A mutational and molecular dynamics study of the cys-loop GABA receptor Hco-UNC-49 from *Haemonchus contortus:* Agonist recognition in the nematode GABA receptor family

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ABSTRACT

The UNC-49 receptor is a unique nematode γ-aminobutyric acid (GABA)-gated chloride channel that may prove to be a novel target for the development of nematocides. Here we have characterized various charged amino acid residues in and near the agonist binding site of the UNC-49 receptor from the parasitic nematode *Haemonchus contorts*. Utilizing the *Caenorhabditis elegans* GluCl crystal structure as a template, a model was generated and various charged residues [D83 (loop D), E131 (loop A), H137 (pre-loop E), R159 (Loop E), E185 (Loop B) and R241 (Loop C)] were investigated based on their location and conservation. These residues may contribute to structure, function, and molecular interactions with agonists. It was found that all residues chosen were important for receptor function to varying degrees. Results of the mutational analysis and molecular simulations suggest that R159 may be interacting with D83 by an ionic interaction that may be crucial for general GABA receptor function. We have used the results from this study as well as knowledge of residues involved in GABA receptor binding to identify sequence patterns that may assist in understanding the function of lesser known GABA receptor subunits from parasitic nematodes.

1. Introduction

The ligand-gated ion channel family or more specifically cys-loop receptors are an important family of proteins which are targets for current insecticides and anthelmintics. Several of these receptors are targets for anthelmintics that are used to treat a variety of parasitic nematodes that infect animals including humans (Wolstenholme, 2011). The two most widely studied cys-loop receptors are the glutamate-gated chloride channels (GluCls) which are targets for macrocyclic lactone anthelmintics such as ivermectin and the nicotinic acetylcholine receptors which are targets for cholinergic anthelmintics such as levamisole (Holden-dye et al., 2014). However, the cys-loop receptor family of nematodes is quite large with several subtypes having no mammalian orthologues (Jones and Sattelle, 2008). This highlights their potential as targets for novel anthelmintics.

Cys-loop GABA receptors are well-studied targets for insecticides and also play an important role in nematode biology. These receptors are also targets for the anthelmintic piperizine (Accardi et al., 2012). The nematode GABA receptor most studied is the UNC-49 receptor which plays a role in muscle contraction essential for locomotion (Bamber et al., 1999). The importance of this receptor to the biology of nematode parasites is evident from the fact that genes encoding the various subunits of the UNC-49 receptor family are found in many parasitic nematode genomes (Accardi et al., 2012). Moreover, the UNC-49 receptor is not analogous to GABA receptors found in mammals and exhibits a unique pharmacology suggesting structural differences in the binding pocket (Bamber et al., 2003; Kaji et al., 2015). In several cases, differences in binding site residues between the UNC-49 receptor and mammalian GABA receptors have partially explained differences in function (Kaji et al., 2015; Kwaka et al., 2018). However, there is much to be learned about how various residues in and around the binding pocket contribute to the overall function of the nematode UNC-49 receptor as well as other nematode GABA receptors and GABA receptors in general. Such knowledge is important for our understanding of the evolution and function of GABA neurotransmission and the potential of these receptors as targets for future nematocides.

The crystal structure of the *Caenorhabditis elegans* GluCl (Hibbs and Gouaux, 2011) has provided the means to begin to examine the structure of the UNC-49 receptor from *H. contortus* which has been key to understanding the residue requirements for receptor function and

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pharmacology (Kaji et al., 2015). There are many residue types that play key roles in overall receptor function. However, charged residues are particularly important in that they play essential roles in the structure and function of the various binding loops and they have been shown to interact directly with GABA (Ashby et al., 2012; Newell et al., 2004). While many of these residues have been studied in other GABA receptors there is little information on their role in GABA receptors from nematodes.

Molecular dynamic (MD) simulations have been extremely useful in visualizing and quantifying inter- and intramolecular interactions between residues (Ashby et al., 2012; Kwaka et al., 2018). In addition, when coupled with mutational analysis this approach can provide a deeper understanding of receptor function, particularly receptors from parasitic nematodes where there has been limited study. Here we have investigated the function of various charged residues in the UNC-49 receptor from the parasitic nematode *H. contortus* using mutational analysis, homology modelling and molecular dynamic simulations. We show that this approach can provide novel insight into the function of cys-loop receptors from parasitic organisms which may aid research that focuses on the development of novel anthelmintics. In addition, using the results from this study along with knowledge of human GABA_A receptors, we have examined other nematode GABA receptor subunits for the presence or absence of key residues that can be used to explain or predict function.

2. Materials and methods

2.1. Homology modelling

The *C. elegans* GluCl crystal structure (PDB 3RIF) was used as a template in MODELLER 9.14 (Sali and Blundell, 1993) for the generation of a Hco-UNC-49B extracellular domain homodimer (GenBank # EU939734.1). The most energetically favorable models were determined based on their DOPE and molpdf scores as well as a PROCHECK Ramachandran plot analysis as described in Kaji et al. (2015). The final model chosen was visually inspected to ensure the binding loops were in the proper positions.

2.2. Computational agonist docking

The energetically reduced zwitterion form of GABA was obtained from the Zinc database, http://zinc.docking.org/ (Irwin et al., 2012). The GABA molecule was prepared for docking using AutoDock Tools and was docked using AutoDock Vina (Trott and Olson, 2010). The center of the $30 \times 30 \times 30$ Å search box located in the aromatic box of the agonist binding site was used for agonist docking. A maximum of 50 binding models all within a range of 5 kcal mol^{-1} from the best scoring pose was generated (Kaji et al., 2015).

2.3. Site-directed mutagenesis and cRNA production

The coding sequence of *hco-unc*-49b, subcloned into the pT7TS vector was used as template for mutagenesis. Fourteen mutants were generated using Stratagene's web-based QuikChange® Primer Design program (www.stratagene.com/sdmdesigner/decault.aspx) and the QuikChange® site-directed mutagenesis kit. Each mutation was verified by DNA sequencing (Genome Quebec). cRNA was produced by *in vitro* transcription using T7 RNA polymerase in the mMessage Machine Kit (Ambion) and precipitated using lithium chloride and resuspended in H$_2$O at a concentration of 0.5 ng/L.

2.4. Expression of unc49b mutants in Xenopus oocytes

Expression of *unc-49* receptor cRNA was according to methods outlined in Abdelmassih et al. (2018) and adhered to the guidelines of the Canadian Council of Animal Care. *Xenopus laevis* were anesthetized

using 0.15% 3-aminobenzoic acid ethyl ester, methane sulphate salt buffered to pH 7 with sodium bicarbonate. Oocytes were surgically removed and cut into small clumps of approximately 20 eggs per clump. These were incubated in OR-2 (82 mM NaCl, 2 mM KCl, 1 mM MgCl$_2$, 5 mM HEPES, pH 7.5) with type-II collagenase (Sigma Aldrich, Canada) for 2 h while shaking. These were then placed in ND96 supplemented with pyruvate and gentamycin. Oocytes were injected with 50 nL of either wild-type (*hco-unc*-49b/c) or mutant *hco-unc*-49b/ wildtype *hco-unc-49c* cRNA, using the Drummond Nanoject microinjector. These were stored at 20ºC for 48 h, replacing the supplemented ND96 twice before electrophysiology.

2.5. Two-electrode voltage clamp electrophysiology

Approximately 48 h after injection, electrophysiology was performed using the Axoclamp 900A voltage clamp. Responses were recorded using varying concentrations of GABA dissolved in non-supplemented ND96. Microelectrodes were made using Ag|AgCl wires and filled with 3M KCl. Injected *X. laevis* oocytes were clamped and held at −60 mV and left for 1–2 min to stabilize before GABA was washed over the oocytes at a flow rate of 8 mL/min. For dose-response analysis, oocytes were washed with ND96 for 1–2 min between agonist applications. Data was recorded and analyzed using Clampex 10.3 software and graphs were produced using Graphpad Prism Software 5.0. Data was collected from at least five individual oocytes. To assure consistency, two different batches of oocytes were used.

2.6. Statistical analysis

Dose-response curves were generated using Prism 5.0 (Graphpad Software, San Diego, CA, USA) using the log (agonist) vs. normalized response-variable slope equation:

$$Imax = \frac{1}{1 + (\frac{EC50}{D})^h}$$

Where I_{max} is the maximal response, [D] is concentration of agonist, EC$_{50}$ is the concentration of agonist required to produce a half maximal response and h is the Hill coefficient. Responses that generated dose-response curves were normalized as a percentage of the maximal current produced by individual oocyte's maximal response to GABA. Statistical analysis comparing EC$_{50}$ and hill values between wildtype and mutants was performed using Students t-test with the Bonferonni correction. *P*-values $\leq$ 0.01 was considered significant.

2.7. MD simulations

MD simulations of the Hco-UNC-49B homodimer with docked GABA were conducted as described in Kwaka et al. (2018). The GROMACS 2016.4 software package (Abraham et al., 2017), employing the CHARMM36 force field (Best et al., 2012), was used to run the simulations. The CHARMM General Force Field (CGenFF) program was used to acquire the CHARMM force field parameters for the GABA molecule (Vanommeslaeghe et al., 2010; Yu et al., 2012; Vanommeslaeghe and MacKerell, 2012; Vanommeslaeghe et al., 2012; CGenFF interface https://cgenff.paramchem.org). The simulation box was solvated with ~21,000 tip3p water molecules and Cl$^-$ counter ions to neutralize the total charge of the system. After a brief energy minimization, a 2 ns equilibration MD simulation was performed under the NVT conditions at 300 K and a time step of 2 fs. The positions of the protein and the ligand were restrained to prevent any structural changes during the equilibration process. In the production run, under the NPT conditions, the system was kept at 300 K and 1 atm with a time step of 2 fs for 500 ns. The Parrinello-Rahman isotropic pressure coupling (with τp = 5 ps and compressibility 4.5×10^{-5} bar^{-1}) and the Nose-Hoover thermostat (with a time constant of 0.1 ps) were employed for these

production runs. Long-range electrostatics were calculated with the particle mesh Ewald method. The trajectory was printed every 10 ps.

The gromacs utilities, such as gmx_mpi mindist, gmx_mpi gyrate and gmx_mpi rms (Humphrey et al., 1996; http://www.ks.uiuc.edu/Research/vmd/) were used to calculate the results. Ionic bonds between oppositely charged residues were determined at $d < 3.0$ Å.

2.8. Sequence analysis

Amino acid sequences of various GABA receptor subunits were retrieved from GenBank. Sequences were aligned using the Clustal Omega: Multiple Sequence Alignment Tool via the European Bioinformatics Institute portal (www.ebi.ac.uk).

3. Results

3.1. Modelling results

A model of the binding site of Hco-UNC-49B homodimer was generated using the *C. elegans* GluCl (PDB 3RIF) as a template. This model was used for determining which residues may have functional significance and/or their proximity to residues of opposite charge or the GABA molecule. Based on our model D83, E131, H137, R159, E185 and R241 were chosen for analysis (Fig. 1).

3.2. Electrophysiology of mutants

Each residue was first examined by alanine mutagenesis. Each mutated receptor was exposed to GABA in increasing concentrations to determine the EC_{50}. Upon recording, it was found that most receptors with mutated subunits responded to GABA, and clean tracings were established for comparisons and determination of the EC_{50} (Fig. 1; Table 1). Of the six residues that were mutated to alanine, D83A and E185A both had complete lack of function and were unresponsive to

Table 1

Summary of EC_{50} values and hill slopes of each mutation introduced into Hco-UNC-49B. Each EC_{50} represents the mean $\pm$ standard error using n $\geq$ 5 independent oocytes.

Mutation	$EC_{50} \pm$ SE (μM)	n^H	Mut/WT	N
Wild-Type	68 $\pm$ 17	2.056 $\pm$ 0.2	1	6
D83A	n.r. < 20,000	–	–	10
D83E	276 $\pm$ 37[a]	2.064 $\pm$ 0.2	4.1	5
D83C	454 $\pm$ 47[a]	1.239 $\pm$ 0.1[a]	6.7	5
D83N	342 $\pm$ 30[a]	2.285 $\pm$ 0.2	5.0	5
D83T	520 $\pm$ 34[a]	1.549 $\pm$ 0.1	7.6	5
E131A	219 $\pm$ 21[a]	0.884 $\pm$ 0.1[a]	3.2	5
H137A	2174 $\pm$ 173[a]	1.123 $\pm$ 0.1[a]	32.0	5
H137K	4166 $\pm$ 745[a]	1.045 $\pm$ 0.1[a]	61.2	5
R159A	5469 $\pm$ 396[a]	1.931 $\pm$ 0.2	80.4	7
R159K	n.r. < 20,000	–	–	11
E185A	n.r. < 20,000	–	–	13
E185D	n.r. < 20,000	–	–	5
R241A	5174 $\pm$ 473[a]	2.168 $\pm$ 0.2	76.1	6
R241K	3793 $\pm$ 435[a]	1.279 $\pm$ 0.1[a]	55.8	6

[a] Indicates statistically different from WT (P $\leq$ 0.005).

GABA concentrations up to 20 mM. The other mutants were functional and EC_{50} values were determined. E131A exhibited an EC_{50} of 219 $\pm$ 21 μM or a 3.2 fold decrease in sensitivity and had a significant decrease in hill slope (n = 5). H137A was found to have an EC_{50} of 2174 $\pm$ 173 μM for GABA, or a 32 fold decrease in sensitivity (n = 5). R159A was found to have an EC_{50} of 5469 $\pm$ 396 μM, or an 80 fold decrease in sensitivity (n = 7), and R241A was found to have an EC_{50} of 5174 $\pm$ 472.5 μM, or a 76 fold decrease in sensitivity (n = 6). The results are summarized in Fig. 1D and Table 1. From the results of the alanine mutagenesis H137, R159, R241, E185 and D83 were chosen for further analysis since they all appeared to have a major impact on GABA sensitivity.
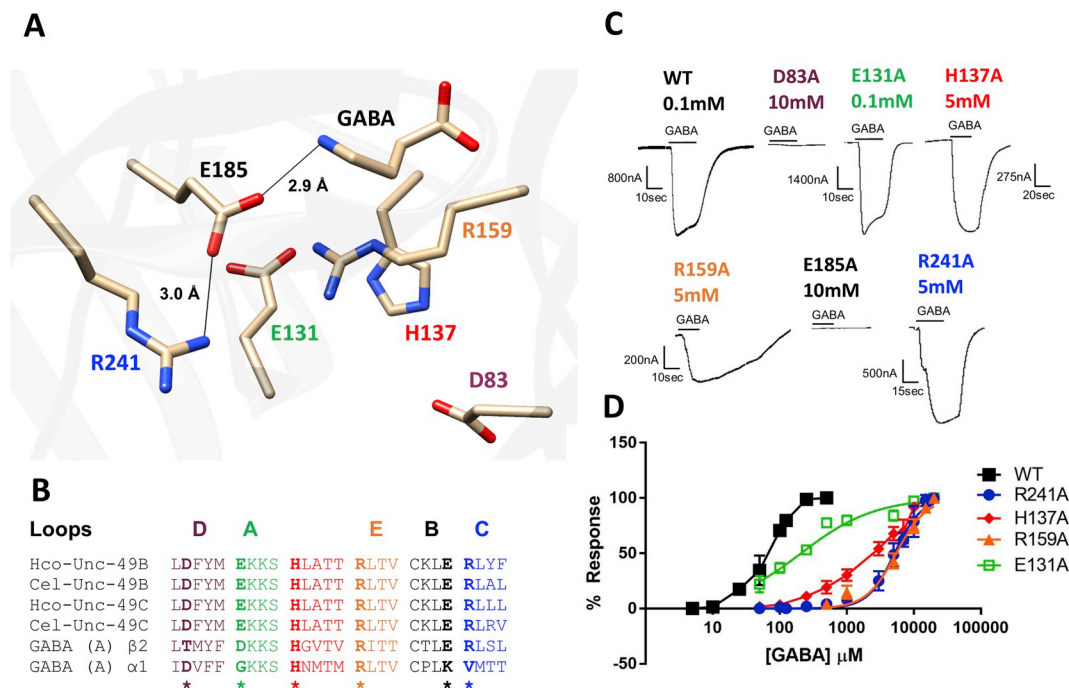

Fig. 1. (A) Model of Hco-UNC-49B homodimer with docked GABA molecule with residues that were examined in this study. Representative distances are shown in angstroms (B) Sequence alignment of the location of the residues within the major binding loops. The various binding loops corresponding to each residue are indicated by color coding. Residues examined in this study are indicated by * (C) Representative electrophysiological tracings of Hco-UNC-49BC with alanine mutated UNC-49B subunits D83A, E131A, E185A, H137A, R159A, R241A (D) Dose-response curves of all functional Hco-UNC-49B alanine mutants showing differences in GABA sensitivity, with normalized currents. Each point represents a mean $\pm$ SE (n $\geq$ 5). Residue numbering refers to the first methionine in the Hco-UNC-49B subunit (EU939734.1). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. Mutational analysis of residues by conserving charge

As a further analysis of charged residues in and around the binding pocket, we mutated each residue to a different amino acid that was similar in charge. In this case there were two mutants, R159K and E185D, which were non-functional even though the replacement amino acid was the same charge. On the other hand, H137K and R241K produced functional channels but had a 61 and 55-fold reduction in GABA sensitivity, respectively. These results suggested that the original residue is crucial for receptor function and each may have complex roles in the nematode UNC-49 receptor. On the other hand, D83E exhibited only a slight reduction in GABA sensitivity (4-fold reduction) (Table 1). Because D83 appeared to be the most tolerant to mutagenesis this position was further investigated using a series of amino acid changes.

3.4. Examination of D83

While removal of the functional group of D83 through the D83A mutation resulted in non-functional receptors, the introduction of other residues particularly those with a polar group maintained some receptor function. Aside from the D83E mutation, the introduction of similarly sized but polar asparagine (D83N) resulted in only a 5 fold reduction in GABA sensitivity. Similarly, D83T and D83C resulted in an 8 and 6 fold decrease in sensitivity, respectively (Fig. 2; Table 1).

3.5. MD simulations

MD simulations were conducted to provide a deeper examination of the above residues and their interactions. During the entire simulation GABA remained with the binding pocket and interacted with nearby residues. First, our simulations revealed that E185 forms an ionic bond with GABA 94% of the time over the course of the simulation (Kwaka et al., 2018). It is not surprising therefore that both E185A and E185D produced non-functional receptors. Simulations also revealed an ionic bond between E185 and R241 which occurred 91% of the time over the course of the simulation (Fig. 3a). These values are similar to a MD study of the *Drosophila* RDL receptor (Ashby et al., 2012). D83 also interacts with R159 via an ionic bond 45% of the time over the course of the simulation. The simulation shows a tight coupling of D83 and R159 between 80 and 150ns then again from 350 to 500ns (Fig. 3b). However, the simulation did reveal electrostatic interaction between D83 and R159 (ie remained < 1 nm distance from each other) during the entire 500ns simulation (Fig. 3b).

3.6. Identification of key residues in other nematode GABA receptors important for function

The mutational results presented here along with knowledge of agonist recognition residues identified in mammalian GABA binding sites and other studies on the UNC-49 receptor has provided tools to help characterize key features in the nematode GABA receptor family.
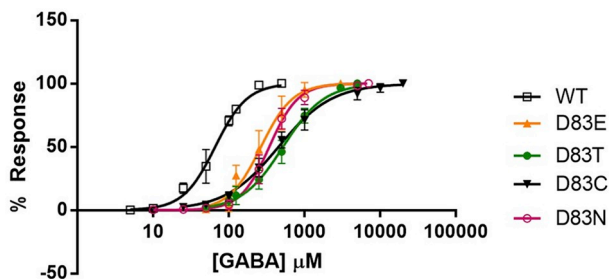

Fig. 2. Dose-response curves of D83 mutations of Hco-UNC-49B in comparison to WT showing differences in GABA sensitivity, with normalized currents. Each point represents a mean ± SE (n ≥ 5).
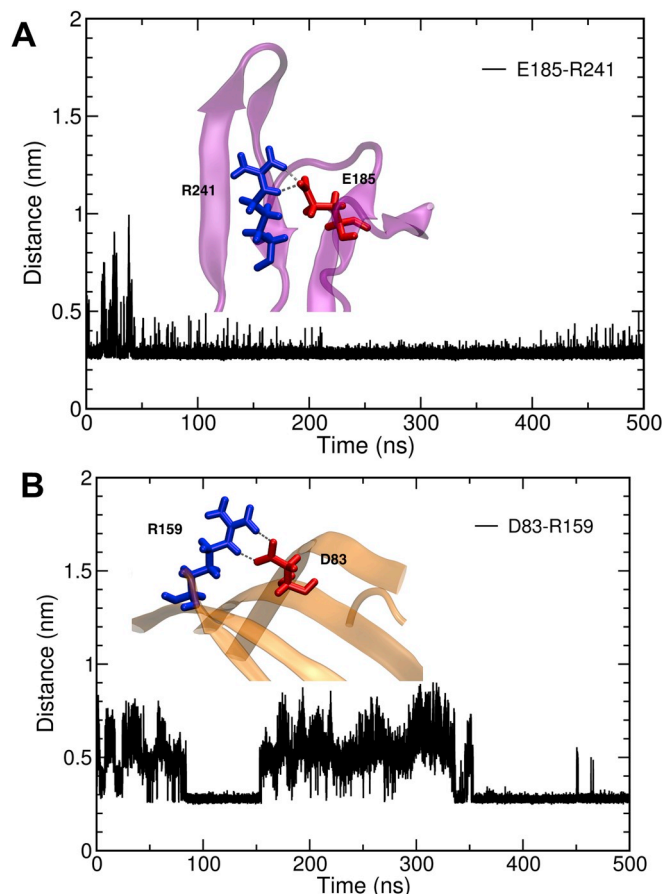

Fig. 3. Molecular simulations of residue interactions in the Hco-UNC-49 receptor. (A) Interactions between E185 and R241 over the course of the simulation. (B) Interactions between D83 and R159 over the course of the simulation. *Insets*: snapshots of the residues during the simulation. Dashed lines represent distances ≤ 3 Å. The bonds with the shortest distance are used to calculate bond percentages.

Fig. 4. Sequence alignment of the major binding loop of Hco-UNC-49B with other Cys-loop GABA receptors. The amino acid residues mutated in this study is indicated by (●) and are numbered above. Residues discussed that are important for agonist recognition described in Table 2 are indicated by (★).

Fig. 4 provides an alignment of various GABA receptor binding loops from other nematodes along with mammalian αβγ receptors. First, results from the D83 (loop D) mutational analysis revealed that changes from aspartic acid to other polar residues were fairly well tolerated in Hco-UNC-49. Alignments shown in Fig. 4 also suggests variability in amino acids at this position. It appears that the most common residue is D while other nematode GABA receptor subunits exhibit either T, E or

Table 2
Residues important for agonist recognition and receptor function and used to classify the various cys-loop GABA receptor subunits found in parasitic nematodes.

	Principle Subunit		Complementary Subunit		Comment
	Loop B	Loop C	Loop D	Loop E	
Hco-UNC-49B	E	R	R	S	UNC-49 subunit/RDL-like
Hco-GAB-1	E	R	H	G	Beta-like subunit
Ace-LGC-36	G	A	R	S	Alpha-like subunit
Hco-LGC-37	G	V	R	S	Alpha-like subunit
Hco-LGC-38	E	R	R	S	RDL-like subunit
Bma-EXP-1	E	R	R	S	EXP-1 (GABA cation channel)
Dme-RDL	E	R	R	S	RDL
Hsa-β2	E	R	Q	G	Beta-subunit
Hsa-α1	G	V	R	T	Alpha-subunit
Hsa-γ1	S	I	A	T	Gamma-subunit
Hsa-γ2	S	V	A	T	Gamma-subunit

Explanation of residues:

Glutamic acid (E) (Loop B): ionic bond with amine group of GABA (current study; Newell et al., 2004).

Arginine (R) (Loop C): ionic bond the loop B glutamic acid (current study; Ashby et al., 2012).

Arginine (R) (Loop D): ionic bond with carboxyl group of GABA (Accardi and Forrester, 2011; Kwaka et al., 2018).

Serine (S) (Loop E): hydrogen bond with carboxyl group of GABA (Ashby et al., 2012; Kwaka et al., 2018).

NOTE: naming of subunits uses the system outlined in Beech et al. (2010) (Hco-H. contortus).

N. Interestingly, Ace-LGC-36 from *Ancylostoma ceylanicum* and Cel-LGC-36 are the only subunits exhibiting an asparagine at this position which we also found to be tolerated in Hco-UNC-49. H137 which was highly sensitive to mutagenizes is highly conserved among GABA receptors with the exception of the EXP-1 receptors which either have an R (Cel-EXP-1) or a K (Bma-EXP-1a). We found that H137K resulted in a large reduction in GABA sensitivity in Hco-UNC-49 so it remains to be seen if Bma-EXP1a is a functional receptor. R159 is highly conserved which is consistent with our results that show that a change to a similarly charged residue (R159K) produced nonfunctional receptors. E185 (Loop B) is a residue that is essential as it interacts with the positive amine group of GABA. All mutations to this position in Hco-UNC-49 produced nonfunctional receptors. LGC-36 and 37 both exhibit a glycine at this position which would explain why Hco-LGC-37 required co-expression with Cel-GAB-1 to produce a functional GABA receptor (Feng et al., 2002). Likewise, R241 (Loop C) was highly sensitive to mutagenesis and is highly conserved among nematode GABA receptors except LGC-36 and 37. Table 2 highlights several of the known key agonist recognition residues that can explain and possibly predict receptor function in parasitic nematodes. First, Hco-UNC-49B, Hco-LGC-38, EXP-1 and Dme-RDL all contain the residues ERRS and all readily form functional homomeric channels in oocytes (Beg and Jorgensen, 2003; Siddiqui et al., 2010; Ashby et al., 2012) (Fig. 5). Cel-GAB-1 (or Hco-GAB-1) and Hco-LGC-37 have been shown to be obligate heteromeric receptor subunits (Feng et al., 2002) and contain ERHG and GVRS respectively. If GAB-1 functions as a beta-subunit (principle subunit) and LGC-37 as an alpha-like subunit (complementary subunit) then the GABA binding site would contain the residues ERRS essential for GABA receptor binding and function. Therefore, we would predict that like LGC-37, Ace-LGC-36 (and other nematode LGC-36 subunits) would require a GAB-1 like subunit to be functionally expressed. Based on the criteria outlined above, Table 2 provides a summary of how these four functionally important resides were used to classify various nematode GABA receptor subunits.

4. Discussion

This study has confirmed that a glutamic acid residue in loop B (E185) is absolutely essential for GABA receptor activation in nematode UNC-49 GABA receptor. The analogous residues of E185 are E155 in the mammalian GABA$_A$ receptor and E204 in the *Drosophila* RDL. In both case these residues have been shown to be crucial channel function (Newell et al., 2004; Ashby et al., 2012; Miller et al., 2014). In the *Drosophila* RDL receptor, like the UNC-49 receptor, all mutations to E204 resulted in channels that were unresponsive. It has been suggested that this loop B glutamic acid acts as a control element where it interacts with the positive amine group of GABA coupling binding to the channel activation (Newell et al., 2004). Not only does E185 form a critical interaction with GABA but it also interacts with an arginine in loop C (R241). Mutations of R241 showed drastically impaired function of the channel, with R241A showing an approximately 80 fold decrease in sensitivity and R241K showing an approximately 59 fold decrease in sensitivity. The crucial role of R241 is also supported by our MD simulations which revealed an ionic bond between R241 and E185. This interaction between analogous residues has also been observed in the human GABA$_A$ receptor (Bergmann et al., 2013; Miller et al., 2014) and it may function in the closure of the channel (Miller et al., 2014). It appears therefore that a glutamic acid in loop B and a loop C arginine and their potential interaction are crucial for GABA receptor function across phyla.

D83 appears to be important for receptor function as a mutation to an alanine produced a non-functional channel. Interestingly, maintaining the charge as in D83E produced only a small reduction in GABA sensitivity. Other mutations at this position which included residues capable of hydrogen bonding such as D83N, D83T and D83C also produced functional channels. It had been shown in both MD simulations of the RDL receptor and a homology model of the human GABA$_A$ receptor that there is a salt bridge between residues analogous to D83 and R159 which likely play a key role in the stability of binding loops D and E (Ashby et al., 2012; Bergmann et al., 2013). R159 seems to be particular sensitive to mutations to either alanine (current study) or cytosine (Kwaka et al., 2018) which caused severely impaired receptors. Surprisingly, R159K produced non-functional receptors while the same mutation (R178K) in the *Drosophila* RDL receptor produced a channel that was similar in sensitivity to WT (Ashby et al., 2012). This suggests that in Hco-UNC-49, R159 may have other functions other than interacting with D83. This is also supported by the results of our MD simulations which found that while D83 and R159 interact by an ionic bond, the strength of this interaction and potential frequency was not as high as the equivalent residues in the RDL receptor.

The genomes of parasitic nematodes appear to contain at least five distinct types of genes that encode various subunits of cys-loop GABA-
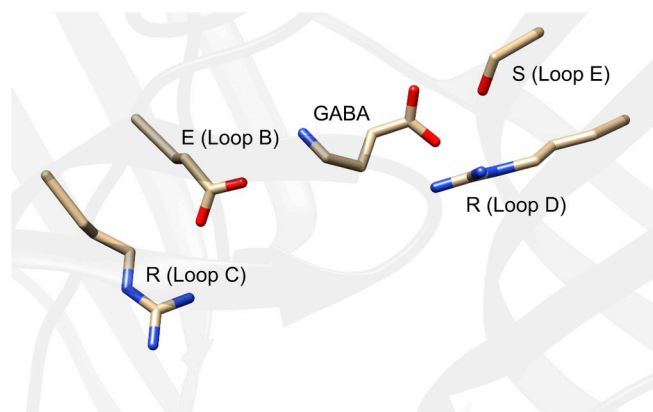

Fig. 5. Molecular model of Hco-UNC-49 homodimer with GABA docked. The residues ERRS and their positions are indicated.

gated chloride channel receptors (Accardi et al., 2012). Of these subunits only two, UNC-49B and LGC-38 have been shown to form functional homomeric channels in *Xenopus* oocytes which is similar to the *Drosophila* RDL receptor (Siddiqui et al., 2010, 2012). On the other hand, subunits such as GAB-1 and LGC-37 have been shown to be unable to form functional homomeric channels but can combine to form a GABA sensitive heteromeric channel (Feng et al., 2002). However, the somewhat weak sequence similarity with classical mammalian αβγ GABA receptors makes it difficult to classify with certainty nematode subunits using mammalian nomenclature. Thus, the naming system utilized in Jones and Sattelle (2008) and Beech et al., 2010 is the most practical and useful method for classifying nematode GABA receptors. However, the knowledge that we now have gained though mutational studies, modelling and comparisons to mammalian αβγ GABA receptors can help better describe these subunits and the key functional motifs they possess. Thus, using criteria based on the presence or absence of key functional residues we suggest that GAB-1 is the only true β-like subunit in the nematode genome that provides essential GABA binding residues from loops B and C but lacks the key binding residues in the complementary loops D and E. The loop D and E residues would be provided by LGC-37 which when co-expressed with GAB-1 produces a functional heteromeric receptor (Feng et al., 2002). The LGC-37 residues indicated in Table 1 resulted in our description of LGC-37 as α-like. While both GAB-1 and LGC-37 are not direct orthologues of mammalian β or α subunits, they both appear to share several of the key residues that are essential for the function of each of these subunits. A similar rational can be used to describe the currently uncharacterized subunit LGC-36 as also α-like. This is what makes the UNC-49 receptors different compared to GABA_A receptors, in that one subunit, (in this case UNC-49B) possessed all the essential requirements for GABA binding and receptor activation. However, it's important to note that the residues described here do not predict for certainty the function of uncharacterized subunits such as LGC-36 nor provide the complete story about the other nematode subunits as there are several other residues that are essential for agonist recognition and receptor function (Lynagh and Pless, 2014).

In conclusion, this study has uncovered several residues that are important for Hco-UNC-49 receptor structure and agonist recognition. It appears that residues such as E185 and R241 in the nematode UNC-49 receptor play a similar role as GABA receptors from other phyla. On the other hand while R159 appears to be interacting with D83, it may play other roles in the function of the Hco-UNC-49 receptor.

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