] Rufener et al. (2010).
[38] Courtot et al. (2015).
[39] Buxton et al. (2014).
[40] Choudhary et al. (2019).
[41] Kaji et al. (2020).
[42] Hansen et al. (2020).

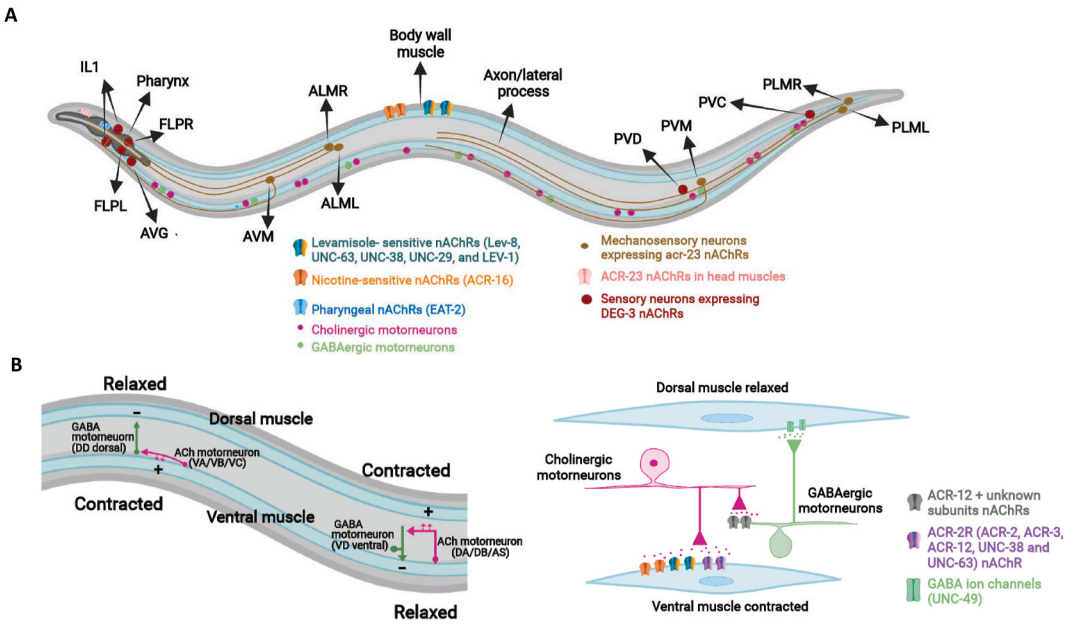

Fig. 3. *C. elegans* nAChRs. **A.** Major type of nAChRs and their tissue expression in *C. elegans*. Each subtype of the receptor is highlighted in different color. **B.** Locomotion circuit of *C. elegans* showing cholinergic motor neurons forming synapses onto both body wall muscles to cause contraction and onto GABAergic motoneurons that lead to release of GABA and cross inhibition of opposite muscles (Adapted from wormatlas.org). The nAChRs subtypes involved in the locomotion circuit are shown in different colors at muscle cell level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

recombinant expression studies (Boulin et al., 2008). These proteins are important molecular components required for the assembly and trafficking of mature nAChRs. RIC-3 is a small endoplasmic reticulum transmembrane protein that acts as a chaperone to promote receptor folding, assembly, or maturation (Halevi et al., 2002; Millar, 2008). The mutants of genes *unc-50* and *unc-74* lack high-affinity levamisole binding (Lewis et al., 1980a). UNC-50, a transmembrane protein of the Golgi complex, prevents lysosomal targeted destruction of the subunits of levamisole sensitive nAChR (Eimer et al., 2007). UNC-74 is a thioredoxin-containing protein required for trafficking the nAChR subunits to the synapses (Haugstetter et al., 2005). Heterologous expression of an active levamisole-sensitive channel requires co-expression of eight genes, five nAChR subunits, and three ancillary proteins. The precise stoichiometry and assembly order of the five subunits are still unclear, but expression and electrophysiology studies suggest that UNC-29 and LEV-1 most likely assemble with either UNC-63 or UNC-38 (Fleming et al., 1997; Jones and Sattelle, 2004). The expressed L-type receptor was more sensitive to levamisole (EC_{50} = 10.1 μM) than acetylcholine (EC_{50} = 26 μM), but levamisole produced smaller inward currents in comparison. Acetylcholine currents were strongly inhibited by d-tubocurarine, methyllycaconitine, and hexamethonium. In agreement with the *in vivo C. elegans* L-type receptors, dihydro-β-erythroidine and α-bungarotoxin produced only modest blocking effects on the acetylcholine responses of L-type nAChR (Richmond and Jorgensen, 1999; Boulin et al., 2008). MOLO-1 (MOdulator Of LevamisOle receptor-1) is a single-pass transmembrane protein that functions as an auxiliary protein for the L-type receptors (Boulin et al., 2012). Auxiliary proteins are non-pore forming components of channels that interact with the receptor directly and modulate the channel properties; they can also assist in trafficking the receptor in heterologous cell systems (Yan and Tomita, 2012). An auxiliary subunit must have a demonstrable biological impact on certain aspects of native channel function *in vivo*. MOLO-1 affects synaptic transmission in the worms and promotes channel opening in the receptors expressed in heterologous systems. It also regulates the levamisole-sensitive nAChRs function through direct physical interaction. Null mutants of the protein result in impaired locomotion and loss of sensitivity to levamisole. The auxiliary proteins for nAChRs further amplify their heterogeneity and pharmacological diversity while expanding the range of potential drug targets for these channels. *C. elegans* body wall muscles also express the ACR-8 subunit (Touroutine et al., 2005; Holden-Dye et al., 2013). It is thought to act as a spare subunit for the levamisole sensitive nAChRs and may replace LEV-8 in its absence (Hernando et al., 2012). Blanchard et al. (2018) replaced *Cel*-LEV-8 of the L-type receptor with ACR-8 subunit from various parasitic nematodes (*H. contortus/O. dentatum/A. suum/D. immitis/Meloidogyne incognita*) and expressed the nAChR in oocytes. Each of the combination of *C. elegans* nAChR complemented with either *Hco*-ACR-8/*Ode*-ACR-8/*Asu*-ACR-8/*Dim*-ACR-8 nAChR produced functional channel gated by acetylcholine and levamisole, albeit with a drastic reduction in acetylcholine EC_{50} values. However, substitution of either *Cel*-UNC-63 or UNC-38 with *H. contortus* orthologs did not modulate the sensitivity of acetylcholine for the receptor, suggesting that ACR-8 subunit is an important structural determinant of acetylcholine activity of the complemented *C. elegans* channel (Blanchard et al., 2018). In the same study two additional *C. elegans* L-type receptors (*Cel*-L-AChR-2.1: *Cel*-ACR-8, *Cel*-UNC-63, *Cel*-UNC-38, *Cel*-UNC-29, and Cel-LEV-1 and *Cel*-L-AChR-2.2: *Cel*-ACR-8, *Cel*-UNC-63, *Cel*-UNC-38, and *Cel*-UNC-29) were expressed. Both *Cel*-L-AChR-2.1 and *Cel*-L-AChR-2.2 assembled into functional channels with decreased efficacy and sensitivity for levamisole but increased potency for pyrantel. The study showed that ACR-8 and LEV-8 subunits contribute to different subsets of L-type nAChRs with different pharmacological sensitivities (Blanchard et al., 2018). Bephenium is another anti-nematodal drug that targets L-type nAChRs in *C. elegans* (Turani et al., 2018). An L-type nAChR mutant strain was resistant to bephenium-induced paralysis, while wild-type worms' exposed to a combination of bephenium with levamisole produced an additive paralysis. Turani and colleagues recorded channel activity to demonstrate that bephenium is an agonist of the receptor and produces open-channel block at higher concentrations (Turani et al., 2018).

C. elegans nicotine-sensitive nAChRs (N-type nAChRs) are homopentamers formed by co-assembly of ACR-16 subunits (initially designated Ce21), a closely related homolog of the vertebrate α-7 subunit (Ballivet et al., 1996; Raymond et al., 2000; Touroutine et al., 2005).

Electrophysiology studies on *C. elegans* confirmed the presence of another type of levamisole insensitive receptor in the body wall muscles (Richmond and Jorgensen, 1999). In *unc-29* mutants, dihydro-β-erythroidine completely blocked nicotine response. The acetylcholine response in wild-type worms was only about 50% blocked by dihydro- β-erythroidine. *unc-38* and *unc-29* mutants displayed locomotory defects but were responsive to both acetylcholine and nicotine, suggesting an equal contribution of the N-type receptors to the cholinergic ionotropic responses (Richmond and Jorgensen, 1999). Two different studies have established ACR-16 as a component of N-type ion channels and identified its contribution to body wall muscles' excitability (Francis et al., 2005; Touroutine et al., 2005). The fast cholinergic current is eliminated in *unc-29*:*acr-16* nulls while there is an almost complete elimination of nicotine-dependent current in *acr-16* mutants (Francis et al., 2005). Touroutine et al. (2005) made similar observations with *unc-63*:*acr-16* double mutants and suggested that ACR-16-dependent receptors contribute 85% of the muscle exogenous acetylcholine response. *unc-63*:*acr-16* and *unc-29*:*acr-16* double mutants in *C. elegans* produced a more robust synthetic phenotype with locomotor defects more severe than any single mutants (Francis et al., 2005; Touroutine et al., 2005; Li et al., 2014b). This shows that ACR-16 (nicotine sensitive nAChR) combined with UNC-63 and UNC-29 (components of levamisole-sensitive nAChR) contribute to locomotor behavior in the worms. The ACR-16 subunit, like vertebrate α7 subunits, expresses as a functional homomeric receptor in oocytes (Ballivet et al., 1996; Raymond et al., 2000). The homomeric receptor produces rapidly desensitizing responses to acetylcholine and nicotine, with nicotine being a partial agonist and more potent than acetylcholine. Levamisole and oxantel do not behave as agonists but produce inhibition of the N-type receptor probably through open channel block. The *C. elegans* ACR-16 nAChR is potently inhibited by dihydro-β-erythroidine and d-tubocurarine but is relatively insensitive to methyllycaconitine and α-bungarotoxin (Couturier et al., 1990; Ballivet et al., 1996; Raymond et al., 2000).

Two members of the nematode-specific DEG-3 family, DEG-3 (α subunit) and DES-2 (DEgeneration Suppressor; α subunit), are part of the same operon that co-transcribes the two subunits before splicing into separate mRNAs (Treinin et al., 1998). These subunits generate a functional heteromeric channel formed by the two α subunits when co-expressed. DEG-3/DES-2 nAChRs have a strikingly higher affinity for choline than acetylcholine and produces ~13 fold higher current response (Yassin et al., 2001). DEG-3/DES-2 nAChRs are blocked by d-tubocurare and strychnine. DEG-3 and DES-2 are localized in sensory endings of chemosensory and PVD polymodal nociceptor neurons (Treinin and Chalfie, 1995; Treinin et al., 1998; Yassin et al., 2001; Cohen et al., 2014). Mutants of *deg-3* display a neuronal degeneration, uncoordination and touch insensitivity phenotype due to increased channel activity, while *des-2* mutants are suppressors of the *deg-3* mutation phenotype (Treinin and Chalfie, 1995; Treinin et al., 1998). Thus, DEG-3 and DES-2 possibly have a notable role in regulating chemotaxis behavior and responses to noxious stimuli (Yassin et al., 2001; Jones and Sattelle, 2004; Cohen et al., 2014). Another member of the DEG-3 group, ACR-23, is the target site for an aminoacetonitrile derivative, monepantel (AAD-1566). ACR-23 activation causes hypercontraction of body wall muscles and spasmodic contractions of the anterior portion of the pharynx leading to worm death (Kaminsky et al., 2008; Kaminsky and Rufener, 2012). ACR-23 nAChRs are expressed in the *C. elegans* body wall muscles, six mechanosensory neurons, and multiple interneurons (Peden et al., 2013; Rufener et al., 2013). Mutants of *acr-23* exhibit swimming defects, molting defects, display necrosis characteristics, and are resistant to monepantel (Kaminsky et al., 2008). ACR-23 subunits assemble into a homopentamer when expressed in *Xenopus* oocytes. These ion channels are sensitive to monepantel, choline, and betaine, with monepantel strongly potentiating choline and betaine mediated responses (Peden et al., 2013; Rufener et al., 2013). ACR-20, another member of the DEG-3 family, expresses as a betaine- and choline-gated homomeric nAChR in *Xenopus* oocytes. Monepantel acts as a positive allosteric modulator of this nAChR at low concentrations (<1 nM) and as an agonist at high concentrations (>0.1 μM) (Baur et al., 2015).

C. elegans pharyngeal receptors are composed of a non-alpha nAChR subunit, EAT-2 (EATing: abnormal pharyngeal pumping), and a small protein EAT-18 (McKay et al., 2004; Choudhary et al., 2020). The nematode pharynx is a neuromuscular organ that undergoes rhythmic peristalsis controlled by excitatory and inhibitory neurotransmission and is essential for feeding (Albertson and Thomson, 1976; Raizen et al., 1995). The excitatory component of a pharyngeal muscle action potential is under MC neurons control that synapse onto marginal cells. This neurotransmission is dependent on cholinergic inputs and requires *eat-2* and *eat-18* gene expression in the pharyngeal muscles (Avery, 1993a; Raizen et al., 1995; McKay et al., 2004). A selective drug that targets pharyngeal nAChR could lead to pharyngeal paralysis and starvation or sluggish behavior in worms, helping with faster expulsion from the host. Choudhary et al. (2020) co-expressed the *C. elegans* EAT-2 nAChR subunit with EAT-18 in *Xenopus* oocytes and reported active expression of a noncanonical channel composed of a non-alpha subunit. *eat-18* encodes for two splice variants, EAT-18c and EAT-18d, which differ in the C-terminal region. Both the isoforms were able to form a functional receptor when expressed with EAT-2. Eat-18 was shown to function as an auxiliary protein for the pharyngeal receptor. It was essential for the assembly of a functional nAChR, directly interacted with the mature receptor and modified pharmacological properties. The receptor's pharmacological properties were distinct from the somatic muscle and mammalian nAChRs with many cholinergic anthelmintics (morantel, levamisole, bephenium, tribendimidine, pyrantel) failing to activate the EAT-2/EAT-18 cation channel. The nicotinic antagonists, α-bungarotoxin and DhβE, were unable to show any blocking effect on the receptor. The ACR-7 subunit, an ACR-16 group member, is also expressed in *C. elegans* pharyngeal muscles, and *acr-7* mutants show a slight reduction in pharyngeal pumping (Saur et al., 2013). Interestingly, *lev-8* mutants display a decreased pharyngeal pumping phenotype; however, the subunit is not expressed on the pharynx or associated neurons (Towers et al., 2005).

ACR-2 and ACR-3 are non-alpha subunits belonging to the UNC-29 family, encoded by a single operon's genes (Squire et al., 1995; Baylis et al., 1997). ACR-2 expresses in the ventral nerve cord cholinergic motor neurons while ACR-3 localizes in the body wall muscles and nervous system (Squire et al., 1995; Baylis et al., 1997; Jospin et al., 2009; Holden-Dye et al., 2013). Co-expression of either ACR-2 or ACR-3 with UNC-38 in *Xenopus* oocytes formed a levamisole sensitive cation channel with mecamylamine behaving as an antagonist of the channel (Squire et al., 1995; Baylis et al., 1997). ACR-2 has been shown to provide excitatory inputs to the body muscles and plays a vital role in the coordinated excitation and inhibition of body muscle movement (Jospin et al., 2009). *acr-2* loss of function mutants have decreased excitability of cholinergic motor neurons resulting in mildly sluggish locomotion. Conversely, the *acr-2* gain of function mutants hyperactivates the release of neurotransmitters from the cholinergic motor neurons and additionally indirectly silences downstream GABA (γ-aminobutyric acid) motor neurons. The resulting imbalance in the motor neuron circuit disrupts coordinated body muscle contraction. In the same study, Jospin et al. (2009) expressed a heteromeric receptor, ACR-2R, composed of the two non-α subunits, ACR-2 and ACR-3, with three α-subunits, UNC-38, UNC-63, and ACR-12. The ACR-2R composition partially overlaps with the L-type receptor but showed distinct pharmacology. The nAChR was gated by nicotine but not levamisole and was efficiently blocked by mecamylamine. *acr-12* expression is localized in GABA motoneurons and cholinergic motoneurons and the ACR-12 subunit is possibly a part of nAChR complex other than ACR-2R that are present on GABA motoneurons (Petrash et al., 2013). The loss-of function mutants of the gene increase sensitivity to drugs that enhance cholinergic excitability, decrease inhibitory motoneuron activity and alter locomotion behavior. The primary function of ACR-12 nAChRs is to

accomplish synaptic coupling between cholinergic and GABA signaling to limit hyperactive motor output (Petrash et al., 2013). While majority of the information on nematode nAChRs arise from studies in *C. elegans* subsequent studies on parasitic nematodes reveal significant differences in receptor expression, function, and properties.

2.1.2. *Ascaris suum* nAChRs

The recent completion of genome and transcriptome studies of many parasitic nematode species have identified a plethora of new nematode-specific ion channel subunits (Blaxter et al., 1998; Parkinson et al., 2004; Ghedin et al., 2007; Williamson et al., 2007; Jex et al., 2011; Laing et al., 2013). Parasitic nematode nAChRs have different subunit composition, biophysical and pharmacological properties from the *C. elegans* receptors (Table 1). *A. suum* (Clade III) is extensively used in the characterization of the nAChRs present at neuromuscular junctions. *A. suum* has large muscle cells making them suitable candidates for electrophysiological manipulation as well as for analysis of receptor subunit distribution. The first evidence for the presence of acetylcholine receptors in *A. lumbricoides* was shown by Baldwin and Moyle (1949) through demonstration of contraction responses to acetylcholine in muscle. In 1959, intracellular recordings from *A. lumbricoides* muscle cells and their spontaneous activity were recorded (Jarman, 1959). The nAChR channels were described for the first time by Pennington and Martin (1990) in *A. suum* muscles using inside-out patch-clamp recordings. They demonstrated the presence of at least two types of acetylcholine-activated channel currents based on single-channel conductance. Robertson and Martin (1993) also used the same technique in *A. suum* muscle vesicles to examine the levamisole-activated channels with a conductance range of 18–50 pS and brief (~1 ms) open times. Levamisole behaved as an agonist of the channel at a lower concentration (1–10 μM) and caused channel block at higher concentrations (30 and 90 μM). These nAChRs were gated by pyrantel and oxantel which also caused an open channel block of the nAChRs at higher concentrations (Robertson et al., 1994; Dale and Martin, 1995). Robertson et al. (2002) studied the effect of combinations of several agonists and antagonists on muscle contraction to identify different types of nAChRs in *A. suum*. The single-channel properties and their distinguishable pharmacology was characterized by Qian et al. (2006). The three subtypes of nAChRs expressed in *A. suum* muscles are L-type preferentially activated by levamisole and antagonized by paraherquamide, the N-type preferentially activated by nicotine, and the B-type preferentially activated by bephenium and antagonized by paraherquamide and derquantel. The single-channel conductance is 24 pS for the N-type, 35 pS for the L-type, and 45 pS for the B-type. The channels' mean open times are also different for channel subtypes, 0.6 msec for N-type, 0.8 msec for L-type, and 1.2 msec for B-type channels. While nicotine and levamisole produce nAChR subtype-selective responses in *C. elegans*, in *A. suum,* pharmacology differences are more subtle. Levamisole can open all three subtypes of *A. suum* nAChRs at high concentrations but activates L-type channels preferentially. Bephenium prefers gating B-type channels and can also open some L-type channels. The presence of different nAChR subtypes likely contributes to varying sensitivities to anthelmintic drugs and, besides, may be exploited by nematodes to develop drug resistance.

Williamson et al. (2009) identified homologs of *unc-29* and *unc-38* in *A. suum* as possible components of the native L-type nAChR. Both the genes were localized on muscle cell membranes and expressed as levamisole- and nicotine-gated nAChR in *Xenopus* oocytes. The relative expression levels and stoichiometry of the two subunits altered the ligands' sensitivity for the receptor. When the oocytes were injected with 5:1 of UNC-38:UNC-29, nicotine acted as a full agonist; oxantel produced robust responses, levamisole was a partial agonist, and pyrantel showed weak responses. When the ratio was reversed to 1:5, levamisole behaved as a full agonist, pyrantel induced a strong response, nicotine was a partial agonist, and oxantel failed to induce measurable currents. Subunit-ratios of 10:1 or 1:10 produced minimal current responses that could not be characterized. Williamson et al. (2009) suggested that $(Asu\text{-}UNC\text{-}38)_2(Asu\text{-}UNC\text{-}29)_3$ possibly constitutes the L-subtype nAChR and $(Asu\text{-}UNC\text{-}38)_3(Asu\text{-}UNC\text{-}29)_2$ constitutes the N-subtype in *A. suum.* However, as with initial heterologous studies on the *C. elegans* levamisole receptor, current responses were relatively modest suggesting other subunits may contribute to the receptors *in vivo*. McHugh et al. (2020) used RT-PCR and RNAscope *in situ* hybridization to establish the expression of putative *A. suum* levamisole receptor, *Asu-unc*-38, *Asu-unc*-29, *Asu-unc*-63, and *Asu-acr*-8, in the intestinal and muscle cells of the nematode. In the same study, calcium flux fluorescent assays identified functional nAChRs in the intestine. These channels were activated by acetylcholine and levamisole while mecamylamine blocked the intestine's nAChRs, suggesting a possible paracrine role in the tissue's receptor.

Abongwa et al. (2017) cloned orthologs of ACR-16 in *A. suum* and reconstituted a homopentameric nAChR in the *Xenopus* oocytes. Unlike *C. elegans*, the *Asu*-ACR-16 has a more widespread distribution and is expressed in the somatic muscle, pharynx, ovijector, and head, suggesting the nAChR may have various tissue-related functions. The *A. suum* channel was most sensitive to nicotine, insensitive to levamisole and pyrantel, as was observed with the *C. elegans* ACR-16 nAChR. Morantel behaved as a non-competitive antagonist of the *A. suum* nAChR but was less potent in comparison to *C. elegans* (Raymond et al., 2000; Abongwa et al., 2016a). There were differences in the antagonist pharmacology between the two homologs, with the *A. suum* channel being susceptible to mecamylamine and moderately sensitive to hexamethonium and DhβE. ACR-16 homologs from both species were nearly insensitive to α-bungarotoxin, which produced a potent antagonist effect in α-7 vertebrate receptors (Ballivet et al., 1996; Zhao et al., 2003; Abongwa et al., 2016b). Ivermectin, genistein, and PNU120596, positive allosteric modulators of α-7 vertebrate nAChRs, produced inhibitory effects on *Asu*-ACR-16 (Ballivet et al., 1996; Abongwa et al., 2016b). The pharmacological properties of the Asu-ACR-16 receptor are distinct from previously characterized nAChRs.

ACR-26, a vertebrate α-7 like receptor subunit, is exclusively present in parasitic nematode species (Bennett et al., 2012). The subunit is expressed in head muscle arms, but not body-wall muscle cells of *A. suum*. ACR-26 can be expressed as an active homomeric receptor gated by acetylcholine and nicotine, albeit with an unreliable expression suggesting that either the subunit requires additional chaperone proteins or contributes to the function of other heteromeric muscle nAChRs in the worm. The receptor was very sensitive to acetylcholine (EC_{50} between 10 and 100 nM), suggesting that a lower level of cholinergic signals may activate the ACR-26 nAChRs *in vivo* but its function has yet to be elucidated (Bennett et al., 2012).

The pharynx of *A. suum* has contractile elements and functions as a feeding pump that undergoes rhythmic peristalsis and is an anthelmintic drug target (Brownlee et al., 1995; Martin, 1996). del Castillo and Morales (1967a, 1967b); Byerly and Masuda (1979) used current-clamp and voltage-clamp techniques to record responses from the *A. suum* pharyngeal muscles. The pharyngeal cholinergic component was determined by measuring significant depolarization in response to acetylcholine application on the pharynx preparation (Choudhary et al., 2020). Several muscarinic agonists showed negligible changes in membrane conductance, establishing nAChRs as major mediators of cholinergic response in *A. suum* pharynx. The functional spectrum of cholinomimetic agonists and antagonists on the pharynx was shown to be distinct from that of somatic nematode nAChRs and vertebrate nAChRs. Choudhary et al. (2020) identified and cloned orthologs of EAT-2 and EAT-18 from *A. suum*. Unlike *C.* elegans, the functional recapitulation of the unique non-α homomeric EAT-2 nAChR in *Xenopus* oocytes required co-expression of the auxiliary protein, *Asu*-EAT-18, along with the ancillary protein, *Asu*-RIC-3. The comparative pharmacological agonist rank order series acquired from both *in vivo* pharyngeal *A. suum* recordings and heterologous expressed EAT-2/18 recordings revealed a similar profile. This suggests that EAT-2 and EAT-18 most

likely constitute the pharyngeal nicotinic response in the parasitic nematode. The pharmacological properties of the EAT-2 nAChR were also distinct from the somatic muscle and mammalian nAChRs, making it a potential drug target. Additionally, the *eat-2* transcript was expressed in reproductive tissues, head region, as well as pharynx. The mRNA for *eat-18* was described in the same tissues and the gut region. This widespread distribution of EAT-2 and EAT-18 mRNA implies additional, non-feeding, roles for the proteins and the possibility of interaction with other subunits.

2.1.3. Other parasitic nematode nAChRs

Haemonchus contortus, a clade V nematode, is phylogenetically related to *C. elegans,* but the heteromeric levamisole receptor has a different subunit composition in the sheep nematode (Table 1). *H. contortus* does not have an ortholog of *lev-8* and the predicted subunit product of *Hco-lev-1* is not incorporated into the levamisole sensitive receptors as it lacks a signal peptide (Boulin et al., 2011). *H. contortus* does possess the orthologs of nAChR subunit encoding genes, viz, *unc-29, unc-38,* and *unc-63*, that comprise the L-type ion channels. *unc*-29 has undergone multiple duplications in the trichostrongylid worm. It encodes for four paralogs of the subunit (UNC-29.1, UNC-29.2, UNC-29.3, and UNC-29.4), with UNC-29.1 being most similar to *C. elegans* UNC-29 ((Neveu et al., 2010; Duguet et al., 2016). Neveu et al. (2010) have identified a truncated variant of *unc-63*, *Hco-unc-63b*, which encodes for a non-functional UNC-63 subunit that may contribute to levamisole resistance. The *H. contortus* ACR-8 subunit is similar to *C. elegans* ACR-8 and also shares typical agonist binding amino acid signatures with *C. elegans lev-8* (Boulin et al., 2011). *Hco-acr-8b*, a truncated variant of *acr-8*, was identified in levamisole resistant strains of the nematode and likely acts as a marker for levamisole resistance (Fauvin et al., 2010; Williamson et al., 2011). Boulin et al. (2011) functionally reconstituted two L-type receptors of *H. contortus*, *Hco*--L-AChR1, and *Hco*-L-AChR2, by co-injecting nAChR subunits with conserved ancillary proteins *Hco*-RIC-3.1, *Hco*-UNC-50, and *Hco*-UNC-74. *Hco*-L-AChR1 is composed of *Hco*-ACR-8, *Hco*-UNC-29.1, *Hco*-UNC-38, and *Hco*-UNC-63a, and to assemble *Hco*-L-AChR2 ACR-8 is removed from the combination. The absence of ACR-8 from *Hco*--L-AChR1 generated pronounced differences in their pharmacological properties. *Hco*-L-AChR1 was more sensitive to levamisole than acetylcholine and weakly sensitive to pyrantel and nicotine (Boulin et al., 2011). *Hco*-L-AChR2 showed a lower expression level than *Hco*-L-AChR1 and was more sensitive to pyrantel and nicotine than levamisole. Bephenium selectively activated *Hco*-L-AChR1 in a dose-dependent manner, and *Hco*-L-AChR2 was insensitive to the compound, highlighting the requirement of ACR-8 to form a bephenium selective binging site. Blanchard et al. (2018) used electrophysiology recordings and gene silencing techniques to demonstrate the critical role of the ACR-8 subunit *in vivo* and *in vitro* levamisole sensitivity. ACR-8 from parasitic species including *H. contortus* and *A. suum* can functionally complement the *C. elegans* L-AChR lacking LEV-8, suggesting that ACR-8 subunits are an important element of pharmacological responses. In the same study, *H. contortus* ACR-8 restored the sensitivity of the body wall muscles of *C. elegans lev-8* mutants to levamisole, showing the importance of the subunit for levamisole sensitivity *in vivo.* Three additional functional L-type receptors with different pharmacological profiles have been reconstituted using the other paralogs of the UNC-29 (UNC-29.2, UNC-29.3, and UNC-29.4) with UNC-38, UNC-63, and ACR-8, which adds to the complexity and diversity of the nAChRs in the parasitic nematode (Neveu et al., 2010; Duguet et al., 2016). *Hco*-UNC-29.2 did not assemble into a functional receptor. Substitution of UNC-29.1 (L-AChR 1.1) by either UNC-29.3 (L-AChR 1.3) or UNC-29.4 (L-AChR 1.4) resulted in an operational acetylcholine-gated channel. L-AChR1.1 was more responsive to levamisole than acetylcholine, and mecamylamine was a potent antagonist of the nAChR. L-AChR1.3 channel was less responsive to acetylcholine, and mecamylamine was a less potent antagonist. With L-AChR1.4, levamisole and acetylcholine responses were both equipotent, and mecamylamine was a powerful antagonist.

Rufener et al. (2009) identified mutations in members of DEG-3 superfamily, *Hco-mptl-1* (MonePanTeL)*, deg-3,* and *des-2* from *H. contortus* with reduced monepantel sensitivity, implicating them as likely targets for the monepantel. *H. contortus* DEG-3/DES-2 nAChRs expressed as choline-gated ion channels in *Xenopus* eggs (Rufener et al., 2010). Monepantel did not act as an agonist of the DEG-3/DES-2 receptor but produced type II positive allosteric modulation of choline-mediated currents at micromolar concentrations. The addition of MPTL-1 to DEG-3/DES-2 nAChR did not alter the pharmacology of choline or monepantel on the receptor, suggesting that the combination of these three subunits is unlikely to constitute the native monepantel target. Baur et al. (2015) expressed *H. contortus* MPTL-1, a close homolog of *C. elegans* ACR-20, homomeric receptors in *Xenopus* oocytes. MPTL-1 nAChRs were gated by betaine and choline, while monepantel directly opened the channels at >100 nM concentrations and potentiated betaine or choline currents at 1 nM. Thus, as observed with *C. elegans*, *the* DEG-3 family contributes to monepantel sensitivity in *H. contortus.*

Courtot et al. (2015) and Bennett et al. (2012) have identified homologs of ACR-26, an α subunit, and ACR-27, a non-α subunit, from parasitic nematodes belonging to Clade III, IV, and V. In *H. contortus,* both subunit encoding genes express in various developmental stages on the parasite and co-express in body wall muscle and nerve ring of the worms. *H. contortus* ACR-26 or ACR-27 did not express as functional homomeric channels but formed a novel class of active heteromeric nAChRs when co-expressed in *Xenopus* oocytes (Bennett et al., 2012; Courtot et al., 2015). *H. contortus* ACR-26/27 nAChRS were more sensitive to morantel and pyrantel than acetylcholine and were relatively insensitive to levamisole. *Hco*-L-AChR2 muscle nAChRs are also very sensitive to pyrantel but showed poor responses to morantel, demonstrating ACR-26/27 receptors as a major target for morantel. In the same study, *Parascaris equorum* ACR-26/27 heteromeric receptors were expressed in oocytes. The pharmacological properties of the channel were similar to the *H. contortus* channel with conserved sensitivity to morantel. *P. equorum* ACR-26/27 channel showed a higher sensitivity for acetylcholine, pyrantel, and morantel than *H. contortus* channel. Courtot et al. (2015) also reported that *Hco*-26/27 and *Peq*-26/27 increase the sensitivity of morantel and pyrantel in the transgenic *C. elegans* worms resistant to a high concentration of the drugs. Thus ACR-26/27 constitutes a novel class of nAChRs that are potential pharmacological targets of morantel and pyrantel.

As observed in *A. suum*, *Oesophagostomum dentatum*, a clade V strongyle parasite, expressed multiple biophysical subtypes of muscle nAChRS defined by their single-channel conductance. Robertson et al. (1999) used patch-clamp techniques and identified four main subtypes if nAChRs, G25 pS, G 35 pS, G 45 pS, and G 40 pS in levamisole-sensitive (SENS) *O. dentatum* nematodes. Interestingly, the G35 channel type was not present in levamisole resistant (LEVR) parasites, and possibly represents the L-type nAChR in *O. dentatum*. In pyrantel-resistant *O. dentatum*, four conductance states of the nicotinic receptors were present. Still, there was a reduction in the percentage of active channels compared to wild-type worms suggesting mechanisms of resistance may vary depending on the specific anthelmintic (Robertson et al., 2000). Buxton et al. (2014) cloned and expressed homologs of subunit genes, *unc-38, unc-29, unc-63,* and *acr-8* from *O. dentatum* to reconstitute the nAChRs in oocytes. Four pharmacologically different receptor subtypes were identified by varying subunit combinations of four *O. dentatum* nAChR subunits. A combination of *Ode*-UNC-29, *Ode*-UNC-63, *Ode*-UNC-38, and *Ode*-ACR-8 subunits reconstituted the L-type receptor, which responded to levamisole and had a single-channel conductance of 35.1 ± 2.4 pS, which is close to G35 pS type channels observed *in vivo*. The pyrantel/tribendimidine-sensitive nAChR was composed of *Ode*-UNC-29, *Ode*-UNC-63, and *Ode*-UNC-38 subunits, while a receptor consisting solely of *Ode*-UNC-29 and *Ode*-UNC-63 formed a pyrantel-sensitive nAChR. The acetylcholine nAChR was composed of UNC-29, UNC-63, and ACR-8 subunits. The LEV-8 subunit has not been

detected in the strongyle parasite. Buxton et al. (2014) altered the subunit stoichiometry of the pyrantel-sensitive nAChR from 1:5 ratio of UNC-29 and UNC-63 subunits to 5:1 and observed a change in agonist pharmacology. With a 1:5 ratio, pyrantel and tribendimidine responses were significantly increased, and when the reversed stoichiometry was used, the nicotine and levamisole responses became considerably larger than the acetylcholine response.

Sequence and bioinformatics studies have identified a small number of orthologs of nAChR encoding genes, including *unc-38, unc-63, unc-29, acr-8, acr-11, acr-26, acr-12, acr-16, deg-3,* and *des-2*, in the clade V filarial nematode, *Brugia malayi* (Ghedin et al., 2007; Williamson et al., 2007; Verma et al., 2017). No orthologs of LEV-1 and LEV-8 have been identified in the worm, suggesting a comparatively less complex composition of L-type muscle nAChRs in *B. malayi*. The genes known to form L-type receptors (*unc-38, unc-63, unc-29),* and *acr-12* have overlapping cellular localization in adult *B. malayi* worms' body muscles. At the same time, *acr-26* was only expressed in males' body muscles (Li et al., 2015). It is possible that in the filarial worm, ACR-12 contributes to a subset of levamisole-sensitive receptors. Also, these subunit genes were expressed in early embryos and gametes in reproductive organs, suggesting a functional role in embryogenesis, spermatogenesis, and microfilariae release. This could explain the embryotoxic effects of levamisole in *B. malayi* (Li et al., 2015). Robertson et al. (2011) reported the single-channel properties of nAChRs from *B. malayi* muscle preparations, gated by acetylcholine and levamisole with conductance values of 26.3–28.5 pS. Levamisole produced a reversible reduction in filarial worm motility (Robertson et al., 2013). In the same study, whole-cell patch-clamp experiments from *B. malayi* muscle preparations revealed the presence of nAChRs sensitive to tribendimidine, pyrantel, levamisole, and bephenium. Verma et al. (2017) detected *Bma-acr-8, Bma-acr-16, Bma-acr-21, Bma-acr-8, Bma-unc-63, Bma-unc-38, Bma-unc-29, Bma-acr-26,* and *Bma-acr-27* transcripts in somatic muscle cells. They also reported the presence of four diverse nAChRs distinguishable by pharmacology and functional in *B. malayi* muscles, namely, morantel sensitive (M-type), L-type, P-type, and N-type nAChRs. L-type and P-type receptors were identified by knockdown of *unc*-38 and unc-29 mRNA, which led to inhibition of motility. In contrast, knockdown of *acr-16* and *acr-26* did not affect the motility in the worms suggesting a different physiological role for M- and N-type receptors. Derquantel produced the most potent competitive inhibition of morantel mediated responses and non-competitive inhibitory effects on nicotine, thus identifying M-type and N-type receptors. Additionally, monepantel failed to exhibit any activity on the muscle preparations suggesting a lack of monepantel sensitive nAChRs (DEG-3 members?) in *B. malayi*.

Multiple nAChR encoding genes have been identified in *Ancylostoma caninum*, hookworms belonging to clade V, namely, *aar-38* (now designated *Aca-unc-38*), *aar-63* (*Aca-unc-63*), *aar-29* (*Aca-unc-29*), *aar-8* (*Aca-acr-8*), *aar-15* (*Aca-acr-15*), and *aar-19* (*Aca-acr-19*) (Kopp et al., 2009). Three of these genes, *aar-38*, *aar-63*, *aar-29*, which are components of pyrantel sensitive receptors, have been shown to play a role in pyrantel resistance through reduced transcription in resistant isolates. ACR-16 orthologs from *A. caninum*, *A. ceylanicum,* and *Necator americanus* have been cloned and expressed as homomeric nAChRs in *Xenopus* oocytes (Choudhary et al., 2019; Kaji et al., 2020). Nicotine behaved as a partial agonist on *Aca*-ACR-16 and *Nam*-ACR-16 but was more potent on *Ace*-ACR-16. Oxantel produced weak activation of *Ace*-ACR-16 nAChR but failed to activate either *Aca*-ACR-16 or *Nam*-ACR-16. Levamisole produced inhibition if acetylcholine-mediated currents of *Ace*-ACR-16 and *Nam*-ACR-16 nAChRs. Although the ACR-16 subunit from different hookworm species shared high sequence similarity, these observed differences in pharmacological profiles could explain the species-based observed disparity in anthelmintic efficacy.

A novel oxantel sensitive (O-type) ACR-16-like nAChR has been expressed from *Trichuris suis*, a clade I whipworm (Hansen et al., 2020). Oxantel acted as a potent agonist on the receptor, consistent with the high sensitivity of whipworms to the drug. Pyrantel behaved as a partial agonist of the receptor, while nicotinic agonists such as nicotine, epibatidine, and cytisine produced weak responses.

2.2. Acetylcholine-gated chloride channels

A unique class of invertebrate-specific chloride-selective AChRs (acetylcholine receptors), known as acetylcholine-gated chloride channels (ACCs), have been identified in mollusks and nematodes (Tauc and Gerschenfeld, 1962; Kehoe, 1972; Gardner and Kandel, 1977; Kehoe and McIntosh, 1998; Putrenko et al., 2005; Laing et al., 2013). ACCs exhibit all the typical features of cys-loop LGIC subunits with low sequence identity to the extracellular ligand-binding domains of nAChRs and lack of conservation of vicinal cysteines in loop-C. In addition, the residues flanking the TM-2 domain of these receptors, which influence the ion selectivity of cys-loop LGICs, are positively charged (Galzi et al., 1992). ACCs mediate inhibitory cholinergic neurotransmission in nematodes (Putrenko et al., 2005). These are most similar to mammalian GABA and glycine receptors suggesting an evolutionary adaptation of anion-selectivity and ligand-binding site in the acetylcholine receptor family (van Nierop et al., 2005). Putrenko et al. (2005) identified and characterized the first ACCs in the model nematode *C. elegans*. Eight subunit genes have been identified in the *C. elegans* ACC family so far: *acc-1*, *acc-2*, *acc-3*, *acc-4*, *lgc-46* (Ligand Gated ion Channel), lgc-47, lgc-48, and *lgc*-49 (Putrenko et al., 2005; Wever et al., 2015). These genes have been identified across the phylum, and orthologs of various subunits are present in many species, including *H. contortus*, *B. malayi*, and *A. ceylanicum* (Wever et al., 2015). Members of the ACC receptor family are considered a potential source of anthelmintic targets as they have not been found in mammals to date, are expressed in tissues with pharmacological relevance, and display distinct pharmacological profiles from previously characterized receptors.

In *C. elegans*, the ACC family subunits have variable expression patterns. *acc-1* is expressed in the ventral nerve cord, the retrovesicular ganglion, pharyngeal neurons, and a few head neurons (Pereira et al., 2015). *Cel-acc-2* is localized in ventral cord neurons and a small set of cholinergic and glutamatergic neurons (Pereira et al., 2015; Wever et al., 2015). ACC-2 subunit likely forms a postsynaptic receptor in cholinergic AIZ interneuron and mediates reversal behavior output with worms lacking *acc-2* displaying an increased reversal frequency (Li et al., 2014b). *acc-3*, *lgc-47*, and *lgc-49* are localized in the ventral cord and extrapharyngeal neurons (Wever et al., 2015). *Cel-acc-4* is expressed in all cholinergic neurons, except ASJ and RIB neurons, and non-cholinergic AVF interneurons (Pereira et al., 2015). *Cel-lgc-46* is expressed in the cholinergic motor neurons, GABAergic motor neurons, head and tail neurons (Cinar et al., 2005; Liu et al., 2017). Takayanagi-Kiya et al. (2016) showed that both ACC-4 and LGC-46 are localized to presynaptic terminals of cholinergic neurons and function together to regulate synaptic vesicle release kinetics. LGC-46 acts as an auto-receptor to regulate neurotransmitter release from cholinergic motor neurons, with ACC-4 working as its functional partner (Takayanagi-Kiya et al., 2016). This suggests that ACC-4 interacts with LGC-46 directly or indirectly to regulate cholinergic release. LGC-46 has also been implicated as the major postsynaptic AChRs that mediate synaptic transmission from AVA command interneurons to A-type cholinergic motor neurons (A-MN) (Liu et al., 2017). The *lgc-46* mutants and A-MN-specific *lgc-46* knockdown worms exhibit defective backward locomotion, and this defect is rescued completely by wild-type LGC-46 (Liu et al., 2017). Overall, the *C. elegans* ACC family members have diverse expression patterns and functions in the nervous system, making them potential anthelmintic targets.

Putrenko et al. (2005) expressed the ACC subunits from *C. elegans* and performed pharmacological characterization for the first time to establish them as ionotropic receptors. *Cel*-ACC-1 and *Cel-ACC-2* assembled as acetylcholine- (EC_{50} = 0.26 μM for ACC-1; EC_{50} = 9.54 μM for ACC-2) and arecoline-sensitive (EC_{50} = 4.7 μM for ACC-1; EC_{50} = 754 μM for ACC-2) homomeric receptors. Nicotine and cytisine

produced weak activation of both *Cel*-ACC-1 and *Cel*-ACC-2 homomeric channels. Furthermore, nicotine was an antagonist of *Cel*-ACC-1 while cytisine produced inhibition of acetylcholine-mediated responses of both ACC-1 and ACC-2. These channels were potently blocked by D-tubocurarine, strychnine, and DhβE but not α-bungarotoxin. The overall pharmacological spectrum of the ACC-1 and ACC-2 homomeric channels is distinct from nAChRs and suggests ACCs have a unique ligand-binding site. *C. elegans* ACC-3 and ACC-4 homomers did not exhibit a robust response to acetylcholine application but co-assembled with ACC-1 and ACC-2 to form heteromeric channels (Putrenko et al., 2005). ACC-1/ACC-3 heteromers expressing oocytes were approximately 200-fold less sensitive (EC_{50} = 39.6 μM) to acetylcholine than ACC-1 homomeric receptors. ACC-1/ACC-4 channels did not show a significant shift in the potency of acetylcholine (EC_{50} = 0.36 μM) from homomeric ACC-1 receptors. A combination of ACC-2 with either ACC-3 or ACC-4 resulted in a functional channel with weaker acetylcholine responses than the homomeric channel, suggesting the interaction of ACC-2 with ACC-3 and ACC-4 results in negative regulation of the receptor (Putrenko et al., 2005). LGC-46 forms an acetylcholine-sensitive homomeric receptor (EC_{50} = 50 μM) (Liu et al., 2017). The expressed AChR was not activated by choline and nicotine, suggesting the LGC-46 channel has unique pharmacology. LGC-46 is also expressed in the presynaptic terminals and functions together with ACC-4 to control synaptic vesicle kinetics in cholinergic motor neurons (Liu et al., 2017). Further studies are needed to determine the interaction between ACC-4 and LGC-46 as a heteromeric channel and help understand the properties of these AChR channels.

H. contortus expresses homologs of all the subunit genes of the ACC family except *lgc-48* (Laing et al., 2013). *Hco-acc-1* is expressed in the pharyngeal muscle cells of the anterior half of the pharynx, while in *C. elegans, acc-1* is localized in the pharyngeal neurons (Pereira et al., 2015; Callanan et al., 2018). Overexpression of *C. elegans acc-1* or *H. contortus acc-1* in *C. elegans acc-1* null mutants produces similar effects on the time taken to initiate aversive responses to 1-octanol, showing conservation of putative functional orthology of the receptor (Callanan et al., 2018). *Hco*-ACC-1 failed to express a homomeric channel in *Xenopus* oocytes, but *Hco*-ACC-2 formed an acetylcholine-sensitive (EC_{50} = 21 μM) channel (Callanan et al., 2018; Habibi et al., 2018). The channel was also activated by acetylcholine derivatives including, carbachol (EC_{50} = 43 μM), methacholine (EC_{50} = 100 μM), urecholine (EC_{50} = 747 μM), choline (EC_{50} = 1276 μM) (Habibi et al., 2018). The anthelmintics, pyrantel (EC_{50} = 72 μM), and levamisole (EC_{50} = 98 μM) behaved as partial agonists of the *Hco*-ACC-1 channel (Habibi et al., 2018). *H. contortus* ACC-1 formed a heteromeric channel with ACC-2 with higher sensitivity to acetylcholine (EC_{50} = 5.9 μM) and carbachol (EC_{50} = 32.5 μM) in comparison to ACC-2 homomeric AChRs (Callanan et al., 2018). Habibi et al. (2020) established that *H. contortus lgc-46* could partially rescue aldicarb hypersensitivity in *C. elegans lgc-46* null mutants. Furthermore, *Hco*-LGC-46 expresses as an acetylcholine-sensitive (EC_{50} = 893 μM) homomeric channel and forms a functional heteromeric channel with *Hco*-ACC-1 (Habibi et al., 2020). The heteromeric channel was approximately five times more sensitive to acetylcholine (EC_{50} = 893 μM) than the homomeric LGC-46 AChR (EC_{50} = 166 μM). Carbachol was a partial agonist of the LGC-46/ACC-1 channel but failed to activate the homomeric channel (Habibi et al., 2020). *Hco*-ACC-4 alone or in combination with LGC-46 failed to assemble into functional AChRs but expressed as a functional heteromeric channel with ACC-2 (Habibi et al., 2020). The sensitivity of the heteromeric ACC-2/ACC-4 mixture was comparable to ACC-2 homomers, but there was a significant decrease in the maximum current response to acetylcholine in the ACC-2/ACC-4 combination. This apparent negative regulation of expression by ACC-4 was also observed in *C. elegans* ACC-2/ACC-4 mixtures (Putrenko et al., 2005; Habibi et al., 2020). The functional characterization of the members of the ACC channel family has shown unique pharmacology; however, the composition of *in vivo* native channels in parasitic nematodes is still unknown and requires further exploration.

2.3. Glutamate-gated chloride channels

Glutamate-gated chloride channels (GluCls), another member of the cys-loop ligand-gated ion channel family, mediate fast inhibitory neurotransmission. GluCls are invertebrate-specific membrane channels confined to protostome phyla rendering them attractive drug targets (Kehoe et al., 2009). These supramolecular complexes were first identified in locust muscle fibers and eventually confirmed in other members of protostome phyla, including nematodes, flatworms, and insects (Cull-Candy, 1976; Gration et al., 1979; Bidaut, 1980; Cully et al., 1994, 1996a; Cleland, 1996). Phylogenetic analysis has shown that GluCls are similar to ionotropic glycine receptors, while their pharmacological and physiological properties closely resemble GABA channels (Cully et al., 1994; Cleland, 1996; Vassilatis et al., 1997b). These membrane-bound cylindrical protein complexes open an intrinsic chloride-channel pore upon glutamate binding, normally resulting in hyperpolarization and dampening of neuronal excitability. Each GluCl subunit consists of an N-terminal extracellular domain, ligand-binding loops (Loop-A – Loop-G), a cys-loop disulfide motif, four transmembrane domains (TM1–TM4), and a C-terminal cytoplasmic domain (Hibbs and Gouaux, 2011). GluCl channels are composed of either five identical (homomeric) or homologous (heteromeric) membrane-spanning subunits. This generates various GluCl receptor subtypes with diverse functional spectra and pharmacological profiles (Wolstenholme, 2012). GluCls are expressed in the membranes of pharyngeal muscles, motor neurons, reproductive tissues, sensory neurons, and the excretory-secretory (ES) pores of nematodes (Dent et al., 1997, 2000; Moreno et al., 2010; Wolstenholme, 2012; Li et al., 2014a; Martin et al., 2021).

Macrocyclic lactones are a group of broad-spectrum hydrophobic antinematodal, and insecticidal compounds derived from soil microorganisms of the genus *Streptomyces* (Campbell et al., 1983, 1984). Ivermectin (IVM), a semi-synthetic macrocyclic lactone introduced by Merck in the 1980s, is one of the most successful anti-parasitic agents used in human and veterinary medicine. In humans, IVM is used to treat onchocerciasis (river blindness) and lymphatic filariasis (elephantiasis) that affects hundreds of millions of people globally (Molyneux et al., 2003; Omura, 2008). IVM produces a potent anti-parasitic effect with exceptionally low toxicity to host by targeting GluCl channels at nanomolar concentrations (Fig. 4) (Cully et al., 1994; Etter et al., 1996; Dent et al., 1997; Vassilatis et al., 1997a; Cheeseman et al., 2001; Horoszok et al., 2001; McCavera et al., 2009). Initial electrophysiological experiments showed that invertebrate muscles' relaxation was associated with an increased chloride conductance, and IVM increased that chloride conductance while picrotoxin blocked the effect (Cleland, 1996; Martin et al., 2002). This led to the assumption that IVM produced its action by opening GABA channels as these channels played a role in nematode locomotion and were potently blocked by picrotoxin (Supavilai and Karobath, 1981; Pong and Wang, 1982; Kass et al., 1984; Scott and Duce, 1986, 1987; Holden-Dye et al., 1988; Cleland, 1996). However, Scott and Duce (1987), Martin and Pennington (1989) and Holden-Dye and Walker (1990) detected inconsistencies with the hypothesis that IVM behaved as an agonist of GABA channels to produce its effects. Scott and Duce (1987) identified specific locust muscles that were not sensitive to GABA but induced increased Cl^- conductance in response to IVM. Martin and Pennington (1989) and Holden-Dye and Walker (1990) observed that IVM behaved as an antagonist of the GABA channel in *Ascaris* muscle. Martin and Pennington (1989) also showed that IVM activated a non-GABA Cl^- permeable channel. GABA receptor hypothesis could not support the observed selective effects of IVM as it did not produce any effects on the mammalian peripheral GABA receptors (Martin et al., 2002). The focus was then shifted to alternate nematode-specific chloride gated channels, GluCls. The GluCls were first described in locust leg muscle fibers as the extrajunctional "H-receptors" by Cull-Candy (1976), followed by their identification in others

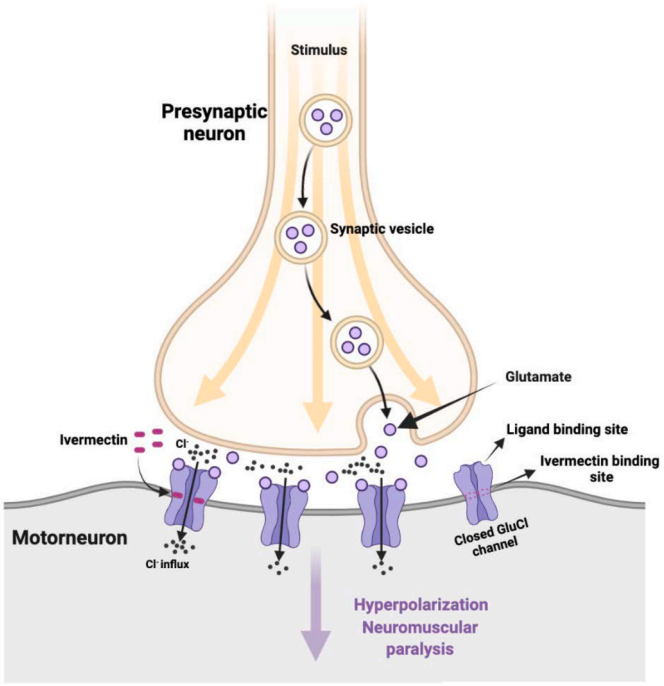

Fig. 4. Mechanism of action of glutamate and ivermectin on the GluCls at the nematode synapse. Glutamate is released from presynaptic neuron and binds to the extracellular domain of GluCl which leads to chloride ion influx, and hyperpolarization. Binding of ivermectin in the transmembrane channel domain of GluCl amplifies the effects of glutamate, allowing more influx of chloride ions, leading to hyperpolarization and paralysis of the invertebrate neuromuscular system.

invertebrates including nematodes (Cully et al., 1994, 1996a; Cleland, 1996). IVM activates GluCls present in nerve and muscle cells which causes hyperpolarization of the neuronal membranes and consequently persistent paralysis of the musculature, and death of parasite (Wolstenholme and Rogers, 2005; Wolstenholme, 2012). IVM treatment's biological effects include inhibition of motility and feeding, reduced fecundity of adult worms, and loss of host immunosuppression (Martin et al., 2021).

2.3.1. *C. elegans* GluCls

Schaeffer and Haines (1989); Schaeffer et al. (1989) showed that IVM binds to specific membrane receptors in *C. elegans*. Subsequently, *C. elegans* total mRNA was collected, fractionated, and expressed in oocytes for screening by Merck scientists (Arena et al., 1991, 1992). This led to the identification of the first nematode GluCls that were sensitive to IVM. Cully et al. (1994) identified and expressed two GluCl subunits, termed α and β, through further fractionation of total mRNA isolated from *C. elegans* into smaller pools. The α subunit (later designated as GLC-1 or GluClα1; GLutamate-gated Chloride channel) formed an IVM-sensitive but glutamate-insensitive channel, while the β subunit (later named as GLC-2 or GluClβ) expressed as an IVM insensitive and glutamate-gated homomeric GluCl. GLC-1 does possess a binding site for glutamate and cannot couple ligand binding with the opening of the channel (Etter et al., 1996). The gene is expressed in the intestine, pharyngeal and body wall muscles, head neurons, and *glc-1* mutants showed defects in foraging behavior (Cook et al., 2006; Ghosh et al., 2012). When both *α* and *β* subunits were co-expressed in *Xenopus*, the assembled heteromeric receptor was sensitive to IVM and glutamate. The pharmacological properties of the channel expressed using total mRNA were comparable to this heteromeric receptor and helped identify GluCls as a target for the nematocidal effects of IVM (Cully et al., 1994; Arena et al., 1995). Depending on the concentration, IVM produced dual effects on the heteromeric receptor. IVM produced slow and irreversible direct activation of the receptor when used in the micromolar range, and at submaximal concentrations, it potentiated glutamate-mediated currents. GluCls α and β channels were also activated by ibotenate, a structural analog of glutamate, and blocked by picrotoxin (Cully et al., 1994). Hibbs and Gouaux (2011) reported the first 3-D structure of the *C. elegans* GluCl-α1 channel at 3.3 Å resolution through crystallographic studies. They delineated the classic agonist binding site of glutamate and the allosteric binding pocket of IVM located within the transmembrane region of the ion channel. Successively, an additional four GluCl encoding genes were identified from *C. elegans*, *viz, glc-3* (GluClα4), *glc-4, avr-14* (GluClα3; AVermectin Resistance), and *avr-15* (GluClα2), and multiple splice variants are encoded by *glc-4*, avr-*14*, and *avr-15* genes (Table 2) (Dent et al., 1997; Laughton et al., 1997a; Vassilatis et al., 1997a; Horoszok et al., 2001; Pellegrino, 2003).

GluCls have a well-understood role in regulating feeding behavior and locomotion in *C. elegans* (Avery, 1993b; Raizen and Avery, 1994; Dent et al., 2000; Wolstenholme and Rogers, 2005; Cook et al., 2006; Wolstenholme, 2012). IVM has been shown to produce its nematocidal effects primarily through inhibition of pharyngeal pumping and body musculature paralysis, which are mediated through GluCls expressed in the pharynx and motor nerves (Schaeffer and Haines, 1989; Avery and Horvitz, 1990; Dent et al., 1997, 2000; Cook et al., 2006). *avr-15* is expressed in the pharyngeal muscles and controls its activity by encoding a GluCl subunit involved in mediating inhibitory M3 neurotransmission. This gene also confers sensitivity to the inhibitory effects of IVM in *C. elegans*. *avr-15* encodes for two splice variants, AVR-15A (GluClα2A) and AVR-15B (GluClα2B), with differences in the amino-terminal, which when expressed in oocytes forms glutamate- and ivermectin-sensitive GluCl channel (Dent et al., 1997; Vassilatis et al., 1997a). An AVR-15B homomeric channel showed higher sensitivity (EC_{50} = 208 μM) to glutamate in comparison to AVR-15A channels (EC_{50} = 2 mM). GLC-2 (GluCl β) subunits are also expressed in the pharyngeal muscles and form a functional heteromeric channel with AVR-15B (Laughton et al., 1997b; Vassilatis et al., 1997a). The heteromeric channel showed comparable sensitivity to IVM with an EC_{50} of 103 nM and higher sensitivity to glutamate (EC_{50} = 62 μM) than the homomeric AVR-15B. This suggested that AVR-15B and GLC-2 could form a native heteromeric receptor and correlated well with the observed biological effects of IVM in the pharynx (Vassilatis et al., 1997a). However, the natural composition of GluCls in worms is not yet known. Direct electrophysiological recordings from the pharynx of wild type and *avr-15* mutant *C. elegans* also indicated that AVR-15B and GLC-2 might associate to form the pharyngeal receptor (Pemberton et al., 2001). In this study, the pharyngeal preparations of *avr-15* null mutants were insensitive to ivermectin. However, the pharynx of the mutant worms was activated by glutamate, albeit at a higher concentration. One explanation for this glutamate effect is the presence of GLC-2 receptors in *avr-15* null mutants. However, glutamate has a lower affinity on GLC-2 than that observed, and picrotoxin behaved as a weaker inhibitor than expected on GLC-2 receptors. Furthermore, IVM was remarkably potent on the pharyngeal receptor of the wild-type worms. The potency was much higher than that observed for any other GluCl combination expressed in *Xenopus* oocytes. This suggested that the pharyngeal GluCl is either not composed of GLC-2 subunit or is formed by a combination of AVR-15 and GLC-2 with another GluCl subunit (Pemberton et al., 2001) or an additional auxiliary protein. Loss of function mutations in *avr-15* reduced the binding affinity for IVM but did not render the worms resistant to ivermectin; a combination of a mutation in *avr-15* with another α subunit encoding gene, *avr-14*, confers moderate ~13-fold resistance to ivermectin while a simultaneous mutation in *glc-1* confers ~4000-fold ivermectin resistance (Dent et al., 2000). Other genes involved in regulating cuticle permeability (*osm-1*; OSMotic avoidance abnormal) and gap junctions (innexins encoding genes, *unc-7* and *unc-9*) also contribute to IVM sensitivity (Dent et al.,

Table 2
A summary of nematode GluCl subunits and receptor pharmacology.

Organism	GluCl gene	Subunit	Tissue distribution	Receptor	Pharmacology		
					Glutamate (EC_{50})	IVM (EC_{50})	PTX (IC_{50})
C. elegans	*glc-1*	GLC-1 (GluClα1)	intestine, pharyngeal and body wall muscle, head neurons[1]	Expresses as IVM-gated homomeric channel[2]	No response[2]	140 nM[2]	59 μM[3] for IVM sensitive current
	glc-2	GLC-2 (GluClβ)	Pm4 pharyngeal muscle cell[4]	Forms a glutamate sensitive homomeric channel[2]	380 μM[2]	No response[2]	77 nM[3] for glutamate-sensitive current
				GLC-1 and GLC-2 assemble to form heteromeric channels[2]	1.36 mM[2]	190 nM[2]	42 μM for glutamate-sensitive current and 52 μM for IVM-sensitive current[3]
	glc-3	GLC-3/ GluClα4	AIY interneurons[5,6]	Expresses as glutamate-gated homomeric channels[7]	1.9 mM[7]	0.4 μM[7]	PTX (0.01–1000 μm) failed to inhibit the L-glutamate (3 mM) agonist response[7]
	glc-4	GLC-4A (full length variant) GLC-4B (truncated variant)	intestine, AIY interneurons, anal depressor muscle, head mesodermal cell, nerve ring, head and tail neurons[8,9]	GLC-4A failed to form a functional homomeric channel[8]	No response	No response	Not determined
	avr-14	AVR-14A/ GluClα3A	neurons in ring ganglion, ventral cord, and mechanosensory neurons[10]	AVR-14A failed to show functional channel expression[10]	No response	No response	Not determined
		AVR-14B/ GluClα3B	neurons in ring ganglion, ventral cord, and mechanosensory neurons[10]	AVR-14B expresses as functional homomeric channels[10]	Robust response to 10 mM glutamate[10]	Robust response to 10 μM IVM[10]	Not determined
				AVR-14B forms a heteromeric receptor with GLC-4A[8]	~21 fold reduction in 1 mM glutamate current amplitude[8]	~19 fold reduction in 10 μM IVM current amplitude[8]	Not determined
	avr-15	AVR-15A/ GluClα2A	pharyngeal muscle (pm4 and 5), head neurons, sublateral nerve cords and mechanosensory neurons[11]	AVR-15A forms a homomeric receptors[11]	2 mM[11]	Robust response to 10 μM IVM[11]	Not determined
		AVR-15B/ GluClα2B	pharyngeal muscle (pm4 and 5), head neurons, sublateral nerve cords and mechanosensory neurons[11]	AVR-15B forms a homomeric receptors[12]	208.3 μM[12]	107.8 nM[12]	Weak inhibition of glutamate-mediated currents by 100 PTX μM[12]
				AVR-15B assembles into a heteromeric receptors with GLC-2[12]	62 μM[12]	103 nM[12]	Not determined
H. contortus	*GluClα*	GluClαA/ GLC-5A	Motor neuron commissures[13]	GLC-5A forms a functional homomeric receptors[14]	8.4 μM[14]	131 nM[14]	weak inhibition of IVM and moxidectin induced currents by 100 PTX μM[14]
		GluClαB/ GLC-5B	Motor neuron commissures[13]	Not determined	Not determined	Not determined	Not determined
	glc-2/ HG4	GLC-2 (GluClβ)	Motor neuron commisures, lateral and subventral nerve cords[13,15]	GLC-2 forms a homomeric receptors[16]	394 μM[16]	>10 μM[16]	Not determined
	avr-14/ gbr-2/ GluClα3	AVR-14A/ GluClα3A	amphidial neurons, motor neuron commissures, lateral and ventral nerve cords, and nerve ring[13,17]	No functional homomeric channels have been reported for AVR-14A	No response	No response	No response
		AVR-14B/ GluClα3B	pharyngeal neurons, amphidial neurons, motor neuron commissures, lateral and ventral nerve cords, and nerve ring[13,17,18]	AVR-14B forms a homomeric receptors[16]	15.3 μM[19] 28 μM[16] 27.6 μM[20]	39 nM[19] 22 nM[16] ~0.1 ± 1.0 nM[20]	1 mM PTX was a potent blocking of glutamate- and IVM- current response[20]
				AVR-14B forms a heteromeric channel when co-injected with GLC-5A in the ratio of 1:1 or 1:50[16]	40 μM (1:1) [16] 44 μM (1:50)[16]	86 nM (1:1) [16] 141 nM (1:50)[16]	Not determined

(*continued on next page*)

Table 2 (*continued*)

Organism	GluCl gene	Subunit	Tissue distribution	Receptor	Pharmacology		
					Glutamate (EC_{50})	IVM (EC_{50})	PTX (IC_{50})
	glc-3	GLC-3/ GluClα4	Not known	Not characterized	Not determined		
	glc-4			GLC-4	Not known	Not characterized	Not determined
	glc-6	GLC-6	Not known	Not characterized	*Hco*-GLC-6 subunit rescues the ivermectin sensitivity of the *C. elegans* DA1316 triple GluCl mutant (*avr-14, avr-15, glc-1*) strain. This suggests that GLC-6 subunit likely expresses an IVM-sensitive channel either alone or in combination with other subunits[21]		
D. immits	*avr-14*	AVR-14A/ GluClα3	Not known	No functional homomeric channels have been reported for AVR-14A	No response	No response	No response
		AVR-14B/ GluClα3B	Not known	AVR-14B expresses as a functional homomeric channel[22]	l-glutamate sensitive channel (1–100 mM) [22]	1 μM IVM produced saturating currents[22]	Not determined
C. oncophora	*GluClβ* from ivermectin sensitive (IVS) and ivermectin resistant (IVR) worms	IVS GluClβ/ GLC-2	Not known	IVS GLC-2 expresses as a homomeric channel	185.6 μM	No response	Not determined
		IVR GluClβ/ GLC-2	Not known	No functional homomeric channel have been reported from IVR GLC-2	No response	No response	Not determined
	IVS and IVR *GluClα3*	IVS AVR-14/ GluClα3	Not known	IVS AVR-14 expresses as a homomeric channel[23]	29.7 μM[23]	0.5 μM[23]	Not determined
				IVS AVR-14/GluClβ express as heteromeric channels[23]	13.4 μM[23]	Not determined	Not determined
		IVR AVR-14/ GluClα3	Not known	IVR AVR-14 expresses as a homomeric channel[23]	96.1 μM[23]	1.3 μM[23]	Not determined
				IVR AVR-14/GluClβ express as heteromeric channels[23]	171.6 μM[23]	Not determined	Not determined
A. suum	*GluCla3*	AVR-14/ GluCla3	Dorsal and ventral nerve cord[17]	Not characterized	Not determined	Not determined	Not determined
B. malayi	*GluCla3*	AVR14A/ GluCla3A AVR14B/ GluCla3B	tissues surrounding ES apparatus, the inner body, and anal pore, reproductive tissue[24, 25]	Not characterized	Not determined	Not determined	Not determined

[1] Ghosh et al. (2012).
[2] Cully et al. (1994).
[3] Etter et al. (1999).
[4] Laughton et al. (1997b).
[5] Wenick and Hobert (2004).
[6] Chalasani et al. (2007).
[7] Horoszok et al. (2001).
[8] Pellegrino (2003).
[9] Wang et al. (2021).
[10] Dent et al. (2000).
[11] Dent et al. (1997).
[12] Vassilatis et al. (1997a).
[13] Portillo et al. (2003).
[14] Forrester et al. (2003).
[15] Delany et al. (1998).
[16] Atif et al. (2019).
[17] Jagannathan et al. (1999).
[18] Yates et al. (2003).
[19] Lynagh and Lynch (2010).
[20] McCavera et al. (2009).
[21] Glendinning et al. (2011).
[22] Yates and Wolstenholme (2004).
[23] Njue et al. (2004).
[24] Li et al. (2014b).
[25] Moreno et al. (2010).

2000). Laughton et al. (1997a) cloned a pair of alternatively spliced subunits, AVR-14A (GluClα3A) and AVR-14B (GluClα3B), encoded by *avr-14* (also referred to as *gbr-2*; GABA Receptor-related), which differ in their C-terminal region. The gene is expressed in the neurons in ring ganglion, ventral cord motor neurons, and mechanosensory neurons (Dent et al., 2000). Mutants of *avr-14* and *avr-15* exhibit a higher frequency of change in direction than wild-type worms (Cook et al., 2006). When expressed in *Xenopus* oocytes, AVR-14A failed to form a functional homomeric receptor while AVR-14B expressed as glutamate- and IVM-gated channel (Dent et al., 2000). As observed with other α GluCls, glutamate produced rapidly desensitizing currents while IVM produced slow, irreversible responses.

GluClα4 (GLC-3) is expressed in the olfactory neurons, and *glc-3* mutants exhibit diminished olfactory behavior (Chalasani et al., 2007). Cook et al. (2006) reported that knockdown of *glc-3* leads to an increase in the frequency of change of direction in worms. As expected of an α GluCl receptor, GLC-3 homomeric channels expressed in oocytes were sensitive to glutamate (EC_{50} = 1.9 ± 0.03 mM), and IVM (EC_{50} = 0.4 ± 0.02 μM) which produced slowly activating irreversible inward responses. Picrotoxin, which blocks the responses in other GluCl channels, failed to have inhibitory effects on the GLC-3 receptor (Horoszok et al., 2001). GLC-4 is another putative GluCl subunit with sequence divergence from previously described α or β subunits and has two splice isoforms, a full-length variant, GLC-4A, and a truncated GLC-4B variant (Cully et al., 1996b; Pellegrino, 2003). *glc-4* is expressed in intestine, anal depressor muscle, head mesodermal cells, nerve ring, head, and tail neurons, and the mutants show hypersensitivity to ivermectin and hyper reversal behavior (Pellegrino, 2003). Wang et al. (2021) reported colocalization of GLC-3 and GLC-4 in AIY interneurons and identified their role in regulating temperature-dependent neurological behavior. Unlike previously expressed GluCl subunits, GLC-4A failed to assemble into a functional homomeric GluCl channel but forms a heteromeric channel with AVR-14B altering its pharmacological properties with decreased current response to glutamate and IVM (Pellegrino, 2003).

2.3.2. Parasitic nematode GluCls

The GluCl gene family of parasitic nematodes is diverse and shows variation between the species, with *avr-14* present in all nematode genomes studied so far (Yates et al., 2003; Wolstenholme, 2012). Among parasitic species, most information regarding GluCl subunit genes is available for *H. contortus* in which six genes have been identified so far: *avr-14* (*gbr-2/GluClα3*), *glc-2* (*GluClβ*), *glc-3* (*GluClα4*), *glc-4*, *glc-5* (*GluClα*), and *glc-6* (Table 2) (Delany et al., 1998; Forrester et al., 1999; Jagannathan et al., 1999; Cheeseman et al., 2001; Martin et al., 2021). No orthologues of *Cel-glc-1* and *Cel-avr-15* have been described in *H. contortus*, while *GluClα* (*glc-5*) and *glc-6* have been exclusively identified in the sheep nematode. As observed with C. elegans, disruption of pharyngeal activity represents an important target for the nematocidal effect of IVM in H. contortus (Geary et al., 1993; Kotze, 1998; Paiement et al., 1999). The pharyngeal paralysis was observed at physiologically relevant concentrations (≥0.1 nM) in *H. contortus*. In contrast, paralysis in the mid-body region was observed at a higher concentration (≥10 nM) of IVM (Geary et al., 1993). IVM also inhibits motility, suppresses the development of the larval stages, and reduces the fecundity of *H. contortus* worms (Gill et al., 1991; Le Jambre et al., 1995). GluCl pharmacology of *H. contortus* has mainly been studied by using radioligand binding assays. High-affinity binding site (KD = 0.07 to 0.6 nM) for IVM with comparable characteristics to that reported from membrane preparations of C. elegans has been identified in membranes prepared from *H. contortus* (Schaeffer and Haines, 1989; Rohrer et al., 1994; Cheeseman et al., 2001). Binding studies conducted on *Hco*-GLC-5 and *Hco*-AVR-14B expressed in COS-7 mammalian cell lines identified a high-affinity GluCl binding site for IVM (Cheeseman et al., 2001; Forrester et al., 2002). As expected, *Hco*-AVR-14A and *Hco*-GLC-2 failed to exhibit specific IVM binding. Glutamate does not compete for IVM binding sites in the radioligand binding assays and modulates the binding affinity of IVM. This shows that glutamate and IVM do not share a binding site and have an allosteric relationship (Cheeseman et al., 2001; Forrester et al., 2002).

Hco-GLC-5 is alternatively spliced into GLC-5A and GLC-5B variants, and GLC-5B is shorter by 69 amino acids at the amino terminus (Forrester et al., 1999). GLC-5A forms functional glutamate- and IVM-sensitive homomeric channels when expressed in *Xenopus* oocytes (Forrester et al., 2003). Glutamate induced fast-acting, desensitizing, and reversible responses while IVM produced slow activating and irreversible inward currents (Table 2). GLC-5A is expressed in the motor neuron commissures, which could explain the paralytic effect of IVM on somatic muscles (Portillo et al., 2003). Unlike *C. elegans*, the *Hco*-GLC-2 subunit is not expressed in the pharyngeal muscles but has an overlapping distribution with GLC-5A in the motor neuron commissures and nerve cords, suggesting a possible co-assembly of the subunits to form a native channel (Delany et al., 1998; Portillo et al., 2003). The expressed *Hco*-GLC-2 channels were less sensitive to glutamate (EC_{50} = 394 ± 57 μM) in comparison to GLC-5A homomeric (EC_{50} = 8.4 ± 0.56 μM). GLC-5 channels were susceptible to IVM (EC_{50} = 131 nM) while as observed with *C. elegans*, the GLC-2 homomeric channels are effectively insensitive to IVM (Forrester et al., 2003; Atif et al., 2019).

Hco-AVR-14 is alternatively spliced to generate *Hco*-AVR-14A and *Hco*-AVR-14B variants that differ in the ion channel and intracellular domain, forming C-terminal regions (Jagannathan et al., 1999). Both the splice variants of *Hco*-AVR-14 can rescue behavioral defects, and motility malfunctions in *avr-14* mutant *C. elegans* and *Hco*-AVR-14B can rescue IVM sensitivity of the triple mutant *C. elegans* (*avr-14*, *avr-15*, *glc-1*) (Cook et al., 2006; Glendinning et al., 2011). *Hco*-AVR-14 variants have an overlapping expression in the motor neuron commissures and nerve ring and have different expression patterns in the rest of the nervous tissues (Jagannathan et al., 1999; Portillo et al., 2003). *Hco*-AVR-14A is expressed in the sensory amphid neurons and has a potential a role in modulating sensory behavior. *Hco*-AVR-14B is detected in three putative pharyngeal neurons suggesting a regulatory role in *H. contortus* feeding behavior and provides evidence for the observed paralytic effect of IVM on pharyngeal pumping (Portillo et al., 2003; Yates et al., 2003). *Hco*-AVR-14A failed to express as a functional receptor, but *Hco*-AVR-14B successfully assembled into an active GluCl receptor when expressed in *Xenopus* oocytes and mammalian cell lines (McCavera et al., 2009; Lynagh and Lynch, 2010; Atif et al., 2017). *Hco*-AVR-14B homomers expressed in *Xenopus* oocytes were very sensitive to glutamate (EC_{50} = 27.6 ± 2.7 μM) and produced slow, irreversible responses to IVM (estimated EC_{50} = 0.1 ± 1.0 nM). Picrotoxin behaved as an antagonist of the receptor (McCavera et al., 2009). Whole-cell patch-clamp recordings from *Hco*-AVR-14B receptor-expressing HEK293 cells were gated by glutamate (EC_{50} = 15.3 ± 1.8 μM) and produced a slow-acting irreversible response to IVM (EC_{50} = 39.0 ± 6.0 nM) (Lynagh and Lynch, 2010). IVM acted as an activator as well as potentiated currents in the presence of glutamate in the macropatch recordings of *Hco*-AVR-14B receptor expressed in HEK293 cells (Atif et al., 2017). In comparison, electrophysiology recordings of glutamate-gated currents from *Hco*-AVR-14B receptor expressing oocytes produced an EC_{50} of 28 ± 6 μM for glutamate and 22 ± 3 nM for IVM (Atif et al., 2019). There is an overlap of the expression of *Hco*-AVR-14 with Hco-GLC-2 and Hco-GLC-5a in the motor neuron commissures indicating that the native GluCl that regulates movement may be composed of either a single subunit or combination of α, α3 and β subunits, however this needs to be explored further (Portillo et al., 2003). Hco-GLC-2 can express as a functional heteromeric channel in combination with the *Hco*-AVR-14B subunit when expressed in HEK293 cells (Atif et al., 2019). Hco-GLC-2 homomeric channels were less sensitive to glutamate in comparison to *Hco*-AVR-14B homomeric receptor and Hco-GLC-2/*Hco*-AVR-14B heteromeric GluCls (EC_{50} = 40 ± 6 μM for 1:1; EC_{50} = 44 ± 3 μM for 1:50). The heteromeric channels showed intermediate sensitivity to IVM (EC_{50} = 86 ± 14 nM, 1:1; EC_{50} = 141 ± 11 nM for 1:50). Interestingly, the presence of a higher expression ratio

of GLC-2 subunits in the heteromeric complex reduces IVM potency, suggesting a possible mechanism for modulation of the receptor responses in the nematodes (Atif et al., 2019). There is no data available for expression pattern and characterization of *Hco-glc-3*, *Hco-glc-4*, and *Hco-glc-6*. Glendinning et al. (2011) reported that the GLC-6 subunit produced an almost complete rescue of the IVM sensitivity of the triple GluCl mutant *C. elegans* worms (*avr-14, avr-15*, *glc-1*).

In *A. suum*, IVM induces a potent paralysis of pharynx and body wall musculature (Kass et al., 1980, 1984; Brownlee et al., 1997). Kass et al. (1980); Kass et al. (1984) used the split chamber technique to demonstrate that avermectin blocked transmission between interneurons and excitatory motor neurons in the ventral cord. IVM sensitive Cl^- currents mediated through a non-GABA channel were recorded on body muscle of *A. suum* (Martin and Pennington, 1989). A fluorescent-labeled IVM (4′-5,7 dimethyl bodipy propriony IVM) injected into adult *Ascaris* produced dose-dependent paralysis in the worm, and the probe was located on the muscle membrane and nerve cord, consistent with the site of action of the drug (Martin et al., 1992). The effect of IVM on *A. suum* pharyngeal muscles was studied by Brownlee et al. (1997) and they found that at higher concentration (1–1000 nM), IVM directly inhibited pharyngeal pumping, while at lower concentration (1–10 pM) it potentiated glutamate-induced inhibition. Martin (1996) used two micro-electrode current-clamp techniques to demonstrate the presence of glutamate-gated receptors in *A. suum* pharyngeal muscle. Glutamate and IVM cause hyperpolarization and an increase in input Cl^- conductance in the pharynx. The channel is potentiated by the avermectin-analog milbemycin. Adelsberger et al. (1997) performed patch recordings of glutamate-induced single-channel openings from *A. suum* pharyngeal muscle membranes. These Cl^- selective channels were activated by glutamate and IVM while picrotoxin behaved as a reversible channel blocker. The channel had a main conductance state of 21 pS and two rare sub-conductance levels. Bioinformatics research and cloning studies have identified three GluCl genes in *A. suum*, *avr-14* (*gbr-2*), *glc-2*, and *glc-4* (Jagannathan et al., 1999; McCavera et al., 2007). The *A. suum avr-14* gene does not undergo alternate splicing. It is more closely related to AVR-14B homologs of other nematodes. The gene is widely expressed in the nematode's nervous system, including nerve cords (Jagannathan et al., 1999).

In *Cooperia oncophora*, a nematode of cattle, two GluCl subunits have been identified, GluClα3 (AVR-14) and β subunit, which are homologous to *C. elegans* AVR-14 and GLC-2 subunit, respectively (Njue et al., 2004; Njue and Prichard, 2004). *avr-14* encodes for two splice variants, AVR-14A and AVR-14B, in the nematode (El-Abdellati et al., 2011). Full-length *Con*-AVR-14B and *Con*-GLC-2 were cloned from IVM-susceptible (IVS) and IVM-resistant (IVR) worms and expressed in *Xenopus* oocytes as homomeric and heteromeric channels (Njue et al., 2004). IVS AVR-14B differed from the IVR subunit in three amino acids (E114G, V235Aand L256F) located in the amino-terminal ligand-binding domain. IVS and IVR GLC-2 also had two amino acid differences (V60A and R101H) in the same N-terminal domain. IVS AVR-14B homomeric channels were more sensitive to glutamate (EC_{50} = 29.7 ± 4 μM) and IVM (EC_{50} = 0.5 ± 0.12 μM) in comparison to IVR AVR-14B channels (EC_{50} = 96.1 ± 4.4 μM for glutamate and 1.3 ± 0.11 μM for IVM). GLC-2 homomeric channels from IVS worms were activated by glutamate (EC_{50} = 185.6 ± 24.9 μM) but were insensitive to IVM, while IVR GLC-2 channels failed to respond to glutamate. Thus, AVR-14B mutations lead to a modest loss of agonist sensitivity, while GLC-2 mutation completely abolishes the receptor's glutamate sensitivity. The heteromeric AVR-14B/GLC-2 channels from both IVS and IVR worms showed higher sensitivity to glutamate (EC_{50} = 13.4 ± 2.5 μM) than either of the homomeric channels. In contrast, the IVR AVR-14B/GLC-2 GluCl receptors were ~13 fold less sensitive to glutamate (EC_{50}=171.6 ± 20.7 μM) than IVS heteromeric receptor. However, since the native receptor composition for GluCls is not yet known, the possibility of co-assembly of AVR-14B and GLC-2 subunits to form a heteromeric receptor needs further exploration.

Avermectins effectively kill larval stages of filarial worms and long-lasting suppression of microfilaria (mf) but have limited efficacy against adult worms. The reason for this lack of adulticidal effect is not yet understood. Homology searching of the genome of *B. malayi* has revealed orthologs of only four subunits, GLC-2, GLC-4, AVR-14A and, AVR-14B (Williamson et al., 2007). AVR-14 homologs have also been identified in the filarial nematodes, *Dirofilaria immitis,* and *Onchocerca volvulus* (Cully et al., 1996b; Yates and Wolstenholme, 2004). The *Bma*-AVR-14 expression has been reported from developing embryos and adult female reproductive tissues, which could address the observed suppression of microfilaria production following avermectin treatment (Li et al., 2014a). *Bma*-AVR-14 is also localized on a muscle that surrounds the excretory-secretory (ES) apparatus in mfs, suggesting a role of the GluCl in regulating protein release from the ES system (Moreno et al., 2010). IVM led to a decrease in the amount of protein release from mf by preventing the ES pore's opening. This, in turn, reduces the parasite's ability to secrete proteins required to neutralize and evade the host immune system. This could explain the microfilaricidal effects of IVM but do not address the drug's limited adulticidal activity. Vatta et al. (2014) reported an increase in the attachment of canine mononuclear cells and neutrophils to *D. immitis* mf after treatment with IVM, which provides support for the still-developing loss of immune modulation theory. *Dim-avr-14* is also alternatively spliced, albeit with a different splicing pattern, with AVR-14A lacking the C-terminal domain of AVR-14B. When expressed in *Xenopus*, AVR-14A failed to form a functional homomeric receptor, and AVR-14B formed glutamate- and IVM-sensitive channels (Yates and Wolstenholme, 2004). The glutamate-induced inward currents were fast desensitizing and fully reversible, while IVM produced very slow activating, irreversible responses. Expression studies of this subunit in various worm stages can further elaborate the subunit's biological role in the worm.

2.4. GABA channels

GABA is a major inhibitory neurotransmitter in the mammalian and invertebrate nervous systems. Inhibitory GABA-gated chloride channels are present at the neuromuscular junction of nematodes. The GABAergic inhibition assists with the nematode's distinctive sinusoidal body movement through contralateral muscle relaxation. The inhibitory effects of GABA on fast synaptic neurotransmission were demonstrated on the somatic muscles of *A. lumbricoides* (Del Castillo et al., 1964). Martin (1980) showed that ionotropic GABA receptors are also present extrasynaptically in the *A. suum* muscle cell bag region. Various researchers have used two-electrode current-clamp technique to show the unique pharmacology of *Ascaris* inhibitory GABA channels than vertebrate receptors (Martin, 1980, 1993; Holden-Dye et al., 1988, 1989; Duittoz and Martin, 1991). The *Ascaris* GABA receptor was insensitive to the vertebrate $GABA_A$ receptor agonists sulphonic acid derivatives such as piperidine-4-sulphonic acid. The $GABA_A$ receptor antagonists such as bicucculline, picrotoxin, securinine, pitrazepine, and dieldrin were either inactive on *A. suum* muscle or produced weak activity. Pentobarbitone and flurazepam which act as potentiators of vertebrate $GABA_A$ channels failed to show similar effects on the *Ascaris* receptors. These investigations suggest that nematode GABA receptors have distinct pharmacological properties from vertebrate receptors and have potential to be exploited as drug targets. These channels serve as a target for piperazine, a GABA agonist, which is used for control of large gastrointestinal nematodes (Martin, 1997). It is an effective chemotherapeutic agent that crosses the worm's cuticle and induces flaccid paralysis.

unc-49 encodes for $GABA_A$-like receptors in nematodes and mediates body muscle relaxation in *C. elegans* (Table 3) (Bamber et al., 1999; Richmond and Jorgensen, 1999). UNC-49 subunits are more closely related to RDL (resistance to dieldrin) receptors of *Drosophila melanogaster* and are not orthologous to any of mammalian $GABA_A$ receptor subunits. *Cel-unc-49* mutants have defective locomotion which result in the "shrinker phenotype" and these mutant worms are resistant to

Table 3
A summary of nematode GABA subunits and receptor pharmacology.

Gene	Species	Subunit	Tissue distribution	Receptor	Pharmacology	
					GABA (EC_{50})	Picrotoxin (IC_{50})
unc-49	*C. elegans*	UNC-49A	Not known	Does not express as a functional receptor[1]		
		UNC-49B	body wall musculature, anal sphincter muscle, dorsal nerve cord, ventral nerve cord and nerve ring[1]	Expresses as a homomeric channel[1,2]	43.7 ± 2.9 μm [1]	0.9 ± 0.2 μm [2]
		UNC-49C	body wall musculature, dorsal nerve cord, ventral nerve cord and nerve ring[1]	Does not express as a functional receptor[1]		
				Cel-UNC-49B and *Cel*-UNC-49C co-assemble to form a heteromeric channel[1,2]	107.5 ± 13.5 μm[1]	166.0 ± 42.0 μm [2]
	H. contortus	UNC-49B	Not determined	Expresses as a homomeric channel[3,4,5]	64.0 ± 4.4 μM[3] 76 ± 6 μm[5] Agonist profile:GABA > trans-4-aminocrotonic acid (TACA) > guanidinoacetic acid (GAA) > imidazole-4-acetic acid (IMA) > isonipecotic acid > isoguvacine > β-alanine > 5-aminovaleric acid (DAVA)[5]	3.65 ± 0.64 μM[4]
		UNC-49C	Not determined	Does not express as a functional receptor		
				Hco-UNC-49B and *Hco*-UNC-49C co-assemble to form a heteromeric channel[3,4,5]	39.9 ± 5.7 μM[3] 59 ± 8 μm[5] Agonist profile:GABA > TACA > isoguvacine > IMA > *R*(−)-4-amino-3-hydroxybutyric acid *R*(−)-GABOB > *S*(+)-GABOB, > GAA > isonipecotic acid > DAVA > β-alanine[5]	134.56 ± 44.12 μM[4]
lgc-37 (hg1)	*H. contortus*	LGC-37A/ HG1A LGC-37E/ HG1E	Ventral nerve cord and in a few neurons associated with the nerve ring around the pharynx[6]	Does not express as a GABA sensitive homomeric receptor[6,7]		
Gab-1	*C. elegans*	GAB-1	Not determined	Does not express as a GABA sensitive homomeric receptor[8]		
				Cel-GAB-1 forms a heteromeric channel when expressed with *Hco*-HG1A and HG1E[8]	*Cel*-GAB-1/*Hco*-HG1A = 17.83 μM[8]	Not determined
					Cel-GAB-1/*Hco*-HG1E = 3.3 μM[8]	Not determined
lgc-38	*H. contortus*	LGC-38	Not known	Expresses as a homomeric channel[8]	19.3 ± 4.3 μM[9]	Not determined. 10 μM PTX produced 97.8 ± 0.9% inhibition of the 20 μM GABA response[9]

(*continued on next page*)

Table 3 (*continued*)

Gene	Species	Subunit	Tissue distribution	Receptor	Pharmacology	
					GABA (EC_{50})	Picrotoxin (IC_{50})
exp-1	*C. elegans*	EXP-1	intestinal and anal depressor enteric muscle[10]	Expresses as a homomeric channel[10]	26.4 ± 1.08 μM[10] 21.0 ± 0.7 μM[11]	Resistant to the channel blocking effects picrotoxin[11]
lgc-35	*C. elegans*	LGC-35	sphincter enteric muscle, a subset of ventral cord acetylcholine motor neurons and the spicule protractor in males[12,13]	Expresses as a homomeric channel[11]	17 ± 0.71 μM[11]	Resistant to the channel blocking effects picrotoxin[11]

[1] Bamber et al. (1999).
[2] Bamber et al. (2003).
[3] Siddiqui et al. (2010).
[4] Brown et al. (2012).
[5] Kaji et al. (2015).
[6] Laughton et al. (1994).
[7] Skinner et al. (1998).
[8] Feng et al. (2002).
[9] Siddiqui et al. (2012).
[10] Beg and Jorgensen (2003).
[11] Nicholl et al. (2017).
[12] Jobson et al. (2015).
[13] Jones and Sattelle (2008).

muscimol's paralyzing effects, a potent mammalian $GABA_A$ receptor agonist (McIntire et al., 1993). *Cel-unc-49* generates three distinct isoforms, UNC-49A, UNC-49B, and UNC-49C, through splicing a common N-terminus to one of the three tandem copies of the C-terminus (Bamber et al., 1999). Of the three variants, only UNC-49B expresses as GABA-sensitive homomeric receptors. *Cel*-UNC-49B is sufficient to form the neuromuscular junction $GABA_A$ receptor in both heterologous cells and worm muscle cells (Bamber et al., 1999, 2005). The subunits, UNC-49B and UNC-49-C, co-localize at the neuromuscular junctions of D-type GABA motor neurons and UNC-49B is essential for synaptic localization of UNC-49C (Bamber et al., 1999, 2005). When co-expressed in heterologous cells, *Cel*-UNC-49B and UNC-49-C assembled into a functional heteromeric channel. The channel displayed decreased sensitivity to GABA (EC_{50}=107.5 ± 13.5 μM) in comparison to UNC-49B (GABA EC_{50}=43.7 ± 2.9 μM) homomeric channels. In addition, effects of several positive (diazepam, pentobarbitone, propofol, and neurosteroids) and negative (bicuculline, dieldrin, PTX, and penicillin G) allosteric regulators of GABA channels were also altered in the heteromeric channel (Bamber et al., 2003). This suggests that UNC-49C is not essential for GABA receptor function although it does have a modulatory functional role in the inhibitory postsynaptic responses. UNC-49B and UNC-49C are 67% identical but have very different functional roles, highlighting the importance of sequence differences in functionally important domains. Bamber et al. (2005) used patch-clamp electrophysiology on *C. elegans* muscle cells to show that the pharmacological properties of the *in vivo* GABA receptors were more similar to the UNC-49B/C heteromeric channel. The *Cel*-UNC-49B/C heteromeric channel has differences in sequence similarity, subunit composition, *in vivo* function, and pharmacological properties from vertebrate receptors. On the other hand, UNC-49A has barely detectable expression levels *in vivo* and doesn't assemble as a functional heteromeric channel with either UNC-49B and UNC-49C (Bamber et al., 1999, 2003, 2005). Hernando and Bouzat (2014) characterized UNC-49 GABA receptors from cultured *C. elegans* L1 muscle cells. In L1 stage of the worm, the GABA receptors are only expressed in the ventral nerve cord (White et al., 1976; Gally and Bessereau, 2003). The *C. elegans* L1 muscle channel was activated by GABA, muscimol and piperazine (Hernando and Bouzat, 2014). The GABA-activated macroscopic currents showed slower desensitization as was observed in UNC-49B/C heteromers expressed in oocytes by Bamber et al. (2003). The single-channel conductance of ~22 pS recorded from L1 muscle cells was in close agreement with the ~30 pS for *C. elegans* UNC-49B/C heteromers expressed in HEK cells and conductance reported for *A. suum* GABA channels (Martin, 1985; Bamber et al., 1999; Hernando and Bouzat, 2014). Thus, it is likely that muscles of L1 stage of *C. elegans* express UNC-49B/C heteromers as reported for adult *C. elegans* muscles (Bamber et al., 1999, 2003). IVM behaved as an antagonist of the channel and produced irreversible inhibition of GABA-mediated activity which has also been observed with *A. suum* GABA channels (Holden-Dye et al., 1988; Martin and Pennington, 1989; Hernando and Bouzat, 2014).

UNC-49B and UNC-49C subunits have been characterized from *H. contortus* by Siddiqui et al. (2010), Brown et al. (2012) and (Table 3). As observed with *C. elegans*, *Hco*-UNC-49B formed a functional GABA-sensitive homomeric channel (EC_{50}=64.0 ± 4.4 μM) and co-assembled into heteromeric receptors (GABA EC_{50}=39.9 ± 5.7 μM) when expressed with *Hco*-UNC-49C which was relatively insensitive to PTX (Siddiqui et al., 2010; Kaji et al., 2015). Interestingly, *Hco*-UNC-49C behaved as a positive modulator of GABA sensitivity in the heteromeric channel, while *Cel*-UNC-49C was a negative modulator of GABA sensitivity. This difference in pharmacological properties was attributed to the *Hco*-UNC-49B subunit (Siddiqui et al., 2010). This highlights the species-based variation in the properties of individual channels even when the receptor subunits are highly conserved among nematodes. Brown et al. (2012) reported direct activation of the *Hco*-UNC-49 channels by piperazine (EC_{50}=6.23 ± 0.45 mM for *Hco*-UNC-49B channels; EC_{50}= 5.09 ± 0.32 mM for heteromeric channels), and this

activity was reversibly blocked by PTX showing the GABA receptor as the target site for the anthelmintic. Kaji et al. (2015) characterized the effect of a diverse panel of agonists to elucidate the distinct pharmacology of *H. contortus* UNC-49B homomeric and UNC-49B/C heteromeric receptors from vertebrate GABA channels (Table 3). For example, the sulphonated molecules P4S and taurine, activators of vertebrate $GABA_A$ receptors, failed to activate *H. contortus* UNC-49 channels. Furthermore, the pharmacological profile of *H. contortus* UNC-49 channel resembles *A. suum* muscle GABA receptor with similar sensitivities to muscimol, activation by piperazine, lack of effect of sulphonated molecules, different sensitivities to the enantiomers of GABOB (4-amino-3-hydroxybutyric acid) and resistance to antagonistic effects of bicuculline (Martin, 1985; Holden-Dye et al., 1988, 1989; Brown et al., 2012; Kaji et al., 2015). Macrocyclic lactones, including IVM and moxidectin, produced moderate potentiation of GABA-mediated responses of UNC-49 channels contrary to inhibition observed in *C. elegans* L1 muscle (Brown et al., 2012; Hernando and Bouzat, 2014). This shows that macrocyclic lactones, which primarily target GluCls, have a poly-pharmacological effect and emphasizes the secondary role of the GABA channels in the paralytic effect of macrocyclic lactones on parasitic nematodes (Brown et al., 2012).

Hco-HG1 (now designated as LGC-37) is another GABA subunit identified in the sheep nematode which appears to be an α-like GABA subunit (Laughton et al., 1994; Foster et al., 2018). The subunit is expressed in the ventral nerve cord and in a few head neurons, including ring motor- and inter-neurons, of the nematode (Skinner et al., 1998). The *Hco-hg1* gene has two alleles associated with IVM susceptibility, *hg1A*, and IVM resistance allele, *hg1E*, which differ in four amino acid residues of the cysteine loop and fourth transmembrane domain (Feng et al., 2002). These alleles can assemble as a GABA-gated heteromeric receptor with *Cel*-GAB-1, a β-like $GABA_A$ subunit, when expressed in *Xenopus* oocytes (Feng et al., 2002). The homomeric receptors of either GAB-1 or HG1 did not respond to GABA application (Laughton et al., 1994; Feng et al., 2002). The sensitivity and efficacy of the receptor for GABA was approximately 5-fold higher with IVM susceptibility allele, HG1E (EC_{50}=3.3 μM) in comparison to *Cel*-GAB-1/HG1A channel (EC_{50}=17.8 μM). Furthermore, IVM potentiated the GABA response of *Cel*-GAB-1/HG1A, but reduced the GABA-mediated current of *Cel*-GAB-1/HG1E receptor (Feng et al., 2002). This suggests the differences in the sequence of HG1A and HG1E, especially in the N-terminal cysteine loop, may play a crucial role in agonist binding. The modulatory effect of IVM on the GAB-1/HG1 channel again highlights the possible interactive role of the GABA channels in the paralysis of nematode motility by macrocyclic lactones. The *gab-1* and *lgc-37* gene is present in the genome of *A. suum* and *L. loa* while only *lgc-37* sequence has been identified in *B. malayi* genome suggesting a lack of conservation in the components of nematode GABA inhibitory neurotransmission (Williamson et al., 2007; Accardi et al., 2012).

H. contortus lgc-38 encodes for another GABA receptor subunit that is related to the nematode UNC-49 group of channel subunits and the RDL insect receptors (Siddiqui et al., 2012). *lgc-38* is widely conserved in nematode genomes, including *C. elegans*, *A. suum*, and *B. malayi*, but it has only been characterized from *H. contortus* (Accardi et al., 2012; Siddiqui et al., 2012). *Hco*-LGC-38 expressed as a GABA sensitive (EC_{50}=19.3 ± 4.3 μM) homomer. Muscimol also produced direct activation of the channel but with reduced efficacy in comparison to GABA which differentiates the channel from vertebrate $GABA_A$ receptors. PTX produced potent blocking of the channel while fipronil and dieldrin produced moderated reduction of the GABA mediated responses (Siddiqui et al., 2012).

In addition to mediating fast inhibitory neurotransmission, GABA has been implicated in excitatory neurotransmission in *C. elegans*. Out of

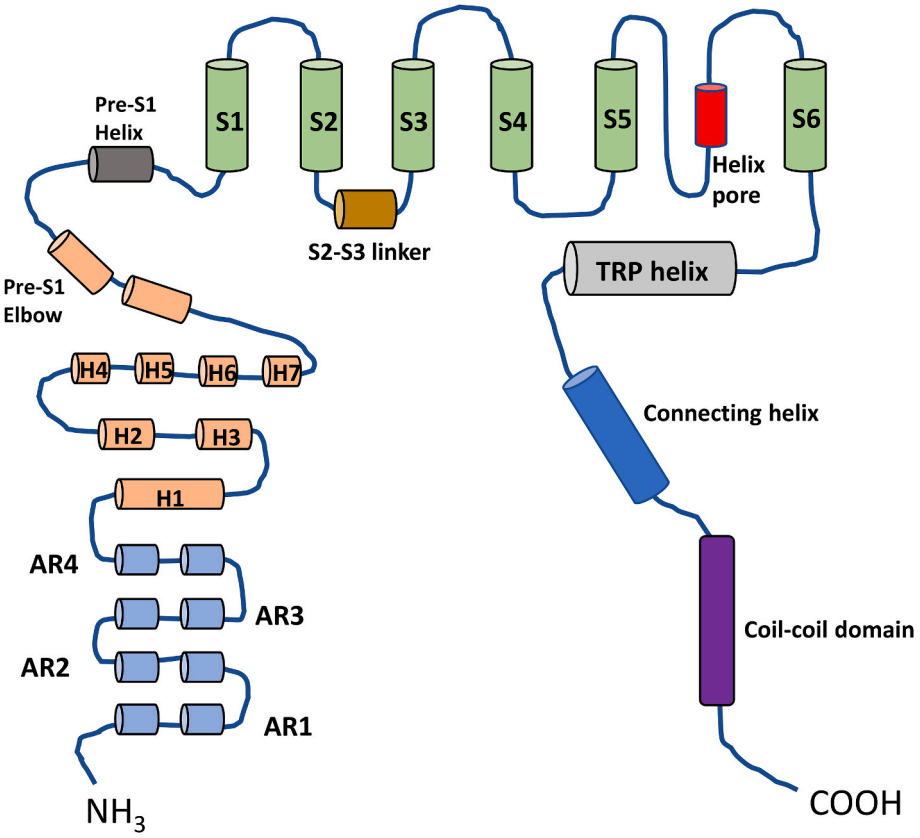

Fig. 5. Secondary structure of TRP-2 channel showing 4 Ankyrin repeat domains (AR-1 to AR-4 highlighted in light blue color), S1 – S6 transmembrane region (shown in green color), helix pore (red color), TRP Helix which contains the EWKFAR domain (grey color), TRP domain located close to S4–S5 linker and coil-coil domain (highlighted in purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the 26 GABAergic neurons of *C. elegans*, DVB (dorsorectal ganglion) and AVL (ventral cord motor neuron) innervate the enteric muscles and GABA release at the synapses of these motor neurons induces muscle contraction resulting in expulsion of intestinal contents (McIntire et al., 1993). Laser ablation of AVL and DVB motor neurons results in severely decreased frequency of enteric muscle contraction and the worms resemble a "constipated" phenotype (McIntire et al., 1993). The anion-selective UNC-49 GABA channel has no effect on enteric muscle contraction, suggesting an involvement of separate module of nematode GABAergic neurotransmission in inhibitory and excitatory actions (Bamber et al., 1999). *C. elegans exp-1* (EXPulsion defective (defecation)) encodes for a cation-selective GABA subunit, EXP-1, which is expressed in the neuromuscular junctions of intestinal and anal depressor enteric muscles and receives inputs from AVL and DVB GABAergic motor neurons (Beg and Jorgensen, 2003). *exp-1* mutants lack enteric muscle contractions and exhibit abnormal defecation cycles but have normal locomotory behavior (Thomas, 1990). This suggests the EXP-1 receptor specifically mediates enteric muscle contraction through excitatory GABA neurotransmission. *lgc-35* encodes for another excitatory GABA channel that is expressed in sphincter muscle, a subset of ventral cord acetylcholine motor neurons and the spicule protractor in males (Jones and Sattelle, 2008; Jobson et al., 2015). LGC-35 channels mediate sphincter muscle contraction in the worms. *lgc-35*:*exp-1* double mutants show a greater decrease in enteric muscle contractions than *exp-1* single mutants (Jobson et al., 2015). This suggests that both LGC-35 and EXP-1 mediate GABA-dependent enteric muscle contraction. Furthermore, LGC-35 has functions as a spillover receptor and stimulates acetylcholine release from motor neurons to modulate locomotory behavior (Jobson et al., 2015). In male worms, LGC-35 mediated excitatory GABA signals stimulate spicule extension involved in male-specific copulatory behavior (Jobson et al., 2015). EXP-1 and LGC-35 subunit have all the characteristic domains of a typical ionotropic GABA receptor subunit but with specific amino-acids in the pore-forming domain that confer cation selectivity (Beg and Jorgensen, 2003). EXP-1 and LGC-35 share 53% sequence identity and express as cation-permeable homomeric channels in *Xenopus* oocytes that were more sensitive to GABA (EXP-1 EC_{50}=26.4 ± 1.08 μM; LGC-35 EC_{50}=17 ± 0.71 μM) in comparison to muscimol (EXP-1 EC_{50}=250.0 ± 22.0 μM; LGC-35 EC_{50}=348.0 ± 17.0 μM) (Beg and Jorgensen, 2003; Nicholl et al., 2017). The pharmacological profile of EXP-1 and LGC-35 channels were more similar to $GABA_A$-ρ family than $GABA_A$ receptors with notable pharmacological differences that distinguishes them from vertebrate and other nematode GABA channels (Nicholl et al., 2017). For example, the cation-selective channels were resistant to the channel blocking effects picrotoxin which is potent blocker of vertebrate $GABA_A$-ρ channels. The synthetic neurosteroid, alphaxalone, a potent modulator of $GABA_A$- and $GABA_A$-ρ -induced currents failed to modulate the activity of EXP-1 channels and weakly inhibited LGC-35 mediated currents (Jobson et al., 2015; Nicholl et al., 2017). *exp-1* and *lgc-35* genes have been identified in the genomes of parasitic nematodes, including *H. contortus* and *A. suum,* but they remain uncharacterized (Accardi et al., 2012).

3. Other ion channels

3.1. Transient Receptor Proteins

Transient Receptor Proteins (TRP) channels belong to a highly diverse family of voltage-gated like ion channel proteins (Yu and Catterall, 2004). Based on their secondary structure, they are classified into subfamilies (TRPC, TRPM, TRPV, TRPN, TRPP, and TRPML; Fig. 5) (Venkatachalam and Montell, 2007; Nilius and Owsianik, 2011; Peng et al., 2015). TRP channels are usually non-selective cation permeable, although the mammalian TRPM4 and TRPM5 are Ca^{2+} activated but impermeable to calcium (Launay et al., 2002; Liman, 2014). TRP channels play a crucial role in sensory signaling where they respond to a large number of cellular and environmental stimuli like calcium, osmotic pressure, membrane lipids, heat, light, stretch, nociceptive compounds like capsaicin, among others (Venkatachalam and Montell, 2007; Gees et al., 2010; Zheng, 2013; Hoffstaetter et al., 2018).

The nematode model *C. elegans* has 15 TRP channels encoded in its genome with 3 TRPCs (*trp-1, trp-2* and *spe-41*), 4 TRPMs (*gon-2, gtl-1, get-2* and *ced-11*), 5 TRPVs (*ocr-1, ocr-2, ocr-3, ocr-4* and *osm-9*), 2 TRPAs (*trpa-1* and *trpa-2*) and one each of TRPP, TRPN and TRPML (*pkd-2, trp-4* and *cup-5* respectively). A diverse repertoire of TRP channels is expected in *C. elegans* as it is a free-living nematode and is subjected to a wide variety of environmental cues. However, in the filarial nematode, *B. malayi, trp-1, gtl-1, trpa-1, pkd-2, ocr-3,* and *ocr-4* are absent (Wheeler et al., 2020b). *B. malayi* also has two *ocr-1/2* like genes (*Bm5691* and *Bm14098*). Interestingly, in clade III nematodes, *A. suum*, homologs can be found for *trp-1, pkd-2, ocr-3,* and *ocr-4* (Wheeler et al., 2020b). The expression pattern of TRP channels differs between *C. elegans* and the filarial nematode *B. malayi*. According to curated data from wormbase.org in *C. elegans, trp-2* is expressed in neurons and intestinal muscles, *osm-9* in sensory neurons, *gon-2* in the gonad, and intestine *ced-11* in the hypodermis. However, a recent study found expression of these TRP genes in muscle cells of *B. malayi*, indicating that the functions of TRP channels could be different in parasitic nematodes (Verma et al., 2020).

The idea that TRP channels could be potential anthelmintic drug targets to combat helminth infections has been proposed in several reviews (Wolstenholme et al., 2011; Bais and Greenberg, 2016, 2018, 2020). More recently, in the flatworm *Schistosoma mansoni,* a TRPM channel ($SmTRPM_{PZQ}$) has been described that is activated by the anti-schistosomal drug praziquantel (Park et al., 2019). In *B. malayi,* the TRPV agonist nicotinamide has been shown to reduce the L3 burden in mosquitoes (Wheeler et al., 2020b). The same study describes RNAi of TRPV, *osm-9*, inhibiting the chemotaxis behavior of infective stage larvae towards host-associated cues (Wheeler et al., 2020b).

One of the more significant findings revealed that diethylcarbamazine (DEC), an old anti-filarial drug, was shown to target TRP channels expressed in *B. malayi* muscles; with loss of TRP-2, GON-2, and CED-11, significantly reducing the effects of DEC (Verma et al., 2020). TRP-2 regulates ACR-15 and ACR-16 in interneurons to modulate nicotine-dependent behavior in *C. elegans (Feng* et al., *2006)*. ACR-16 is a potential cholinergic drug target and is extensively studied in parasites including *A. suum* and *A. caninum* (Abongwa et al., 2016b; Zheng et al., 2016; Choudhary et al., 2019).

The nematode intestine is slowly being characterized as a potential target for future ion channel targeting anthelmintics (Wang et al., 2015; Jasmer et al., 2019, 2020). The TRPM, *gon-2,* is generally expressed in the intestine, regulating calcium homeostasis in *C. elegans* (Teramoto et al., 2005; Xing et al., 2008). Targeting GON-2 and intestinal calcium signaling could be a novel mode of action for potential anthelmintic drugs.

Several TRP channels in *C. elegans* have been implicated in mechanosensation and locomotory circuits (Schafer, 2015). TRP-4 is involved in stretch receptor-mediated proprioception (Li et al., 2006). TRP-1 and TRP-2 are required in proprioceptive responses in *C. elegans*' head steering locomotion (Yeon et al., 2018). TRPA-1 is required in OLQ sensory neurons for behavioral responses to nose touch (Kindt et al., 2007) and functions as a thermosensory receptor for cold (Chatzigeorgiou and Schafer, 2011; Xiao et al., 2013). A fourth TRP channel involved in mechanosensation, OSM-9, is expressed in several touch neurons, ASH, FLP, and OLQ (Colbert et al., 1997). An anthelmintic targeting these channels, thereby disrupting the locomotory circuit, could effectively eliminate parasitic nematode infections.

3.2. Potassium channels in parasitic nematodes

Large conductance (BK – Big K^+) potassium channels are potential targets for an anthelmintic drug that could be used against parasites resistant to current anthelmintics. SLO-1 belongs to this family of channels that are involved in inhibitory signaling in nematodes. They

can be activated by changes in the membrane potential and/or by depolarization due to increased cytosolic calcium (Lee and Cui, 2010). In nematodes, *slo-1* is expressed in the body wall musculature and neuronal system and has a role in regulating pharyngeal pumping and motility (Bull et al., 2007; Holden-Dye et al., 2007). It has also been shown to be expressed in parasitic nematodes like *Trichuris* spp. (Kulke et al., 2014; Yilmaz et al., 2015), and in the body wall muscle of the filaria *B. malayi* (Kashyap et al., 2019).

Emodepside, a drug developed by Bayer® as a broad spectrum anthelmintic, is a potent activator of SLO-1 in nematodes (Guest et al., 2007; Welz et al., 2011). The human ortholog of *slo-1*, *kcnma1,* was shown to be capable of rescuing the phenotype of *slo-1* null mutants in *C. elegans* but worms expressing the KCNMA receptor were 10 times less sensitive to emodepside when assayed for motility and pharyngeal activity (Crisford et al., 2011, 2015). SLO-1 channels of parasitic nematodes, *A. caninum* and *C. oncophora,* could compensate for the endogenous SLO-1 channels in a null mutant background in *C. elegans* (Welz et al., 2011). Emodepside was shown to elicit an outward potassium currents in the muscle flaps from *A. suum* (Buxton et al., 2011). These results indicate that emodepside could activate SLO-1 channels in parasitic nematode muscle. However, data from heterologous expression systems including *Xenopus* oocytes expressing a parasitic nematode SLO-1 channel was lacking. Whilst heterologously expressed *C. elegans'* SLO-1 channels were shown to be activated by emodepside, SLO-1 channels of the parasitic nematode *T. muris* did not respond to emodepside treatment (Kulke et al., 2014). Also, the potency of emodepside on filarial nematodes like *L. sigmodontis, A. vitae, Brugia* spp., *O. gutturosa* and *O. lienalis* was quite varied with *Brugia* spp. being the least sensitive *in vitro* (Kulke et al., 2017).

B. malayi is an excellent *in vitro* filarial model for pharmacological and molecular characterization of drug targets. Adult *B. malayi* paralyze upon treatment by emodepside but a sex-specific difference was first reported by Kashyap et al. (2019). This difference was also seen in the emodepside evoked outward potassium currents in muscle cell preparations. The males were more sensitive to emodepside than females (Kashyap et al., 2019). This difference was attributed to sex-specific expression of splice variants; *slo-1a* and *slo-1f* were expressed in female *B. malayi* while only *slo-1f* was expressed in males. *In vitro* studies in *L. sigmodontis* also revealed sex-dependent differences in its sensitivity to emodepside with the males being 10 times less sensitive than the females (Kulke et al., 2017). The sex-specific expression of splice variants in *L. sigmodontis*, however, is unclear.

In the free-living nematode *C. elegans*, there are 13 predicted splice variants of *slo-1* and they exhibit varied channel properties when expressed in a heterologous system (Johnson et al., 2011). It is understood that the differential expression of these splice variants is critical for the functioning of the BK channels (Glauser et al., 2011). However, the relative expression of these splice variants *in vivo* is unknown. Splice variants of SLO-1 channels of *B. malayi* expressed in *X. laevis* oocytes elicited an outward potassium currents due to their activation by emodepside (Kashyap et al., 2019). This study also revealed the differences in the EC_{50} of expressed SLO-1f and SLO-1a receptors with SLO-1f more sensitive to emodepside than SLO-1A. The EC_{50} of the combination of SLO-1A and SLO-1F was similar to SLO-1A indicating that SLO-1A could be the dominant splice variant expressed in the female worm rendering it less sensitive to emodepside.

BK channels contain two Regulators of K^+ Conductance (RCK) domains, 1 and 2 in their cytoplasmic region. These domains regulate the channel by responding to nucleotides and ions like Ca^{2+}. In *C. elegans*, the splice variation in RCK1 and RCK2 domains as well as the linker between these domains alters the calcium permeability and conductance of the channel (Johnson et al., 2011). In *B. malayi,* alternative splicing near the RCK1 domain leads to a reduced sensitivity to emodepside (Kashyap et al., 2019). Homology modelling also suggests that the RCK1 domain could contribute to the binding site of emodepside (Kashyap et al., 2019) suggesting that mutations in the RCK domain may confer

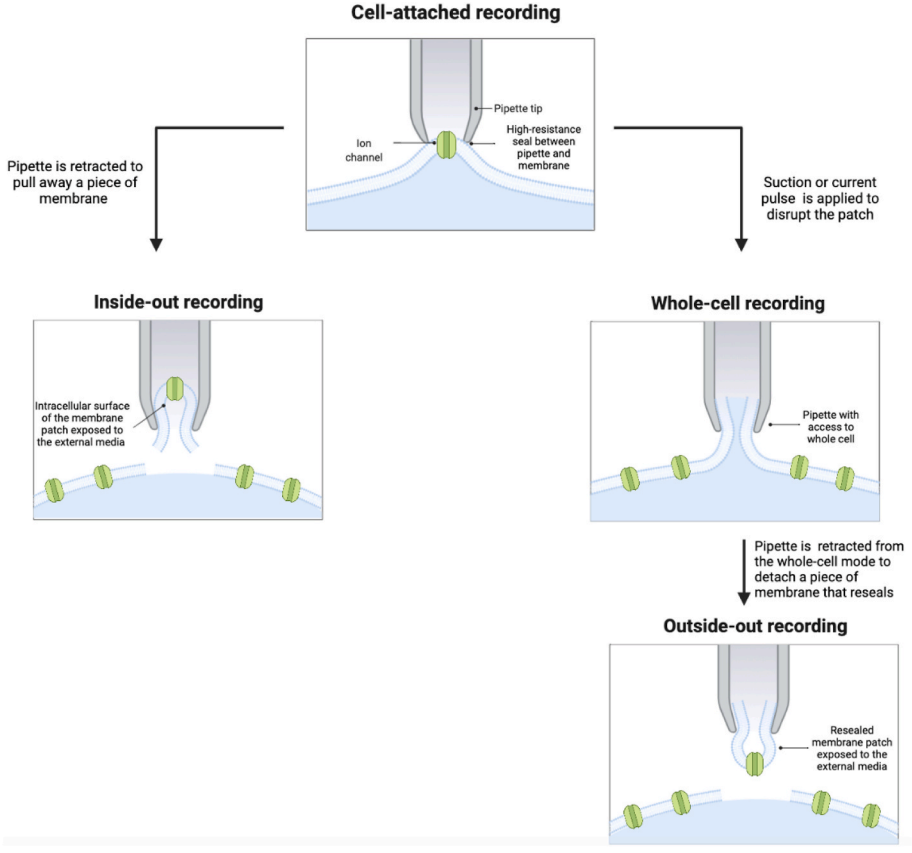

Fig. 6. Schematics of various patch-clamp configurations. In Cell-attached configuration, a pipette is attached to the surface of the cell by gentle suction to form a giga-ohm seal. This allows recording of activity of any ion channels active in patched membrane. When this pipette is retracted, it pulls away the original small patch of membrane that has its cytoplasmic surface facing the external solution. This forms an inside-out patch. Alternatively, if suction or a large brief current pulse is applied to the original sealed patch, it leads to disruption of the patch with pipette still attached to the cell. This allows the pipette to gain access to the interior of the cell, allowing whole-cell recordings. If the pipette is retracted in the whole-cell mode to detach a patch of membrane, an outside-out patch can be formed; the cell membrane reseals and is exposed to the external media.

resistance to emodepside. Recent studies have identified a third splice variant that contains both the exons that show alternative usage in the a and the f isoforms. This suggests existence of a complex *in vivo* genomic regulatory mechanisms that can combat the insult caused by anthelmintics like emodepside (Wheeler et al., 2020a).

One way to counter natural anthelmintic resistance mechanisms of parasites is by combination therapy. Diethylcarbamazine (DEC) is an antifilarial drug used against human infections. DEC on muscle preparations in both *A. suum* and *B. malayi* elicits an outward potassium current similar to emodepside (Buxton et al., 2011; Verma et al., 2020). This suggests DEC could be involved in the activation of BK channels in parasitic nematodes. DEC causes a transient muscle contraction phenotype in *B. malayi* and this is thought to be mediated via TRP channels (Verma et al., 2020). Many TRP channels conduct calcium ions and an increase in calcium levels in the cytoplasm could have an effect on the SLO-1 channels. SLO-1 can be regulated by internal calcium concentrations and the L-type calcium channel, EGL-19, regulates SLO-1 channels by associating itself close to SLO-1 channels in *C. elegans* (Kim et al., 2009). In *A. suum*, DEC significantly increased the hyperpolarizing effects caused by emodepside suggesting a synergistic effect of the combination (Buxton et al., 2011). This is particularly important in lymphatic filarial infections as the bioavailability of emodepside is lower in the regions where the worms reside. This could be one of the reasons why emodepside is not very efficient in clearing *B. malayi* in *in vivo* models (Zahner et al., 2001). In conclusion, BK potassium channels act as a good anthelmintic target but the efficacy of the drugs targeting BK channels depend on alternative splicing with even small changes in amino acid sequences leading to a significant change in drug efficacy.

4. Techniques for studying ion channels

4.1. Electrophysiology techniques

Electrophysiological assays are widely used to study ion-channel behavior as they allow measurements of ionic currents across the cell membrane from cells or tissue. The science of electrophysiology began towards the end of the 18th century in Italy with Luigi Aloisio Galvani, in collaboration with his wife, Lucia Galeazzi (Piccolino, 2008; Verkhratsky and Parpura, 2014). They discovered that on the application of electric shock to the relevant nerves of a dead frog, the musculature would twitch in response. In the nineteenth century, the German physiologist Emil Heinrich du Bois-Reymond, known as the father of experimental electrophysiology, and his student Julius Bernstein discovered the action potential and recorded its time-course (Finkelstein, 2015). Alan Lloyd Hodgkin and Andrew Huxley were awarded the Nobel Prize in Physiology and Medicine in 1963 for their outstanding work providing insights into nerve cell excitability (Hodgkin et al., 1952; Schwiening, 2012). They used the voltage-clamp technique to study the ion channel events giving rise to propagating action potentials and provided a mathematical framework for analyzing the kinetics of ion-channels. Their work led to an explosion of interest in electrophysiology and use of the voltage-clamp technique in a wide range of cells. Various tools and methods have gradually developed that constitute modern electrophysiology approaches. Electrophysiology techniques involve measuring voltage changes or electric currents, offering direct and accurate characterization of the ion-channels various functional states. This technique can be used to study the biophysical activity of ion-channels, resolve their interactions with drug molecules, and assess a new chemical entity's activity. Among the various electrophysiological measurement assays, patch-clamp electrophysiology is considered the "gold-standard" for studying physiological, pharmacological, and biophysical behaviors of ion channels from native cells and heterologous expression systems. Developed in the 1970s by Neher and Sakmann, this toolbox can stimulate, modulate, and record ion channel activity from membrane fragments and whole cells (Neher and Sakmann, 1976). Neher and Sakmann shared the Nobel Prize in Physiology and Medicine in 1991. The patch-clamp technique was further refined to record single-channel currents, allowing the discrete opening and closing of individual channel macromolecules to be recorded (Hamill et al., 1981). The patch-clamp technique is frequently used for characterizing the biophysical properties of a single ion channel type while providing unrivaled signal-to-noise ratio, excellent time resolution (microsecond range), and sensitivity (picoampere range) (Fig. 6). This technique involves patching a glass micropipette to the surface of the cell membrane. A small suction is then applied to the back of the pipette, causing a high-resistance seal (giga-ohm range) to form between the glass and the cell membrane. The tight seal allows the electric isolation of the membrane patch and ensures that all current to be recorded flows through the pipette without leaking through other regions of the membrane. This conformation is called the cell-attached mode and helps with high fidelity recording from single ion channels (Ogden and Stanfield, 1994; Molleman, 2003; Carter and Shieh, 2009).

In the whole-cell mode, the most commonly used patch-clamp conformation, the membrane patch within the micropipette, is disrupted by applying strong suction briefly, giving the pipette electrical access to the whole cell (Molleman, 2003). This arrangement can be used in two configurations: voltage-clamp or current-clamp mode. In voltage-clamp, voltage is applied, and current is measured, while in current-clamp mode, the current is kept constant, and membrane potential changes are measured. The patch-clamp technique's other variations are inside-out recordings, outside-out recordings, and perforated patch recordings (Yajuan et al., 2012; Linley, 2013) (Fig. 6). In the inside-out mode, a small piece of membrane is pulled away from the cell by retracting a pipette in the cell-attached mode. This exposes the intracellular surface of the membrane patch to the external media or bath solution. This technique can be used to manipulate the environment at the exposed intracellular surface of the single-ion channel. An outside-out patch is complimentary to inside-out clamping and begins with the pipette's retraction from the whole-cell mode. When the electrode is drawn out of the cell, a piece of membrane detaches from the cell and reseals to form a patch exposing its extracellular surface to the bath solution. This arrangement helps study the influence of extracellular signals on channel activity. Electrophysiologists sometimes use a different arrangement called a perforated patch. In this technique, a chemical (usually an antibiotic such as amphotericin-B or nystatin) is applied with a glass pipette to cause perforations in the cell membrane to transition from cell-attached to whole-cell mode (Linley, 2013). This methodology is used to prevent dialysis of cytoplasmic contents into the pipette but has the disadvantage of generating a smaller signal. In certain recordings, a low electrical resistance (loose seal) is used, allowing multiple areas of the membrane of the same cell to be tested with the same pipette (Almers et al., 1983; Ogden and Stanfield, 1994). This technique is called loose-patch clamp configuration and is analogous to cell-attached mode. This procedure doesn't alter or causes minimum perturbation of the membrane surface, which permits recording for longer periods. A loose-patch clamp is an appropriate choice for examining channel distribution and experiments in which the same population of channels needs to be sampled multiple times. This technique is also useful for cell membranes covered with basal lamina or other protective layers that make the formation of a tight seal impossible. The pipette does not get close enough to the membrane to form a tight seal (gigaseal) in a loose-patch clamp, and the distance between the membrane and the pipette is ~20–100 nm. The small gap between the glass and cell surface allows the ions to pass outside without entering the pipette. The major disadvantage of the technique is that the loose seal allows a larger leak current and higher noise signal, reducing the resolution of small currents.

Patch-clamp techniques have been widely used for the biophysical analysis of ion channels expressed in nematodes nerves, muscles, and synapses. Jarman (1959) performed the first patch-clamp study in a nematode and analyzed the *A. lumbricoides* muscle cells' electrical activity. Several groups of scientists have since used the patch-clamp

technique in a variety of nematode preparations, including muscle cells, pharyngeal tissue, and nerve cells (Holden-Dye et al., 1988; Martin and Pennington, 1988, 1989; Holden-Dye and Walker, 1990; Martin, 1996; Adelsberger et al., 1997; Brownlee et al., 1997; Francis et al., 2003; Qian et al., 2006; Robertson et al., 2011, 2013; Goodman et al., 2012).

Two-electrode voltage clamp (TEVC) is another conventional electrophysiology approach employed by scientists to study ion channel properties. The technique has become irreplaceable for clamping large cell types, especially *Xenopus* oocytes, which are utilized for heterologous expression of various protein receptors (Stühmer and Parekh, 1995). Gurdon et al. (1971) injected *Xenopus* oocytes with foreign messenger RNA and reported successful translation of the RNA into proteins. The first successful expression of receptors and ion channels in the *Xenopus* oocytes was reported by Gundersen et al. (1983) and Miledi et al. (1983). Cole and Marmont designed the voltage-clamp method, and in the late 1940s, Hodgkin, Huxley, and Katz applied this technique as TEVC to record from giant axons of squids (Hodgkin et al., 1952). The method involves experimental control of the membrane potential and measurement of electric current produced by the flow of ions across the cell membrane. Two intracellular electrodes are used to penetrate the membrane of the oocytes in this technique, a voltage electrode to sense the membrane potential and a current electrode for injecting current to adjust the potential to desired values. The measured membrane potential is compared to command voltage, and the difference is brought to zero by a control amplifier. At the same time, the monitored injected current provides a measure of the total membrane current (Baumgartner et al., 1999; Guan et al., 2013). TEVC has been extensively used to study the function of ion channels and their general characterization. The technique allows clamping of large currents due to the high-current passing capacity of microelectrodes, offers a fast clamp setting resulting in high time resolution and low noise (Hernández-Ochoa and Schneider, 2012). There are technical difficulties observed with TEVC due to the oocytes' large size (Baumgartner et al., 1999). The voltage clamp's speed is constrained due to the limited current provided through a pipette and, in turn, the limited rate at which the large membrane capacitance can be charged. High levels of channel expression in oocytes can result in very high currents (100 μA or more), which results in substantial voltage errors. These problems are addressed by using high-voltage two-microelectrode amplifiers and applying electronic compensation for series resistance (Baumgartner et al., 1999).

4.2. High throughput screening (HTS) methods

Electrophysiology methods are very useful, but their scalability and application are restricted by the requirement for highly skilled and trained operators, complex recording equipment, which results in low throughput. This labor-intensive and time-consuming technique is not suitable for preliminary screening experiments. Various HTS assays, non-electrophysiological and electrophysiology-based, have been extensively developed, which enable ion channel studies. Combining these electrophysiology and non-electrophysiology approaches provides a cost-effective and information-rich measure of ion channel activity.

4.2.1. Ligand binding assays

Ligand binding assays are non-functional tests that detect the binding affinity of a compound to a target (ion channel in this case). These are low-cost screening assays widely used to study ion-channel modulators and measure the ligand's direct interaction with its target (McManus, 2014; Yu et al., 2016). Ligand-binding assays were used to identify the high-affinity binding sites for IVM in *C. elegans* and *H. contortus* (Schaeffer and Haines, 1989; Schaeffer et al., 1989; Rohrer et al., 1994). Forrester et al. (2002) also used radioligands to identify a common high-affinity IVM and moxidectin binding site on *Hco*-GluClα; this site was distinct than the glutamate-binding site. A critical prerequisite for ligand binding assays is knowledge of the target binding site. HTS binding assays require the synthesis of a radio-labeled ligand with known pharmacological activity. The technique can only detect ligands that occupy the same binding site indicated by the labeled probe's displacement. The method cannot identify the functional effect (for example, agonist or antagonist activity) of the ligand on the ion channel and requires low-throughput secondary assays to determine the functional change. The assay is configured for the known binding site and cannot explore weakly coupled allosteric binding sites. Another limitation is for ligands with a binding site affinity dependent on voltage or conformational state of the channel, which cannot be controlled by this platform (Denyer et al., 1998; Yu et al., 2016). Additionally, the assay outcome depends on the affinity of the labeled ligand; a high-affinity ligand may limit the detection of weaker ligands, while a low-affinity ligand may identify non-specific binding. This HTS technique is suitable for the identification of channel activators and modulators but is rarely used for general screening (Bennett and Guthrie, 2003; Picones et al., 2016; Yu et al., 2016).

4.2.2. Flux-based assays

Flux-based assays are cell-based functional assays that detect ionic flux through ion channels that correlate with the channels' activity. These assays are used for screening compounds and have been successfully applied to study ion channel targets. These assays either employ a radioactive ion, a non-physiological ion (e.g., rubidium, Rb+) or fluorescent based native ion-selective probes. Ion flux is detected with a scintillation counter or atomic absorption/emission spectrometry (Bennett and Guthrie, 2003; Yu et al., 2016). Radioactive labeled ions or radiotracers ($^{22}Na^+$ and [^{14}C]-guanidinium for Na^+ channels, $^{45}Ca^{2+}$ for Ca^{2+} channels, $^{86}Rb^+$, for K+ channels, and $^{36}Cl^-$ for chloride channels) are used to measure the influx or efflux of permeable ions (Gill et al., 2003; Terstappen, 2005; Terstappen et al., 2010). In this assay, cells expressing ion channels are incubated with the radiotracer, activated by adding an appropriate ligand, and the radioactivity is counted. The radiotracer flux assay is robust and insensitive to disturbances but has poor temporal resolution. The critical issue with this technique is the inconvenience and cost associated with handling and disposal of radioactive waste material. This method is therefore, quite impractical and is not commonly used. Terstappen (1999) developed a non-radioactive rubidium-based efflux assay that used atomic absorption spectroscopy for detection. This assay has found a more widespread application in the pharmaceutical industry as it is amenable to HTS formats and is more economical (Gill et al., 2003; Terstappen, 2005). The application of rubidium-based flux assays is limited by low temporal resolution leading to lower sensitivity, deficient membrane potential control, and less information than electrophysiology-based assays (Picones et al., 2016; Yu et al., 2016). Ion-selective fluorescent dye-based indicators (calcium indicator dyes: Fura-2, Fluo-3, Fluo-4; potassium indicator dyes: FluxOR and PBF; sodium indicator: SBFI) are used to measure intracellular ionic concentrations for the pharmaceutical screening of ion channels (Kao et al., 1989; Minta et al., 1989; Minta and Tsien, 1989; Meuwis et al., 1995; Beacham et al., 2010; Yu et al., 2016). These indicators are easier to use and offer spatial as well as temporal ion flux measurements. Fluorescent Ca^{2+} probes are the best-developed indicator dyes that are widely used to study Ca^{2+} channels and non-selective cation channels with Ca^{2+} conductivity. These indicators are sensitive, provide a robust measure of fluorescence emission on Ca^{2+} binding, and are available with a range of binding affinities to select the rate of interaction. Ca2+ fluorescence imaging on *C. elegans* patch-clamped muscles was used to demonstrate the role of EGL-19 in muscle activation (Jospin et al., 2002). McHugh et al. (2020) used Fluo-3 dye to show evidence of functioning nAChRs in the *A. suum* intestine. In the study, they reported stimulation of Ca^{2+} signals in response to application of acetylcholine (as a non-selective agonist) and levamisole (as an L-type nAChR agonist). The fluorescent Ca^{2+} probes can be used with fluorescence plate reader instrumentation (for example, fluorescence imaging plate reader, FLIPR) which allows high throughput, low-noise detection of absolute, and measurement of rapid

kinetic changes in fluorescence readouts for Ca^{2+} peaks (Schroeder and Neagle, 1996). The use of indicator dye assays is mainly affected by the limited availability of high-performance ion-specific probes and false-positive hits resulting from interference caused by other cellular processes that change the intracellular Ca^{2+} concentration (such as Ca^{2+} transporters and Ca^{2+} release from intracellular organelles). Furthermore, the lack of voltage control and limited temporal resolution affect the assay's utility (Bennett and Guthrie, 2003; Terstappen, 2005; Terstappen et al., 2010; Yu et al., 2016).

4.2.3. Assays exploiting membrane potential changes

The membrane potential is a biophysical variable that provides a generic readout of ion channel functionality regardless of ion selectivity. It is an indirect and non-linear measure of channel activity since membrane potential is determined by the gating state of all ion channels in a cell, and the gating of a channel is voltage-dependent (Zheng et al., 2004). Fluorescent voltage-sensing dye probes are electrically charged molecules that partition across the cell membrane according to the membrane potential. They measure transmembrane voltage changes using either the potential-dependent redistribution of dye or a fluorescence resonance energy transfer (FRET) between a dye pair (Epps et al., 1994; González and Tsien, 1995; Wolff et al., 2003). The oxonol derivative, bis-(1,3-dibutylbarbituric acid) trimethine oxonol [$DiBAC_4$ (3)], is an example of a lipophilic and negatively charged potentiometric dye that demonstrates voltage-dependent distribution between extracellular medium to cell cytoplasm and displays a corresponding increase in fluorescence signal. Since this dye's response time is slow (minute timescale), oxonol probes excel at detecting slow and steady-state membrane-potential changes but are not suitable to study kinetic changes of membrane potential. Furthermore, a high number of false-positive responses are observed in a HTS format due to test compound autofluorescence and interaction of test compound with cell membranes leading to an increase in intracellular concentration of dye probes (Terstappen, 2005; Terstappen et al., 2010). The FLIPR membrane potential (FMP) kit uses an anionic membrane potentiometric probe, FMP dye, which provides comparatively faster response time (tens of seconds) and has improved sensitivity to detect kinetic signal changes (Whiteaker et al., 2001). However, the lipophilic nature of both the probes makes them non-selective for membranes and can produce fluorescence signals in response to voltage changes from cell surface membranes and the intracellular membranes (Yu et al., 2016).

FRET is another form of potentiometric measurement which uses two molecular probes, a voltage-sensitive dye as an acceptor (for example, membrane-soluble oxonol dyes (bis-(1,3-dialkylthiobarbituric acid) trimethine oxonol [$DiSBAC_n$ (3)]) and a fixed FRET donor fluorophore (for example coumarin-tagged phospholipids (CC2-DMPE)) (González and Tsien, 1995; Whiteaker et al., 2001; Worley and Main, 2002). The FRET donors are stationary as they are integrated into the membrane's outer surface while the acceptor dye probes are mobile. After the cells are loaded with these probes, an increase or decrease in FRET is produced in response to membrane voltage changes, leading to fast, and radiometric responses. The radiometric measurement eliminates many of the limitations observed with the voltage-sensitive dyes. The response time (second's timescale) is much quicker with FRET assays. These assays allow kinetic analysis by producing sub-second temporal resolution (Terstappen, 2005; Terstappen et al., 2010; Yu et al., 2016). Additionally, since the donor molecular probe is anchored to the cell surface, the measured voltage changes occur at the cell surface rather than intracellular compartments. Specialized automated instrumentation such as the electrical stimulation Voltage/Ion Probe Reader (E-VIPR) and FLIPR platform are used for FRET-HTS purposes (Liu et al., 2006; Solly et al., 2008; Picones et al., 2016).

4.2.4. Automated electrophysiological assays

All the HTS screening techniques discussed so far are helpful in identifying and profiling compounds, however, they do not provide a direct measure of the ion-channel activity. Electrophysiology assays encompass the most powerful approach for direct and real-time measurement of ionic currents movement through channels and allow identification of state-dependent ion channel modulators. Automation of the electrophysiology platforms has made these assays simpler, time-efficient, less labor intense, and increased throughput. Current automated patch clamp platforms are based on two core technologies, glass pipettes or planar arrays (Dunlop et al., 2008; Terstappen et al., 2010).

The glass pipette-based automated patch clamp platforms automate the conventional manual patch-clamp approach with differences in cell attaching and gigaseal formation. This technique enables automatic patch-pipette handling, giga-ohm seal formation, obtaining whole-cell or perforated cell configuration, execution of drug application, and data acquisition with increased throughput by a factor of ten. The examples of the robotic micropipette-based instruments systems are Flyscreen (Fyion), AutoPatch (CeNeS Pharmaceuticals) and RoboPatch (Molecular Devices) for mammalian cells, and the Robocyte (IonGate) and OpusXpress 6000A (Molecular Devices) for *Xenopus* oocytes (Lepple-Wienhues et al., 2003; Schnizler et al., 2003; Papke, 2006; Vasilyev et al., 2006). These glass pipette–based platforms reduce the amount of labor needed while maintaining a high quality of the ionic current recording. These systems lead to a moderate increase in throughput that is not suitable for conducting large-scale drug screens.

Planar array based automated systems employ a flat substrate containing a small hole (1–2 μm) in a plate-based or chip-based format to replace the glass pipette (Dunlop et al., 2008; Southan and Clark, 2009; Terstappen et al., 2010; Liu et al., 2019). In general, these automated techniques have integrated machine-based operation for cell, solution, suction, and compound handling. A suspension of cells is added to the recording well containing a microscopic recording aperture, and suction is applied, which leads to the formation of an electrical seal between a cell and substrate surrounding the hole. The membrane over the aperture is disrupted to form the whole-cell configuration by applying negative pressure or by exposing the membrane to a perforating agent. The recordings can be performed between an electrode located below the plate, which is common to all the wells, and individual recording electrodes that are lowered into each well. This system allows parallel recordings from multiple wells in the same plate/chip and thus provides much higher throughput (Picones et al., 2016). IonWorks HT (Molecular Devices), a plate-based platform, is the first widely available system that uses computer-controlled fluid handling, recording electronics, and processing tools (Schroeder et al., 2003). It is now available in its second generation, the IonWorks Quattro™, which allows data collection from a 384-well plate format in two modes, single-hole mode (one cell per well is recorded) and population-patch clamp (PPC) mode (average currents from recordings of up to 64 cells per well are reported) (Bridgland-Taylor et al., 2006; Finkel et al., 2006; John et al., 2007). The PPC mode offers improved speed, robustness, and reproducibility since the cell-to-cell variation is minimized (Dale et al., 2007). PatchXpress (Molecular Devices) is the first planar chip-based electrophysiology system that uses 16 well "glass chips". It allows continuous recordings during compound application (Xu et al., 2003; Tao et al., 2004). Other examples of planar patch-clamp technology include QPatch (Sophion) and Patchliner/Synchropatch (Nanion Technologies), which are suitable for developing structure–activity relationships or screening studies (Asmild et al., 2003; Mathes, 2006; Farre et al., 2007). These systems offer rapid solution exchange time and perfusion, making them suitable for ligand-gated ion channel studies. HTS electrophysiology platforms have many advantages, and their continued evolution for automated channel screening will improve the ion-channel targeted drug discovery.

4.3. Genetics techniques for functional genomics

Genetic methods are a powerful tool for understanding the function of genes in a biological process of interest. Technological advances in sequencing have led to the completion of genome and transcriptome

sequences of various nematodes, including *C. elegans*, *A. suum*, and *B. malayi* (Blaxter et al., 1998; Parkinson et al., 2004; Ghedin et al., 2007; Williamson et al., 2007; Jex et al., 2011; Laing et al., 2013). Genetic techniques are used for comprehensive studies to close the gap between structural and functional genomics to decipher the biological function of genes. The deconvolution of gene function helps identify molecular targets and serves as a tool for hypothesis-based, mechanistic, and pharmacological investigations.

Genetic approaches include forward and reverse genetics strategies that are conceptually based on generating and mapping genetic mutations to identify genes associated with the biological target of interest. Forward genetics is a classical 'phenotype to genotype' approach where studies are initiated to determine the genetic underpinnings involved in the specific mutant phenotype of interest (Jorgensen and Mango, 2002). Sydney Brenner (1974) published the first report of the tractability of *C. elegans* for forward genetic studies and established this approach as a staple for genetic research. Brenner described a method for a forward screen that involved isolation of homozygous recessive mutant worms and mapped genes responsible for observable phenotypes. *C. elegans* is the first metazoan organism to have its whole genome sequenced (Consortium, 1998). It makes an excellent model for comprehensive genetic research and comparative pharmacology studies for the phylum Nematoda as most of the parasitic nematodes are not tractable to genetic manipulation (Gilleard, 2004). Functional genomic studies performed using *C. elegans* have been crucial in identifying nematode genes that are potential targets of anthelmintic drugs (Raymond and Sattelle, 2002; Gilleard, 2004). A mutagenesis screen with a predicted phenotype of interest serves as a starting point in forward genetic analysis. A heritable genome-wide mutagenic lesion with an observable phenotype is created using chemical agents, ionizing radiation, or transposon insertion. Chemical mutagenesis is the simplest and most widely used method to induce DNA mutation with high frequency. Ethyl methane sulfonate (EMS) and trimethylpsoralen with ultraviolet light (UV/TMP) are commonly used chemical mutagens (Anderson, 1995). The targets of monepantel, an anthelmintic used to treat gastrointestinal nematodes of sheep, and emodepside, a broad spectrum anthelmintic, were identified using EMS-induced mutagenesis (Guest et al., 2007; Kaminsky et al., 2008). Forward genetic screens using levamisole have helped in identifying gene function including nAChR subunit encoding genes expressed at the neuromuscular junction (Brenner, 1974; Lewis et al., 1980a, 1980b, 1987; Fleming et al., 1997; Boulin et al., 2012). Hu et al. (2009) also used EMS-induced mutagenesis to identify a subtype of L-type nAChRs as a target of tribendimidine. Optogenetic mutagenesis is another method that uses light and genetics to generate mutations in a defined cell population and has potential application in target identification (Zhang and Cohen, 2017). This technique has been successfully adopted in *C. elegans* to study cell-specific manipulations of neuromuscular activity and functional analysis of ion-channel mutations (Noma and Jin, 2015; Schüler et al., 2015). This technique is well-suited for *C. elegans* due to its compact nervous system, consistent neural morphology, and transparent body; however, its application in other nematode parasites is limited (Husson et al., 2013).

Reverse genetics strategies involve targeted modification of specific genes to analyze the phenotypic impact on biological processes. These techniques establish a direct link between a gene's function and its role in specific biochemical pathways. The reverse genetic strategy provides an important complement to forward genetic approaches as it can investigate the function of all genes in a gene family (Ahringer, 2006). In addition, reverse genetic tools provide an opportunity to study the role of genes of interest that have not been explored yet. In this approach, the genomic study starts with knocking down genes with known sequences. The process of perturbing a gene function in reverse genetic experiments can either be target-selected (for example, RNA-mediated interference (RNAi), Targeting Induced Local Lesions IN Genomes (TILLING)) or gene-targeted random disruptions (for example, chemical mutagenesis or transposon-mediated insertional mutagenesis followed by detection of gene knockouts by PCR, nuclease-based methods such as clustered regularly interspaced short palindromic repeats/CRISPR/Cas9 nuclease) (Kutscher and Shaham, 2014).

RNAi, first described in *C. elegans*, is a technique based on the posttranscriptional endogenous gene silencing mechanisms observed in most eukaryotes that protect cells against viral infection and transposon activity (Fire et al., 1998; Strange, 2003). In this technique, the expression of a target gene is silenced by introducing double-stranded RNA (dsRNA) complementary to the gene of interest. RNAi can be triggered by either injecting worms with interfering dsRNA, soaking worms in a solution of dsRNA, feeding worms with *Escherichia coli* expressing target gene dsRNA, and *in vivo* transcription of dsRNA from the injection of transgenes under the control of a heat shock promoter and hypoxia conditions (Fire et al., 1998; Tabara et al., 1998; Timmons and Fire, 1998; Tavernarakis et al., 2000). It is a robust and reliable tool used for genetic manipulations with the potential to identify novel drug targets and functional genetic analysis. This powerful and efficient strategy has helped create a library of bacterial clones expressing dsRNA corresponding to different *C. elegans* genes (Strange, 2003; Jones et al., 2005). RNAi has been successfully applied to large-scale, automated high-throughput genome-wide screening of the *C. elegans* genome (Maeda et al., 2001; O'Rourke et al., 2009; Squiban et al., 2012). High throughput RNAi screens in a liquid culture used in combination with automated phenotype selection, such as a modified flow cytometer COPAS BIOSORT (complex object parametric analyzer and sorter; Union Biometrica), and integrated computational tools are likely to facilitate the discovery of new drug targets and screening of potential compound libraries (Morton and Lamitina, 2010; Jagadeesan and Hakkim, 2018). RNAi-based gene silencing in intact parasitic nematodes has provided an alternative to the free-living model nematode, *C. elegans,* that lacks the parasitic adaptation and associated functionally conserved genes, which could be potential anthelmintic targets. RNAi has been explored for the discovery and validation of anthelmintic drug targets in parasitic nematodes, including *H. contortus*, *B. malayi, O. volvulus*, *A. suum,* and *Ostertagia ostertagi* (Behm et al., 2005; Kotze and Bagnall, 2006; Dalzell et al., 2011; Maule et al., 2011; McCoy et al., 2015; Verma et al., 2017). Verma et al. (2017) used RNAi in *B. malayi* and other techniques to identify four diverse nAChRs with different drug sensitivities and functions. McCoy et al. (2015) performed successful co-silencing of levamisole receptor subunit genes, *Asu-unc-29* and *Asu-unc-38*, validating the RNAi approach for drug target studies in *A. suum*. The role of latrophilin in the mode of action of emodepside was identified through RNAi of *lat-1* and *lat-2* in *C. elegans* (Willson et al., 2004). *Slo-1* was validated as a target of emodepside in adult *B. malayi* through RNAi knockdown studies (Kashyap et al., 2019). Although RNAi is a widely accepted parasite-based research tool, its utility is limited by species-, tissue-, and target-specific inconsistencies in gene knockdown, lack of heritable phenotypic effects, and potential off-target effects (Maule et al., 2011). Nevertheless, with improved delivery methods for dsRNA, RNAi can serve as an excellent complementary tool in combination with other genetic screening methods. Genome-wide RNAi automated high-throughput screens in parasitic nematodes could help accelerate the discovery of anthelmintic drug targets.

TILLING (Targeting Induced Local Lesions in Genomes) is a relatively new high throughput reverse genetics approach that allows identification of single nucleotide polymorphisms (SNPs) and/or insertions/deletions (INDELS) in the genes of interest (McCallum et al., 2000; Henikoff et al., 2004). The technique is an attractive, functional genomics tool as it does not involve the creation of transgenic modifications, is independent of genome size, and can detect induced and natural variations (Gilchrist and Haughn, 2005). Initially developed to characterize genes in *Arabidopsis thaliana*, the technique also applies to animals, including nematodes, which can be mutagenized (McCallum et al., 2000; Gilchrist et al., 2006). The technique requires prior DNA sequence information and uses a mismatch-specific endonuclease to locate mutations in a target gene through hybridizing a mutated genome to a

non-mutated genome (Gilchrist and Haughn, 2005). Gilchrist et al. (2006) applied TILLING to construct a frozen library of 1500 EMS mutagenized *C. elegans* and identified 71 mutations in 10 genes. Out of the 10 target genes screened, the TILLING approach provided the first genetically heritable mutations in eight genes. In the study, phenotypic analyses were performed on the identified mutants to establish the biological function of the genes. Thus, TILLING adds to the arsenal of reverse genetic tools available in *C. elegans*. It is a practical approach that can complement other genetic strategies and provides an allelic series of the mutant for functional genomic studies. This technique can be applied in both mutagenized and natural populations of parasitic nematodes for which genome- and transcriptome-sequences are available.

The CRISPR RNA-guided Cas9 genetic scissors are the sharpest enzyme-based genome engineering tools used for targeted modification. The technique was pioneered by Emmanuelle Charpentier and Jennifer Doudna, for which they received Nobel Prize in Chemistry in 2020 (Ledford and Callaway, 2020). The CRISPR/Cas mechanism, identified in bacteria and archaea, facilitates an adaptive immune response against foreign DNA by using CRISPR RNAs (crRNAs) to silence the invading nucleic acids (Jinek et al., 2012; Wiedenheft et al., 2012). In the type II CRISPR/Cas system, the transcribed and processed crRNA hybridizes to transactivating CRISPR RNA (tracrRNA) and associates with Cas9 nuclease. The crRNA-tracrRNA:Cas9 complexes recognize and introduce double-stranded breaks (DSBs) in homologous target DNA bearing the protospacer sequences (Jinek et al., 2012; Sander and Joung, 2014). The type II CRISPR/Cas genome-editing mechanism has been adapted to induce double-stranded DNA breaks in *C. elegans* (Chen et al., 2013; Friedland et al., 2013; Katic and Großhans, 2013; Waaijers et al., 2013). It can be used to induce precise heritable targeted mutations in nearly any genome location with fidelity. CRISPR/Cas9 enables rapid modifications and the implementation of conditional knockouts and knockins (Friedland et al., 2013). As a practical example, this genome editing tool has the potential to introduce orthologs of parasitic genes into *C. elegans* to provide a better understanding of anthelmintic activity and identify resistance genes from nematodes that are not tractable to other genetic manipulation. The strategy of using *C. elegans* knockout strains rescued with parasitic nematode transgenes has been successfully employed in previous studies. Cook et al. (2006) used *Hco-avr-14* expressed under the control of the *C. elegans avr-14* promoter to restore motor movement in *Cel-avr-14* knockout worms. Similarly, expression of *H. contortus* GluCl subunits (avr-14b, glc-2, glc-5, glc-6*) rescued ivermectin sensitivity in a highly resistant triple mutant C. elegans strain. The replacement of C. elegans genes with parasitic orthologues using CRISPR/Cas9 genome editing* allows higher precision and better control of spatiotemporal aspects. Hahnel et al. (2018); Kitchen et al. (2019) evaluated benzimidazole (BZ) resistance alleles of gastrointestinal nematodes in the *Cel-ben-1* gene using CRISPR-Cas9 and confirmed their role in conveying high levels of BZ resistance into sensitive wild type *C. elegans* strain. This setup could potentially be applied to other drug classes and genes, such as GluCls and nAChRs subunit encoding genes, to *characterize parasite-resistant alleles*. This experimental setup will not work for parasitic genes that lack a direct ortholog in *C. elegans* or for proteins that require specific chaperones, auxiliary proteins, or post-translational modifiers. The specificities of the CRISPR/Cas9 genetic editing tool remain to be fully explored in parasitic nematodes. With the wealth of genomic and transcriptome data available this toolkit can perform highly efficient and targeted modifications of gene expression. Furthermore, CRISPR/Cas9 has been successfully used in parasitic nematodes such as *S. stercoralis* and *B. malayi*, accelerating the development of this system for future application in nematode functional genomic studies (Gang et al., 2017; Adams et al., 2019; Liu et al., 2020). This system may represent an ideal tool to understand the function of drug target genes in parasitic nematodes that are genetically intractable. The simplicity and broad applicability of the CRISPR/Cas9 system can help transform the parasitic genomics research and spur the identification of novel therapeutic targets and novel intervention strategies for parasitic control.

5. Conclusion

Parasitic nematodes affect billions of people worldwide and threaten not only the health of the host but also pose a serious threat to global food security. Chemotherapeutic intervention continues to be the mainstay for treatment and control of nematode infections as there are no commercial vaccines available for human use. Although chemotherapy has improved health and agricultural economics, livestock production and overall food security, there are reports of widespread resistance in the veterinary field and reduced cure rates in human medicine. There is an incontrovertible need to focus research on identification and development of novel antinematodal targets and new therapeutics. The current advances in our knowledge of parasitic ion channels combined with enhanced screening techniques can help in restoring the depleted drug-discovery pipeline before the existing antiparasitic drugs are rendered unviable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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