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FELLOWSHIP FINAL REPORT

Studies on glc-3, a potential target of Ivermectin in parasitic nematodes

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ABSTRACT

The free-living nematode Caenorhabditis elegans has been used for many years as an expression system for genes from parasitic species. We wished to further develop and improve this system by using CRISPR/Cas9 to delete specific genes from C. elegans and replace them with single copies of orthologous genes from the parasite, Haemonchus contortus. Initial experiments focussed on glc-3 which encodes a subunit of the glutamate-gated chloride channels, the target of the avermectin/milbemycin family of anthelmintics. We cloned the promoters from the glc-3 genes of both species and compared the expression patterns of mCherry under the control of both promoters. The C. elegans glc-3 promoter drove expression in a subset of head interneurons, as previously reported whereas the H. contortus promoter drove expression in a pharyngeal motoneuron, M4. We were able to generate heterozygous worms in which one copy of glc-3 was deleted, but we could never obtain homozygous knock-outs. Further investigation of the mRNAs encoded by glc-3 revealed a novel transcript, glc-3T, which encodes a severely truncated form of GLC-3. The presence of such truncated transcripts may explain the unexpected difficulties encountered in attempting to knock out ion channel genes in C. elegans.

1- Introduction

Ivermectin is one of the most important drugs currently in use for the control of infectious diseases of humans and their domesticated animals, both pets and livestock (Campbell, 2012). Related compounds are also important in the control of agricultural pests. These drugs are effective because they have selective effects on the nervous systems of nematodes and arthropods. This selectivity is due in large part to a high-affinity interaction with glutamate-gated chloride channels (GluCls), a class of neurotransmitter receptor specific to invertebrates (Cleland, 1996;Wolstenholme, 2012). These channels are made up of five subunits and can be either homomeric or heteromeric; the subunit composition of the native channels is largely unknown (Wolstenholme & Neveu, 2022). Several genes encode GluCl subunits in nematodes; this investigation focussed on one of these genes, *glc-3*, from *Haemonchus contortus*, an

important parasite of ruminants, and *Caenorhabditis elegans*, a model organism for which unparalleled resources for genetic manipulation are available. This gene was originally identified in the Wolstenholme laboratory (Horoszok et al. 2001) and forms an ivermectin-sensitive channel when expressed as a homomer in vitro. It is widely conserved among nematode species; in *C. elegans* it is expressed in interneurons and plays an important role in integrating sensory inputs (Chalasani et al. 2007; Mutlu et al. 2020; Wen et al. 2020; Wang et al. 2021), but its functions and expression pattern in parasites are unknown. However, recent studies on GLC-3 from parasitic nematodes has recently shown that it forms both homomeric and heteromeric channels, with GLC-2, and that these channels are sensitive to ivermectin and other macrocyclic lactone anthelmintics (Lamassauide et al. 2022).

C. elegans has long been used as a model for genetic and molecular studies of parasitic nematodes. It was the first multicellular organism to have its genome completely sequenced (Consortium, 1998), and even today the quality of the current genome assembly is unsurpassed. In addition, there are a number of powerful tools for the genetic modification of *C. elegans* that have been used to study the properties of genes and gene products from parasitic species. In particular, cDNA sequences derived from parasite genes have been cloned downstream of a promoter sequence and microinjected into the syncytial gonad, where they are maintained as extrachromosomal arrays and expressed under the control of the promoter (Britton & Murray, 2006). Frequently the host *C. elegans* strain used in these experiments will have a mutation in the orthologous gene to that being studied. Such a procedure has been used in the Wolstenholme laboratory and has demonstrated that parasite GluCl and other ion channel genes can function in *C. elegans* and can restore ivermectin or levamisole sensitivity to drug-resistant mutant strains (Cook et al., 2006; Glendinning et al., 2011; Sloan et al., 2016). However, this approach does have limitations. Firstly, the mutant host strains used nearly always retain at least part of the target gene, and this may be expressed as a non-functional protein, potentially complicating the interpretation of the results. Secondly, the injected gene is present as multiple copies; after injection in the form of an extrachromosomal array, which is inherently unstable, and if the gene becomes integrated into the host genome we have no control over the site of integration. Depending on the exact site at which the transgene is integrated, this may produce unpredictable effects on the host worm.

For these reasons, we wished to develop an improved method for the expression of parasite-derived sequences in *C. elegans*, making use of the recently developed CRISPR/Cas9 system. In essence, this is a system for cutting the genome of *C. elegans*, or other species, at very specific places, directed by guide RNAs (gRNAs). If a gene is specifically cut at either end, it will be excised from the genome; it can then be replaced by the parasite genes using homologous recombination, where the parasite gene in cloned into a vector surrounded by flanking sequences identical to those on either side of the two cut sites (Dickinson & Goldstein, 2016). This should result in a population of worms in which one of the two chromosomes has the parasite sequence inserted in the same place as the *C. elegans* orthologue, and it will be under the control of the same promoter and other regulatory motifs. In theory, these heterozygote *C. elegans* will be self-fertile and this will, via Mendelian genetics, yield 25% progeny with a homozygous deletion of the original gene and with the parasite sequence in its place. Identification of the worms bearing the transgene is simplified by the inclusion of several sequences encoding markers, including resistance to antibiotics such as hygromycin B, a dominant genetic marker, *rol-6*, that produces a very distinct locomotion defect, 'rolling', and a fluorescent protein such as GFP or mCherry.

For this project we decided to use *Haemonchus contortus* as the parasite source of *glc-3*. *H. contortus* is a clade V nematode, fairly closely related to *C. elegans*, that is a serious pathogen

of small ruminants and has become widely resistant to all the current anthelmintic drugs. There is a high quality genome assembly (Doyle et al., 2020) and GluCl sequences from this species have been shown to rescue drug-resistance and behavioural phenotypes of mutant *C. elegans* strains (Couthier et al., 2004; Cook et al., 2006; Glendinning et al., 2011; Welz et al., 2011; Li et al., 2014; Sloan et al., 2016). The INRAE laboratory has access to several drug-resistant isolates of *H. contortus*, which would be valuable in studying whether variation in a specific transcript was directly involved in mediating drug resistance.

2- Experimental details

2.1 Molecular Biology

Unless otherwise stated, all enzymes used in the molecular cloning procedures were from ThermoFisher. Synthetic oligonucleotides were from Eurogentec, DNA sequencing was carried out by Eurofins Genomics, and guide RNAs were supplied by Merck or Millipore/Sigma. Nucleic acid purification was using the relevant kit (Miniprep, genomic DNA) from Qiagen. RNA purification was achieved via extraction using Trizol.

2.1.1 Construction of glc-3 reporter constructs

The genomic DNA sequence upstream of the *C. elegans glc-3* gene was amplified using the oligonucleotide primers Cepglc3Sac1F and Ceglc3Age1R (Table 1) with a proof-reading thermostable DNA polymerase. The resultant DNA product was cloned into the Sac1 and Age 1 restriction sites of pHT101-mCherry (a gift from Casonya Johnson (Addgene plasmid # 61021 ; http://n2t.net/addgene:61021 ; RRID:Addgene_61021). A similar procedure was used to amplify the *H. contortus* genomic sequences upstream of *Hco-glc-3*, this time using the primers Hcopglc3BamF2 and Hcopglc3AgeR1, and the product was cloned into the BamH1 and Age1 sites of pHT101-mCherry.

Construction of CRISPR/Cas9 deletion constructs

The genomic sequences flanking the selected CRISPR/Cas9 target sites were amplified using a thermostable DNA polymerase and cloned into pDD282_GFP_SEC via the In-Fusion cloning system (Takara Bio); the 5' flanking sequence was amplified using primers fus-glc3RH5'fw and fusRH5'glc3rev and the 3' flanking sequence amplified using fusglc3HR3'fw and fusglc3HR3'rev (Table 1). The resulting DNA products were cloned into pDD282 (Dickinson et al., 2015) (Addgene) from which the ccdB poison sequences had been removed and transformed into competent *ccdB* sensitive *E. coli*. The resulting colonies were cultured overnight, the plasmids purified and sequenced.

Name	Sequence
PCR primers	
Ceglc3Sac1F	CATGGAGCTCTTGCCAGCTTACCCTCAGG
Ceglc3Age1R	CATGACCGGTAAGTATTAACCGAAAACGCG
Hcopglc3BamF2	CATGGGATCCTTCAACCAGAACATGGCCT
Hcopglc3AgeR1	CATGACCGGTCCGGAACCCTGCCCGGAA
fus-glc3RH5'fw	AGTCGCCGGCACTAGTCGGTTATAGGACCGACGTCAG
fusRH5'glc3rev	CTCCTTTACTCATCGAAGGACGTACTCTCCAGTCATATC
fusglc3HR3'fw	ATGACAAGAGACTAGCCGGAAGCCAAATGAAATTG
fusglc3HR3'rev	TATGACCATTTATCGATAGGCGGCAATTCCCGTTCG
Glc-3F	CTGTTCCGGGCTGGCTTCCGG
Glc-3TR	CTCCCGTGGAACACTCAGACG
Guide RNAs	
glc-3gRNA2	GUCAAAAAUGAUGAGCUCCG
glc-3gRNA4	GUAUCGUUUUGGCUAGACAG

Table 1. Oligonucleotides used

Identification and cloning of glc-3T

mRNA was purified from adult *Haemonchus contortus* using the Trizol reagent, and then used in the synthesis of double-stranded cDNA using the Maxima first-strand synthesis kit (Fisher) following the manufacturer's instructions, together with an oligo(dT)RoRi primer (Laughton et al., 1994). 3'RACE reactions were used to amplify any *glc-3* transcripts; any products shorter than the predicted full-length sequence were cloned in pCR-Blunt (Invitrogen) and sequenced.

2.2 *C. elegans* culture and manipulation

C. elegans N2 were cultured on NGM agar as described by (Stiernagle, 2006). For microinjection, L4 were picked onto new plates 24 hrs prior to injection. Micro-injection into the gonadal syncytium was carried out as described by (Evans, 2006). When appropriate, injected worms were selected with hygromycin 24-48 hrs (4mg/ml) post-injection.

3- Results and discussion

3.1 Expression of mCherry under the control of the *C. elegans* and *H. contortus glc-3* promoter regions

The first stage in the replacement of the *C. elegans* gene by its parasite orthologue is its deletion by making two cuts in the DNA flanking sequences; the excised *glc-3* gene is then replaced by the fluorescent protein GFP. Since the worms derived in the first stage of this process will be heterozygotes the level of fluorescence is likely to be quite low. In order to confirm the pattern of fluorescence that we would expect at this stage, we expressed mCherry, a red fluorescent protein, under the control of the endogenous *glc-3* promoter sequences as an extrachromosomal array. The intergenic region between *glc-3* and the next gene upstream, ZC317.8 was cloned into the plasmid pHT101-mCherry. This construct was microinjected into N2 *C. elegans* and the progeny worms observed under the fluorescence microscope (Figure 1). Bright fluorescence was observed in a few specific neuronal cell bodies just posterior of the nerve ring, with processes extending towards the anterior. This pattern was consistent with the reported expression of *glc-3* in the head interneurons AIY and AIA (Chalasani et al., 2007; Taylor et al., 2021).

A similar strategy was used to indicate the likely expression pattern of *glc-3* in *H. contortus.* A region of approximately 3kbp upstream of the *H. contortus glc-3* gene was amplified and cloned into pHT101-mCherry, and the resulting plasmid micro-injected into N2. Bright mCherry fluorescence was observed (Figure 2), however the pattern was different from that seen with the endogenous *C. elegans* promoter (Figure 1) in that only a single cell body fluoresced with processes heading towards the posterior of the worm; a pattern reminiscent of the M4 pharyngeal motoneuron.

Figure 1. Expression of mCherry under the control of the *C. elegans glc-3* promoter. Bright fluorescence was observed in the anterior region of the worm, in a pattern strongly reminiscent of the AIA and AIY interneurons. The anatomical position of these interneurons is shown below the image (wormatlas.org).

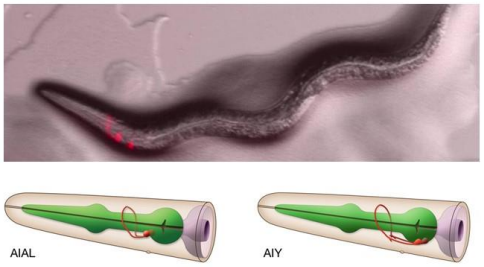
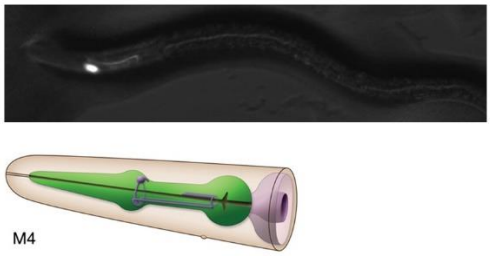


Figure 2. Expression of mCherry under the control of the putative *H. contortus glc-3* promoter. As with the *C. elegans* promoter (Figure 1) fluorescence was observed in the anterior of the worms, but in this case the pattern of expression was more similar to the

pharyngeal M4 motor neuron (wormatlas.org).



3.2 Using CRISPR/Cas9 to knock out *glc-3* from *C. elegans*.

Potential CRISPR/Cas9 cleavage sites were identified in *glc-3* and the appropriate guide RNAs obtained. DNA sequences flanking *glc-3* were amplified from *C. elegans* genomic DNA and cloned into plasmid pDD288; successful cloning of these fragments was confirmed by DNA sequencing. This plasmid, along with the guide RNAs and purified Cas9, was micro-injected into the gonadal syncytium of young adult hermaphrodites and the injected worms maintained on NGM agar plates. After 2-3 days, drug was added and the incubation continued for several more days. Worms that survived the drug treatment and exhibited the 'rolling' phenotype were picked and examined under the fluorescence microscope for expression of GFP. Though some weak fluorescence was observed, and these worms were picked onto new plates to self-fertilise, no stronger fluorescence indicative of a homozygous knockout strain was ever observed. We repeated the procedure with different guide RNAs, to produce a smaller deletion in the genome equivalent to that seen in the *glc-3* mutant strain, but again no homozygous deletions could be obtained.

3.3 A truncated form of GLC-3 from *H. contortus*

The failure to obtain a homozygous *glc-3* knockout was surprising, as the presumed *glc-3* null allele, *ok321*, is viable as a homozygote. However, this allele does leave the 5'portion of the coding sequence intact and there are reports of severely truncated mRNA transcripts from other ligand-gated ion channel genes, in particular *acr-8*, where a truncated transcript caused by an intron retention event has been linked to levamisole resistance in parasitic nematodes (Fauvin et al., 2010; Neveu et al. 2010; Williamson et al., 2011). For that reason we attempted to discover if such a transcript might be encoded by *glc-3*. The strategy employed was 3' RACE, using a primer based on sequences towards the 5' end of the full-length *glc-3* transcript in combination with the Ri portion of the oligo(dT)RoRi primer used to synthesise the cDNA. When this strategy was applied to a cDNA population synthesised from adult *H. contortus* RNA, a smaller PCR product was detected; when this product was cloned into pCR-Blunt and sequenced, it was found to represent a severely truncated *Hco-glc-3* product, formed by an intron retention event at the end of exon 3 (Figure 3).

4- Conclusion

The long-term goal of the experiments reported here is the further development of *C. elegans* as an expression system for studying the role of the ivermectin-sensitive glutamate-gated chloride channels of parasitic nematodes. An important assumption underlying this approach is that the functions of the genes encoding these receptors will be broadly conserved between the species, including the cells in which the genes are expressed. We tested this assumption with our first target gene, *glc*-3. The GLC-3 subunit of nematodes has been implicated in a variety of processes, most notably in the integration of sensory inputs into behavioural outcomes, such as changes in locomotion downstream of chemosensation or thermosensation (Chalasani et al. 2007; Mutlu et al. 2020; Wen et al. 2020; Wang et al. 2021). This is consistent with the reported expression of the *C. elegans glc-3* gene in the head interneurons AIA and AIY, and we able to confirm these reports as a first step in this project (Figure 1). However, the use of the putative *H. contortus glc-3* promoter sequences to drive mCherry expression led to a markedly different result, with the promoter apparently active in the pharyngeal M4 motoneuron (Figure 2), rather than AIA or AIY. At first glance this might indicate that the role of GLC-

3 is different in the two species, however the use of 'foreign' promoter sequences in *C. elegans* has to be interpreted with caution, and the pharynx has often been observed as a 'default' organ for the expression of such constructs. Expression of the *C. elegans* GLC-3 has been reported in M4, albeit at lower levels than the interneurons, and it is well established that ivermectin has a potent paralysing effect on the *H. contortus* pharynx (Geary et al., 1993); part of that effect could potentially be mediated by pharyngeal expression of GLC-3 in the parasite.

but could never breed these to homozygosity. This unexpected result suggests that worms in which the whole *glc-3* gene is deleted are either non-viable, or at least at a severe competitive disadvantage compared to wild-type worms. A re-examination of the *ok321* allele showed that a portion of the gene did remain in worms carrying this allele, including the extreme 5' end of the coding sequence. If this sequence was required for viability, but not the rest of the gene, what could be the potential explanation? We looked for other transcripts that included the

Figure 3. The sequence of GLC-3-T. The nucleotide sequence of the original cDNA clone is shown in red underneath the published sequence of *Hco-glc-3* mRNA. In the lower panel the predicted amino-acid sequence of the polypeptide is shown, along with the N-terminal region of the full-length protein.

cDNA Sequence

```
GLC-3 gtttaattac ccaagtttga ggctttaaag agtcccctct acattctaag agttcgagtg
GLC-3T
      ctgttccggg ctggcttccg gatgatgttc gttcatgaac cattgccttt cgttcataat

      tcttttctgc tcaattcgat tgcctttctg ctgacgattt ttcgggttca attcataaca

      ttatgtgaag cagatgcctc ctcggacacg gagatcatca agaaactgtt gggcaaaggc
                             TCGGACACG GAGATCATCA AGAAGCTATT GGGCAAAGGC
      tatgattgga gagtacgacc gccagggatc aacctcacag caaaaggtag ccacggaccg
      TATGATTGGA GAGTACGACC GCCAGGGATC AACCTCACAG CAAAAGG

      CACTCCATTTCTTTTCTTCACAAAAAGAACAAAAACCGTCTGA
```

Predicted amino-acid sequence

```
GLC-3   MMFVHEPLPFVHNSFLLNSIAFLLTIFRVQFITLCEADASSDTEIIKKLLGKGYDWRVRPPGIN
GLC-3T  MMFVHEPLPFVHNSFLLNSIAFLLTIFRVQFITLCEADASSDTEIIKKLLGKGYDWRVRPPGIN

GLC-3   LTAKG                SHGPVVVNVNMLIRSISKIDDVNMEYSVQLTFR
GLC-3T  LTAKGTPFLFFTKRTKTV*
```

Strains of *C. elegans* homozygous for a deletion allele, *ok321*, are viable and have no obvious deleterious phenotypes. This deletion is predicted to be essentially a null mutation, with no functional protein produced. Therefore, we expected that it should not be too difficult to generate a strain in which the entire coding sequence of *glc-3* had been removed, as reported for many other *C. elegans* genes and also achieved in the Neveu laboratory. However, this proved to be impossible despite repeated attempts with alternative guide RNA sequences. We did obtain evidence of the generation of heterozygotes with the deletion

5' portion of the *glc-3* mRNA and identified a severely truncated transcript, glc-3T, from *H. contortus*, which was derived from an intron retention event and encoded a very short polypeptide carrying a signal peptide, suggesting it should be targeted into the secretory pathway and possibly secreted from the cell. The constraints of time and the pandemic precluded further investigation of this polypeptide. Investigating the genomic sequence of *C. elegans* suggested a similar transcript was potentially present, but no direct confirmation was possible.

Truncated polypeptides have been identified from several nematode ion channel genes, including some produced by intron retention events. In *H. contortus*, such a transcript derived from *Hco-acr-8* has been identified and associated with drug resistance, in this case to levamisole (Fauvin et al, 2010; Neveu et al. 2010; Williamson et al., 2011). Interestingly, the length of the ACR-8b polypeptide, 87 amino-acid residues, is very similar to the predicted length of GLC-3T, 82 amino acids. In *C. elegans*, intron retentions in other ion channel genes result in similar truncated polypeptides and in one gene this truncated protein has been shown to act as a 'dominant negative', affecting ion channel function, nematode behaviour and drug resistance (Neveu, Reaves & Wolstenholme, unpublished). It is possible to speculate that the synthesis of such polypeptides might be a common property of ion channel subunit genes, and that they play a role in controlling the assembly and trafficking of the receptor complex. Though this a speculation at present, the difficulty in generating homozygous knock-outs of other nicotinic acetylcholine receptor and GluCl genes that we and others have observed (unpublished observations) supports the contention that these genes possess unexpected properties that are worthy of further investigation and which might lead to novel insights into the assembly and regulation of the ion channels. Obviously further investigations are required to confirm this, and these are currently planned and were in progress prior to the pandemic.

5- Perspectives of future collaborations with the host laboratory

Future collaborations are likely to continue within the framework of the ARTI initiative, which was set up during the Le Studium conference that we organised in November/December 2021 and which involves a consortium of European and other researchers. Dr. Neveu and myself are co-applicants on an Australian grant, and this should also continue our interactions. I intend to visit Tours several times in the coming years to try and complete the studies started during this fellowship, as well as some pre-existing projects.

6- Articles published in the framework of the fellowship

George, M.M., Vatta, A.F., Howell, S.B., Storey, B.E., McCoy, C.J., Wolstenholme, A.J., Redman, E.M., Gilleard, J.S., Kaplan, R.M. (2021). Evaluation of changes in drug susceptibility and population genetic structure in *Haemonchus contortus* following worm replacement as a means to reverse the impact of multiple-anthelmintic resistance on a sheep farm. *Int. J. Parasitol. Drugs Drug Res.* **15**, 134-143.

Hübner, M.P., Townsen, S., Gokool, S., Tagboto, S., Maclean, M.J., Verocai, G.G., Wolstenholme, A.J., Frohberger, S., Specht, S., Scandale, I., Harder, A., Glenschek-Sieberth, M., Hahnel, S.R., Kulke, D. (2021). Evaluation of the in vitro susceptibility of various filarial nematodes to emodepside. *Int. J. Parasitol. Drugs Drug Res.* **17**, 27-35.

Wilson, N.E., Reaves, B.J., Wolstenholme, A.J. (2021). Lack of detectable short-term effects of a single dose of ivermectin on the human immune system. *Parasites & Vectors*, **14,** 304.

Wolstenholme, A.J. & Neveu, C. (2022). The avermectin receptors of parasitic nematodes. *Pestic. Biochem. Physiol.***181**, 105010.

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8- References

Britton, C., Murray, L. (2006). Using *Caenorhabditis elegans* for functional analysis of genes of parasitic nematodes. *Int. J. Parasitol.* **36,** 651–659.

Chalasani, S.H., Chronis, N., Tsunozaki, M., Gray, J.M., Ramot, D., Goodman, M.B., Bargmann, C.I. (2007). Dissecting a circuit for olfactory behaviour in *Caenorhabditis elegans*. *Nature* **450**, 63-70.

Cleland, T.A. (1996). Inhibitory glutamate receptor channels. *Mol. Neurobiol*. **13,** 97–136.

Consortium, *C. elegans* Sequencing (1998). Genome sequence of the nematode *C. elegans*; a platform for investigating biology. *Science* **282,** 2012-2017.

Cook, A., Aptel, N., Portillo, V., Siney, E., Sihota, R., Holden-Dye, L., Wolstenholme, A. (2006). *Caenorhabditis elegans* ivermectin receptors regulate locomotor behaviour and are functional orthologues of *Haemonchus contortus* receptors. *Mol. Biochem. Parasitol.* **147**, 118- 125.

Couthier, A., Smith, J., McGarr, P., Craig, B., Gilleard, J.S. (2004). Ectopic expression of a *Haemonchus contortus* GATA transcription factor in *Caenorhabditis elegans* reveals conserved function in spite of extensive sequence divergence. *Mol. Biochem. Parasitol*. **133**, 241-253.

Dickinson, D.J., Goldstein, B. (2016). CRISPR-based methods for *Caenorhabditis elegans* genome engineering. *Genetics* **202**, 885-901.

Dickinson, D.J., Pani, A.M., Heppert, J.K., Higgins, C.D., Goldstein, B. (2015). Streamlined genome engineering with a self-excising drug selection cassette. *Genetics* **115**, 178335.

Doyle, S.R., Tracey, A., Laing, R., Holroyd, N., Bartley, D., Bazant, W., Beasley, H., Beech, R., Britton, C., Brooks, K., Chaudhry, U., Maitland, K., Martinelli, A., Noonan, J.D., Paulini, M., Quail, M.A., Redman, E., Rodgers, F.H., Sallé, G., Shabbir, M.Z., Sankaranarayanan, G., Wit, J., Howe, K.L., Sargison, N., Devaney, E., Berriman, M., Gilleard, J.S., Cotton, J.A.(2020). Genomic and transcriptomic variation defines the chromosome-scale assembly of *Haemonchus contortus*, a model gastrointestinal worm. *Commun Biol*. **3,** 656.

Evans, T. C. (2006). Transformation and microinjection, *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.108.1, http://www.wormbook.org.

Fauvin, A., Charvet, C., Issouf, M., Cortet, J., Cabaret, J., Neveu, C. (2010) cDNA-AFLP analysis in levamisole-resistant *Haemonchus contortus* reveals alternative splicing in a nicotinic acetylcholine receptor subunit. *Mol Biochem Parasitol.* **170,** 105–107.

Geary, T.G., Sims, S.M., Thomas, E.M., Vanover, L., Davis, J.P., Winterrowd, C.A., Klein, R.D., Ho, N.F.H., Thompson, D.P., (1993). *Haemonchus contortus*: ivermectin induced paralysis of the pharynx. *Exp. Parasitol.* **77,** 88–96.

Glendinning, S.K., Sattelle, D.B., Buckingham, S.D., Wonnacott, S., Wolstenholme, A.J. (2011) Glutamate-gated chloride channels of *Haemonchus contortus* restore drug sensitivity to ivermectin resistant *Caenorhabditis elegans. PLoS ONE* **6**, e22390.

Horoszok, L., Raymond, V., Sattelle, D.B., Wolstenholme, A.J. (2001). GLC-3: A novel fipronil and BIDN-sensitive, but picrotoxinin-insensitive, L-glutamate-gated chloride channel subunit from *Caenorhabditis elegans*. *Brit. J. Pharmacol.* **132**, 1247-1254.

Lamassaiude, N., Courtot, E., Corset, A., Charvet, C.L., Neveu, C. (2022). Pharmacological characterization of novel heteromeric GluCl subtypes from *C. elegans*

and parasitic nematodes. *Brit J. Pharmacol*. In Press. https://doi.org/10.1111/bph.15703

Laughton, D.L., Amar, M., Thomas, P., Towner, P., Harris, P., Lunt, G.G., Wolstenholme, A.J. (1994). Cloning of a putative inhibitory amino acid receptor subunit from the parasitic nematode *Haemonchus contortus. Receptors and Channels*, **2**, 155-163.

Li, F., Lok, J.B., Gasser, R.B., Korhonen, P.K., Sandeman, M.R., Shi, D., Zhou, R., Li, X., Zhou, Y., Zhao, J., Hu, M. (2014). Hc-daf-2 encodes an insulin-like receptor kinase in the barber's pole worm, *Haemonchus contortus*, and restores partial dauer regulation. *Int. J. Parasitol*. **44**, 485-496.

Mutlu, A.S., Gao, S.M., Zhang, H., Wang, M.C. (2020). Olfactory specificity regulates lipid metabolism through neuroendocrine signaling in *Caenorhabditis elegans*. *Nat Commun,* **11**, 1450.

Neveu, C., Charvet, C.L., Fauvin, A., Cortet, J., Beech, R.N., Cabaret, J. (2010). Genetic diversity of levamisole receptor subunits in parasitic nematode species and abbreviated transcripts associated with resistance. *Pharmacogenet Genomics* **20,** 414–25.

Sloan, M., Reaves, B.J., Maclean, M.J., Storey, B.E., Wolstenholme, A.J. (2015). Expression of nicotinic acetylcholine receptor subunits from parasitic nematodes in *Caenorhabditis elegans*. *Mol. Biochem. Parasitol.* **204**, 44-50.

Stiernagle, T. (2006). Maintenance of *C. elegans*, *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.101.1, http://www.wormbook.org.

Taylor, S.R., Santpere, G., Weinreb, A., Barrett, A., Reilly, M.B., Xu, C.-A., Varol, E., Oikonomou, P., Glenwinkel, L., McWhirter, R., Poff, A., Basavaraju, M., Rafi, I., Yemini, E., Cook, S.J., Abrams, A., Vidal, B., Cros, C., Tavazoie, S., Sestan, N., Hammarlund, M., Hobert, O., Miller III, D.M. (2021). Molecular topography of an entire nervous system. *Cell* **184**, 4329-4347.

Wang, M., Witvliet, D., Wu, M., Kang, L., Shao, Z. (2021). Temperature regulates synaptic subcellular specific mediated by inhibitory glutamate signalling. *PLoS Genet*. **17**, e1009295.

Welz, C., Krüger, N., Schniederjans, M., Miltsch, S.M., Krücken, J., Guest, M., Holden-Dye, L.M., Harder, A., von Samson-Himmelstjerna, G. (2011). SLO-1-channels of parasitic nematodes reconstitute locomotor behaviour and emodepside sensitivity in C*aenorhabditis elegans slo-1* loss of function mutants. *PLoS Pathog* **7**, e1001330.

Wen, X., Chen, Y.H., Li, R., Ge, M.H., Yin, S.W., Wu, J.J., Huang, J.H., Liu, H., Wang, P.Z., Gross, E., Wu, Z.X. (2020). Signal decoding for glutamate modulating egg laying oppositely in *Caenorhabditis elegans* under varied environmental conditions. *iScience*, **23**, 101588.

Williamson, S.M., Storey, B., Howell, S., Harper, K., Kaplan, R.M., Wolstenholme, A.J. (2011) Candidate anthelmintic resistance-associated gene expression and sequence polymorphisms in a triple-resistant isolate of *Haemonchus contortus. Mol. Biochem. Parasitol.* **180**, 99-105.

Wolstenholme, A.J. (2012) Glutamate-gated chloride channels. *J. Biol. Chem*. **287**, 40232-40238.

Wolstenholme, A.J. & Neveu, C. (2022). The avermectin receptors of parasitic nematodes. *Pest. Biochem. Physiol.***181**, 105010.