Avermectins are a class of macrocyclic lactones that is widely used in crop protection and to treat helminth infections in man and animals. Two complementary DNAs (GluCl<sub>a</sub> and GluCl<sub>b</sub>) encoding chloride channels that are gated by avermectin and glutamate, respectively, were isolated from Caenorhabditis elegans. To study the role of these subunits in conferring avermectin sensitivity we isolated a mutant C. elegans strain with a Tc1 transposable element insertion that functionally inactivated the GluCl<sub>a</sub> gene (GluCl<sub>a</sub>\texttt{::Tc1}). GluCl<sub>a</sub>\texttt{::Tc1} animals exhibit a normal phenotype including typical avermectin sensitivity. Xenopus oocytes expressing GluCl<sub>b</sub>\texttt{::Tc1} strain mRNA elicited reduced amplitude avermectin and glutamate-dependent chloride currents. Avermectin binding assays in GluCl<sub>a</sub>\texttt{::Tc1} strain membranes showed the presence of high affinity binding sites, with a reduced B<sub>max</sub>. These experiments suggest that GluCl<sub>a</sub> is a target for avermectin and that additional glutamate-gated and avermectin-sensitive chloride channel subunits exist in C. elegans. We isolated a cDNA (GluCl<sub>2</sub>) encoding a chloride channel that shares 75% amino acid identity with GluCl<sub>a</sub>. This subunit forms homomeric channels that are gated irreversibly by avermectin and reversibly by glutamate. GluCl<sub>2</sub> coassembles with GluCl<sub>b</sub> to form heteromeric channels that are gated by both ligands. The presence of subunits related to GluCl<sub>a</sub> may explain the low level and rarity of target site involvement in resistance to the avermectin class of compounds.

Extensive use of parasiticides and pesticides has resulted in the emergence of resistant strains of target organisms. Characterization of the molecular targets of such compounds would help elucidate their mode of action and mechanisms of resistance. Avermectins are a class of macrocyclic lactones with insecticidal and nematocidal activity. Abamectin (avermectin B<sub>1</sub>) is a miticide and insecticide used in crop protection. Ivermectin (22,23-dihydroavermectin B<sub>1</sub>) is a miticide and insecticide used in crop protection and to treat helminth infections in man and animals. Two complementary DNAs (GluCl<sub>a</sub> and GluCl<sub>b</sub>) encoding chloride channels that are gated by avermectin and glutamate, respectively, were isolated from Caenorhabditis elegans. To study the role of these subunits in conferring avermectin sensitivity we isolated a mutant C. elegans strain with a Tc1 transposable element insertion that functionally inactivated the GluCl<sub>a</sub> gene (GluCl<sub>a</sub>\texttt{::Tc1}). GluCl<sub>a</sub>\texttt{::Tc1} animals exhibit a normal phenotype including typical avermectin sensitivity. Xenopus oocytes expressing GluCl<sub>b</sub>\texttt{::Tc1} strain mRNA elicited reduced amplitude avermectin and glutamate-dependent chloride currents. Avermectin binding assays in GluCl<sub>a</sub>\texttt{::Tc1} strain membranes showed the presence of high affinity binding sites, with a reduced B<sub>max</sub>. These experiments suggest that GluCl<sub>a</sub> is a target for avermectin and that additional glutamate-gated and avermectin-sensitive chloride channel subunits exist in C. elegans. We isolated a cDNA (GluCl<sub>2</sub>) encoding a chloride channel that shares 75% amino acid identity with GluCl<sub>a</sub>. This subunit forms homomeric channels that are gated irreversibly by avermectin and reversibly by glutamate. GluCl<sub>2</sub> coassembles with GluCl<sub>b</sub> to form heteromeric channels that are gated by both ligands. The presence of subunits related to GluCl<sub>a</sub> may explain the low level and rarity of target site involvement in resistance to the avermectin class of compounds.

Extensive use of avermectins has led to the appearance of resistance to this class of compounds. Low level (3–10-fold) ivermectin resistance has only been detected in parasitic nematodes of goats and sheep. In most cases multiple resistance to other groups of drugs is involved (18). These findings suggest that either ivermectin acts on multiple targets or that the target site is involved in essential processes.

The extensive genetic characterization of C. elegans makes it a useful system to study the function of GluCl channels in vivo and the mechanisms of resistance to ivermectin. In an attempt to understand the role of GluCl channels in conferring avermectin sensitivity and their physiological function, we determined the chromosomal localization of the GluCl<sub>a</sub> and GluCl<sub>b</sub> genes and searched for known mutations mapping to these loci. However, mutations attributed specifically to these genes could not be identified. We then identified a C. elegans strain with a Tc1 transposable element insertion in the GluCl<sub>a</sub> gene. Here, we report the biochemical characterization of this mutant strain and the isolation of a novel subunit (GluCl<sub>2</sub>) with properties similar to GluCl<sub>a</sub>.

**EXPERIMENTAL PROCEDURES**

**Chromosomal Localization of the GluCl<sub>a</sub> and GluCl<sub>b</sub> Genes**—Grids with ordered YAC clones were hybridized with <sup>32</sup>P-labeled probes using the

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1 The abbreviations used are: GluCl, glutamate-gated chloride; IVM, ivermectin; IVMPO<sub>4</sub>, ivermectin phosphate; PCR, polymerase chain reaction; cRNA, copy RNA; kb, kilobase(s); nt, nucleotide(s); PTX, picrotoxin.
Random Priming Kit (Boehringer Mannheim). Cosmid clones spanning the regions of the hybridizing YAC clones were kindly provided by Dr. Alan Coulson (Medical Research Council, Cambridge). Cosmid DNA isolation was performed by alkaline lysis using Wizard Mini- and Maxiprep kits (Promega). Hybridizations were done at 42 °C in 50% formamide, 5× SSC, 1% SDS, and a Bio-Rad LightCycler (Fig. 1). The sequence of the insertion point was determined using the dideoxy method. Some of the strains used were obtained from the Caenorhabditis Genetics Center. GluCl:Tc strain isochromosomes were generated by using random and oligo(dT) primers using the reagents of the Superscript kit (Life Technologies, Inc.). PCR reactions were performed using the Superscript kit (Life Technologies, Inc.) and the following primers: 5′-TTCAAACGTATTCTCACTACTGTAATG-3′ and 5′-TCTCCTATTGAGGGCAGCGGTAGT-3′. PCR conditions were 95 °C 1 min, 62 °C 2 min, 72 °C 3 min for 45 cycles. The GluCl:Tc mRNA was synthesized using the T3 promoter of the Bluescript SK vector. A poly(A) tail was added by annealing and ligation of two oligonucleotides containing 25 A to the NotI/Pst1 sites.

Electrophysiology—Xenopus oocytes from the same donor were used in every set of experiments. Oocytes were injected with 50 nl of mRNA (1 µg/ml) or cRNA and voltage-clamped at −80 mV at room temperature using standard two-electrode voltage-clamp techniques described (13, 16). Oocytes injected with mRNA were analyzed 2 days after injection while those injected with cRNA 2–4 days after injection. The water-soluble avermectin derivative, IVOMe, was used for these studies. GluCl:Tc mRNA was synthesized using the T3 promoter of the Bluescript vector as described (16). The reported current amplitudes are average responses of at least four oocytes. All data in text are mean ± S.E.

Ivermectin Binding Assays and Pharmacology—Four different membrane preparations of wild-type and GluCl:Tc animals were tested for [3H]IVM binding as described (23). Wild-type and GluCl:Tc animals were grown in liquid media, membranes were prepared and binding assays were done in parallel for each set (20). Motility assays in solution and plates as well as egg laying assays were done as described (19). Approximately 50–100 animals of mixed developmental stages or single L4 hermaphrodites were used for each ivermectin concentration 0.1–500 nM.

RESULTS

Chromosomal Mapping of GluCl and GluCl– Genes—The GluCl and GluCl– genes were assigned to yeast artificial chromosome clones and corresponding cosmids (Fig. 1). The GluCl gene was found to be within cosmid clone C25D4 of contig 313 on chromosome V, while the GluCl– gene was present within cosmid clone C04E4 of contig 405 on chromosome I. These data indicate that the GluCl gene is located between unc-76 and dpy-21 while the GluCl– gene is distal to unc-63 and next to unc-38. The unc-38 mutation has been assigned to cosmid clone ZZ#11 that overlaps with cosmid C04E4. The nucleotide sequence of unc-38 shows it to be related to the nicotinic acetylcholine receptors and therefore does not represent a mutation in the GluCl– gene (25). No other mutations have been assigned to the cosmids containing the GluCl and GluCl– genes.

Isolation of the GluCl:Tc1 Strain—To study the functional role of the GluCl channels in C. elegans we sought to develop null mutants in the GluCl and GluCl– genes using transposon insertional mutagenesis (19). The C. elegans strain, MT3126, which shows a high frequency of Tc1 transposable element insertions, was used to generate mutants for screening in the GluCl and GluCl– genes. Tc1 insertions in the GluCl gene (strain NL704, allele pk56::Tc1) and GluCl– gene (strain NL705, allele pk56::Tc1) were identified by screening a frozen transposon mutagenesis library bank arranged into an orthogonal manner (19). To identify an insertion in the GluCl– gene that was not identified did not result in a null mutation (data not shown). Our subsequent attempts to obtain a null mutant in the GluCl gene by searching for a Tc1 excision/deletion event by PCR were unsuccessful (data not shown). A GluCl gene Tc1 insertion event identified did result in gene inactivation. A homozygous GluCl::Tc1 strain
with a Tc1 insertion in the GluCl gene was isolated and the majority of the irrelevant Tc1 insertions were eliminated by backcrosses.

Sequencing of the Tc1 insertion region of the GluCl::Tc1 gene revealed that the Tc1 insertion (amino acid 255, Figs. 2 and 7) disrupted the putative extracellular N-terminal domain of the GluCl protein. The Tc1 insertion interrupts a highly conserved, length invariant disulfide loop that is found in all ligand-gated anion channels (Fig. 2). Thus, it is unlikely that the GluCl::Tc1 gene encodes a functional channel subunit.

**Analysis of GluCl::Tc1 Transcripts**—To confirm that the Tc1 insertion resulted in a mutated GluCl gene we compared the GluCl gene products of wild-type N2 and GluCl::Tc1 animals (Fig. 3, lanes 1 and 2, respectively) by Northern blot analysis. A GluCl cDNA probe detected 1.6- and 2.3-kilobase (kb) transcripts in the N2 lane and 0.8-, 1.6-, 2.3-, and 3.2-kb transcripts in the GluCl::Tc1 lane (Fig. 3). The 0.8-kb mRNA that is specific for GluCl::Tc1 hybridized to a probe corresponding to the region 5' of the Tc1 insertion site but not to a probe derived from the region 3' of the Tc1 insertion (data not shown). These results indicate that the 0.8-kb mRNA (denoted with an asterisk in Fig. 3) is an N-terminal truncated transcript of the GluCl gene. The 1.6- and 2.3-kb mRNAs that appear in the wild-type lane were reduced in intensity in the GluCl::Tc1 lane by 15–20 fold, as determined by PhosphorImager intensity quantitation. Expression of GluClβ mRNA in the GluCl::Tc1 strain was unaffected, showing that disruption of the GluCl gene does not affect GluClβ transcription levels (data not shown).

The GluCl::Tc1 strain is homozygous for the Tc1 insertion and appears genetically stable. However, low frequency somatic excision of Tc1 transposable elements has been reported by several laboratories (26, 27). Thus, it is likely that the faint transcripts at 1.6- and 2.3-kb that appear in the GluCl::Tc1 strain are the result of somatic excision of Tc1. This was confirmed by analysis of the Tc1 integration/excision site by reverse transcription of mRNA followed by PCR amplification of the resultant cDNA. Reverse transcriptase-PCR reactions of GluCl::Tc1 strain mRNA with primers flanking the integration site revealed multiple reverse transcriptase-dependent bands that were not present in the N2 mRNA (see “Experimental Procedures” for details) (data not shown). These bands were up to 50 nt larger or up to 20 nt smaller than the wild-type GluCl PCR-derived control fragment. Bands corresponding to the wild-type GluCl PCR-derived control fragment were not detected in the GluCl::Tc1 strain mRNA. These results indicate that the 1.6- and 2.3-kb GluCl transcripts arise from imperfect somatic Tc1 excision and are mutated at the site of excision. Due to small deletions or amino acid additions at this highly conserved region the majority of these (near) full-length GluCl mRNAs are likely to be defective (28). However, it is possible that a small percentage of these transcripts is functional.

In summary, as a result of the Tc1 insertion a truncated GluCl mRNA encoding a nonfunctional protein is generated. A low level of near full-length GluCl mRNA may result in some somatic cells by Tc1 excision that could restore the reading frame in one-third of these rare mRNAs. We therefore conclude that the GluCl gene is functionally inactivated in most cells of the GluCl::Tc1 animals and the amount of functional GluCl mRNA in the GluCl::Tc1 strain is reduced at

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**Fig. 1. Chromosomal map of the C. elegans GluClα and GluClβ genes.** YAC clones and the corresponding cosmids that hybridized to genes. The Tc1 insertion interrupted a highly conserved, length invariant disulfide loop that is found in all ligand-gated anion channels (Fig. 2). Thus, it is unlikely that the GluCl::Tc1 gene encodes a functional channel subunit.

**Fig. 2. Schematic representation of the GluCl::Tc1 allele.** The nucleotide sequence of the junction point is indicated in the lower part of the figure. The Tc1 sequence is in lowercase characters. The approximate positions of DKV1.3, DKV1.4, RI, and RII are indicated.
Phenotypic Analysis and Pharmacology—The GluClα::Tc1 strain was inspected for phenotypic abnormalities and found to lack any visible defects. Since the GluCl channel is thought to be a target of avermectin, we tested the GluClα::Tc1 strain for sensitivity to avermectin and found it to be equal to the wild-type strain. Ivermectin sensitivity was tested in motility assays in liquid (9) plates and egg laying (24) assays (data not shown). This result may indicate that C. elegans expresses additional ivermectin-sensitive GluCl channel(s) that can functionally compensate for the elimination of GluClα.

Expression of GluClα::Tc1 Strain mRNA—Wild-type and GluClα::Tc1 animals were tested for the presence of ivermectin-sensitive GluCl channels by mRNA expression in Xenopus oocytes. The average response to 1 mM glutamate was 266 ± 20 nA for oocytes injected with wild-type mRNA and 84 ± 7 nA for those injected with GluClα::Tc1 strain RNA; the average IVMPO4 response was 218 ± 16 and 75 ± 8 nA, respectively (Fig. 4). Perfusion of Xenopus oocytes with 100 μM picrotoxin (PTX), can distinguish between glutamate elicited currents from homomeric GluClβ (PTX sensitive) and heteromeric GluClα and β (PTX insensitive) GluCl channels (13). The glutamate responses in oocytes injected with either wild-type or GluClα::Tc1 mRNA were insensitive to 100 μM PTX, indicating that the expressed channels were heteromeric (data not shown). These data indicate an approximately 3-fold difference in amplitude of the glutamate and IVMPO4 responses between GluClα::Tc1 and wild-type samples. To exclude that this difference results from an overall decreased ability of the GluClα::Tc1-injected RNAs to be expressed in Xenopus oocytes, we measured the induced amplitude of the endogenous calcium-activated chloride channel currents in the same oocytes. The amplitude of this current was comparable for oocytes injected with either wild-type or GluClα::Tc1 strain mRNA (812.5 ± 77 and 886 ± 83 nA, respectively; control oocytes showed responses of 250 ± 35 nA). If the glutamate and IVMPO4 responses of oocytes injected with wild-type mRNA were dependent on the contribution of GluClα mRNA alone, an approximately 45–60-fold reduction in the current amplitudes would have been expected in oocytes injected with GluClα::Tc1 strain RNA.

In reconstitution experiments, synthetic GluClα and GluClβ cRNAs were expressed in a 1:50 (α:β) ratio, which resulted in a 97% decrease in IVMPO4 and glutamate responses as compared with a 1:1 (α:β) ratio (data not shown). The channels formed in these experiments were insensitive to 100 μM PTX,

![Graph](image)

**FIG. 3.** Northern analysis of the GluClα::Tc1 transcripts. Lane 1, wild-type mRNA; lane 2, GluClα::Tc1 mRNA. The Tc1 insertion has resulted in the truncation of the primary transcript (lane 2, asterisk), however, there remain apparently wild-type GluClα transcripts due to somatic excision of Tc1 (see text).

**FIG. 4.** Expression of C. elegans mRNA in Xenopus oocytes. Glutamate and ivermectin responses of Xenopus oocytes injected with mRNA from wild-type (N2) and GluClα::Tc1. The scale for each of the traces is different and is indicated on the lower right. The responses are similar but of different amplitudes.

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2 A. Etter, personal communication.
3 J. P. Arena, unpublished data.
indicating that the expressed channels were heteromeric. Expression of GluCl::Tc1 mRNA results only in a 3-fold reduction in its response to IVMP4 and glutamate. Since the reduction in the current amplitudes of oocytes injected with GluCl::Tc1 strain RNA is only 3-fold, these experiments predict the presence of additional GluCl-like subunit(s) in *C. elegans*.

**Specific Elimination of GluClα Transcripts from Wild-type mRNA**—Specific elimination of GluClα transcripts by RNase H digestion was employed to confirm that other *C. elegans* genes exist that can encode avermectin-sensitive GluCl channels. mRNA from wild-type and GluCl::Tc1 animals were hybridized with an antisense oligonucleotide (ANTI 1.1) to the GluClα coding sequence, spanning the translation initiation site, and then treated with RNase H. Specific digestion of the DNA-RNA hybrid of the GluClα mRNA was confirmed by Northern analysis (Fig. 5A) (29). Labeled ANTI 1.1 oligonucleotide detected intact GluClα transcripts only in the untreated samples (lane N2, band labeled with arrow in Fig. 5A). RNase H treatment, as expected, eliminated the hybridization to ANTI 1.1 oligonucleotide. The cross-hybridizing bands detected with this probe are unaffected and are of identical size in the RNase H-treated samples and not on the RNase H-treated samples (the major band in the N2 lane is indicated by an arrow; the major band in the lane GluCl::Tc1 by an asterisk). The size markers on the left side are in kilobases. B, the same membrane as on panel B was rehybridized with the GluClα cDNA probe. The bands detected in the RNase H-treated samples appear undegraded and of reduced size by ~60 nt in comparison to the controls. The major bands are indicated by arrows. Size markers in kb are indicated on the left. C, electrophysiological responses of Xenopus oocytes injected with the RNase H-treated GluClα mRNA from wild-type (N2/RNase H) and GluCl::Tc1 (GluCl::Tc1/RNase H) analyzed on panels A and B. Similar responses to both glutamate and ivermectin are observed.

**Equilibrium Binding Analysis of GluClα::Tc1 Membrane-binding Sites**—Membrane preparations of wild-type and GluCl::Tc1 strain animals were tested for [H]ivermectin binding (30). The *B*~max~ of the wild-type membranes was 0.55 ± 0.09 pmol/mg of protein (*n* = 4) while for the GluCl::Tc1 membranes this level dropped ~40% to 0.32 ± 0.02 pmol/mg of protein (*n* = 4) (Fig. 6). These data indicate that high affinity ivermectin-binding sites exist in *C. elegans* membranes in the absence of functional GluClα gene expression. Scatchard plot analysis of the wild-type membrane binding indicated a *K*_D*~α~ (0.111 ± 0.019 nM) while for the GluCl::Tc1 membrane binding of 0.153 ± 0.018 nM (Fig. 6).

**Isolation of a GluClα2 cDNA**—The indications for the presence of RNase H-treated RNA samples were expressed in *Xenopus* oocytes (Fig. 5C). The oocytes expressing either wild-type or GluCl::Tc1 strain mRNA treated with RNase H responded to both glutamate (1 mM) and IVMP4 (1 μM): 18 ± 3 nA glutamate, 24 ± 4 nA IVMP4; 14 ± 4 nA glutamate, 21 ± 4 nA IVMP4, respectively. The channels formed were insensitive to 100 μM PTX, indicating that they were heteromeric. The magnitude of the currents to IVMP4 and glutamate were reduced by about 3-fold when compared with the expression of untreated GluCl::Tc1 strain mRNA, as were the responses of the endogenous Ca^2+^-activated Cl^- channels indicating a nonspecific effect. These experiments indicate that additional chloride channel subunit genes with properties similar to GluClα are expressed in *C. elegans*.
of additional GluCl-like channels in *C. elegans* prompted us to search the databases for related genes. One such gene, GluClα2, that showed homology to GluClα1 (from here on GluClα1) was identified within cosmid T10G3 of contig 313 on chromosome V. The introns of the GluClα2 gene appear in identical positions to the introns of the GluClα1 gene (17). Oligonucleotides corresponding to the 5′- and 3′-ends of the GluClα2 gene were used to isolate a GluClα2 cDNA by reverse transcriptase-PCR. The 5′-oligonucleotide included a 20-nt upstream sequence of the putative initiator Met codon, while the 3′-oligonucleotide was upstream of the putative poly(A) addition signal. Nucleotide sequencing of an isolated GluClα2 cDNA revealed no mismatches to the sequence of the predicted exons of cosmid T10G3. The GluClα2 cDNA revealed 80, 63, and 61% nucleotide identity with GluClα1, GluClβ, and DrosGluCl, respectively (31); the corresponding predicted amino acid identities were 75, 49, and 49%. Alignment of GluClα2, GluClα1, GluClβ, and DrosGluCl-predicted amino acid sequences is shown in Fig. 7. The putative GluClα2 protein has a 289-amino acid long N-terminal extracellular domain followed by three closely spaced putative transmembrane domains (M1–M3), an 83-amino acid cytoplasmic loop and a fourth transmembrane domain (M4). Two pairs of cysteine residues that are conserved in all GluCls and glycine receptors are found in the N-terminal extracellular domain of GluClα2 and a protein kinase C phosphorylation recognition sequence is conserved between GluClα1 and GluClα2.

**GluClα2 Expression—*Xenopus* oocytes injected with GluClα2 cRNA (500 pg/oocyte) responded to both glutamate (1 mM) and IVMPO 4 (1 μM) (Fig. 8A). The glutamate responses were rapid, desensitizing, and reversible while the IVMPO 4 responses were slow and irreversible. The average glutamate response was 5.7 ± 0.5 μA with an EC50 of 208.3 μM and a Hill coefficient of 2.1, indicating that more than 1 glutamate molecule is required to open the channel (Fig. 8A). IVMPO 4 responses of the same oocytes were 21.4 ± 1.8 μA with an EC50 of 107.8 nM and a Hill coefficient of 1.6 (Fig. 8C). Coapplication

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**Fig. 6. Ivermectin binding.** Saturation binding assays of ivermectin to wild-type (A) and GluClα::Tc1 (B) membranes. The average values of four different binding assays for each, mutant and wild-type, were plotted. The corresponding Scatchard analysis for each curve are inserted.

**Fig. 7. GluClα2 predicted amino acid sequence comparisons.** The predicted amino acid sequences of GluClα2, GluClα1, GluClβ, and DrosGluCl were aligned using Pileup and conserved amino acids were boxed using Prettyplot from the GCG package. The putative transmembrane domains M1–4 are underlined (solid lines) and the two putative disulfide bridges are overlined (dashed lines). The protein kinase C phosphorylation recognition sequence found in GluClα1 and GluClα2 are indicated with an inverted filled triangle.
of PTX (100 μM) reduced the average glutamate (1 mM) response to 5.27 ± 0.4 μA indicating a weak PTX block of the GluClα2 homomeric channel. The GluClα2 homomeric channels were insensitive to γ-aminobutyric acid (1 mM), glycine (1 mM), aspartate (1 mM), and kainate (1 mM). Coinjection of *Xenopus* oocytes with GluClα2 and GluClβ (10 and 50 pg, respectively) resulted in heteromeric channel formation that responded to both glutamate (1 mM) and IVMPO4 (1 μM) (Fig. 8A). The kinetics of the glutamate response retained the kinetics attributed to GluClβ, such as reversibility and slow desensitization, but were PTX (100 μM) insensitive. The maximal glutamate response was 3356 ± 341 nA, while the maximal IVMPO4 response was 3574 ± 273 nA. Dose-response curves for ivermectin and glutamate of oocytes coexpressing GluClα2 and β are shown in Fig. 8, B and C. The ivermectin EC50 was calculated to be 103 nM with a Hill coefficient of 1.85 and 62 μM glutamate and 2.4, respectively. The GluClα2 and β heteromeric channels were insensitive to γ-aminobutyric acid (1 mM), glycine (1 mM), aspartate (1 mM), and kainate (1 mM). These experiments indicate that the pharmacological properties of *Xenopus* oocytes injected with GluClα1::Tc1 mRNA could be accounted for by the formation of GluClα2 and β heteromeric channels.

Since GluClα2 is homologous to GluClα1, we tested whether its expression is altered in the GluClα1::Tc1 strain. Northern analysis of mRNA from wild-type and GluClα1::Tc1 revealed two GluClα2 transcripts of approximately 1.7 and 2.3 kb (data not shown). Expression of the two GluClα2 transcripts in the GluClα::Tc1 strain was unaffected (data not shown).

**DISCUSSION**

A comparison of wild-type and mutant organisms can be instrumental in addressing questions about gene function. Despite detailed characterization of the GluClα1 and GluClβ proteins *in vitro*, their *in vivo* function remains unknown (16, 32). We tried to identify previously characterized mutations that map to the GluClα and GluClβ genes by determining their chromosomal localization (Fig. 1). Since such mutations were
not identified, a *C. elegans* strain with an insertion of the Tc1 transposable element in the GluClα1 gene was identified in a frozen transposon mutant bank. The Tc1 insertion occurred in an exon encoding part of the extracellular region of the GluClα1 protein and resulted in truncated GluClα1 transcripts.

Correlation between biological activity, binding affinity to *C. elegans* membranes, and *in vitro* potency of a series of avermectin analogs strongly suggests that GluClα1 and GluClβ represent efficacious ivermectin targets that may have a physiologically essential function (16, 33, 34). However, inactivation of the GluClα1 gene resulted in animals with no obvious phenotype and typical ivermectin sensitivity, suggesting that there could be functional complementarity of GluCl channel subunit genes with similarity to GluClα1. It is also possible that the developmental and spatial expression of GluClα1 is such that interference of ivermectin with its function does not result in a detectable phenotype, or that GluClα1 is expendable. The electrophysiological and biochemical experiments presented demonstrate the existence of ivermectin-sensitive, GluCl channels in addition to GluClα1 in *C. elegans*.

As predicted by the above experiments, a gene (GluClα2) with high degree of homology to GluClα1 was identified in the database. Cloning and expression of a cDNA encoding GluClα2 revealed pharmacological properties similar to GluClα1. However, in contrast to GluClα1, GluClα2 is gated by glutamate. Co-injection of GluClα2 and β in *Xenopus* oocytes resulted in heteromeric channel formation as indicated by the decreased rate of desensitization of the glutamate response; by the PTX insensitivity of the channels formed, and by the shift of the glutamate dose-response curve to lower values (Fig. 8). Interestingly, coexpression of GluClα2 and β reduced the EC50 for glutamate of GluClβ from 380 to 62 μM while coexpression of GluClα1 and β increased it to 1.3 molar (16). This may indicate that the GluClα2 and β subunit combination may be the naturally occurring one. Co-immunoprecipitation and colocalization experiments could prove this association. Our experiments indicate that GluClα2 can account, at least partially, for the ivermectin responses of the GluClα1::Tc1 mRNA, but cannot exclude the existence of additional avermectin-sensitive GluCl channels besides GluClα1 and GluClα2 or that ivermectin acts on other targets in addition to the GluClα1 channels.

The target site involvement in the development of resistance to avermectins is of primary importance. Significant resistance to ivermectin has emerged in the field but target site involvement has not been documented. Four-fold resistant strains of *Haemoncous contortus*, a nematode parasite of sheep, tested for the presence of high affinity ivermectin-binding sites did not indicate an alteration in the *Kd* or *B*max over controls (18, 35). Our data indicate that ivermectin may be acting on multiple related targets. The experiments presented in this paper predict that high level resistance to ivermectin may be rare because of the presence of several avermectin-sensitive GluCl channel subunits, each of which may need to be mutated to confer resistance.

GluCl channels are proteins with interesting pharmacological properties (36). Understanding the physiology of the GluCs can provide insight into mechanisms of anthelmintic and insecticide resistance (37). Furthermore, their study could lead to rational approaches to the discovery of novel anthelmintics and other antiparasitic and insecticidal agents (38). The GluClα1::Tc1 strain, although it does not exhibit an obvious phenotype, could be useful in understanding the physiological function of GluCl channels in *C. elegans* and other invertebrates. Possibly in other genetic backgrounds a phenotype can be detected. Mutations in other GluCl channel subunits such as GluClα2, Cegbr, or GluClβ should help in elucidating the physiological role of the GluCl channels and the action of the avermectin class of compounds.

**Acknowledgments**—We thank Dr. Paul Liberator for discussions, advice, and for the synthesis of numerous oligonucleotides. Dr. Michel Hamelin for help and advice with the backcrosses and critical reading of the manuscript. Drs. Charles Cohen and Wesley Shop for critical reading of the manuscript. Ken Liu and Dr. Reid Leonard in the electrophysiology experiments and Dr. Anna Pomes for critical advice in the binding assay experiments. The transposon insertion mutant was isolated by D. K. V. during a stay in the laboratory of R. H. A. P. and was isolated from a mutant library supported by the National Institutes of Health/NCCR Grant 5 R01 RR0082-02.

**Note Added in Proof**—Dent et al. (Dent, J. A., Davis, M. W., and Avery, L. (1997) EMBO J. 16, 5867–5879) have found that avr-15 encodes GluClα2 and is required for functional M3 synapse.

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