The *Caenorhabditis elegans* unc-63 gene encodes a levamisole-sensitive nicotinic acetylcholine receptor α subunit

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Key Words: *Caenorhabditis elegans*, unc-63, nicotinic acetylcholine receptor, levamisole resistance, cholinergic synaptic transmission, *in vivo* patch-clamp electrophysiology.
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Running Title: Nematode levamisole-sensitive nicotinic receptors

Version: 21 07 04
Summary

The anthelmintic drug, levamisole, causes hypercontraction of body-wall muscles and lethality in nematode worms. In the nematode Caenorhabditis elegans, a genetic screen for levamisole resistance has identified 11 genes, 3 of which (unc-38, unc-29 and lev-1) encode nicotinic acetylcholine receptor (nAChR) subunits. Here we describe the molecular and functional characterization of another levamisole-resistance gene, unc-63, encoding a nAChR α subunit with a predicted amino acid sequence most similar to that of UNC-38. Like UNC-38 and UNC-29, UNC-63 is expressed in body wall muscles. In addition, UNC-63 is expressed in vulval muscles and neurons. We also show that the LEV-1 is expressed in body wall muscle, thus overlapping the cellular localization of UNC-63, UNC-38 and UNC-29 and suggesting possible association in vivo. This is supported by electrophysiological studies on body wall muscle, which demonstrate that a levamisole-sensitive nAChR present at the C. elegans neuromuscular junction requires both UNC-63 and LEV-1 subunits. Thus, at least four subunits, two α types (UNC-38 and UNC-63) and two non-α types (UNC-29 and LEV-1), can contribute to levamisole-sensitive muscle nAChRs in nematodes.
INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) are Cys-loop ligand-gated ion channels (LGICs) formed by 5 polypeptide subunits (1). Typical features of Cys-loop LGICs include an N-terminal extracellular domain where ligand binding occurs, the Cys-loop which is two disulphide-bonded cysteines separated by 13 residues and four transmembrane regions (M1-4), the second of which contains many channel lining residues (2). The nAChR subunits possessing two adjacent cysteines in the acetylcholine (ACh) binding site are referred to as α subunits whereas those with no such motif are referred to as non-α subunits (β, γ, δ and ε). Birds and mammals possess 17 subunits characterized as either ‘muscle’ (α1, β1, γ, δ and ε) or ‘neuronal’ (α2-10, β2-4) subtypes (3,4). Recent analysis of the pufferfish genome has revealed a larger nAChR gene family consisting of 28 subunits that probably arose through genome duplication (5). One of the most extensive and diverse nAChR gene families currently known is that of the nematode *Caenorhabditis elegans* which consists of at least 27 subunits (6).

Early investigations of *C. elegans* mutants identified 11 loci that mediate resistance to the anthelmintic drug, levamisole, which causes paralysis of nematode body wall muscles (7-9). Among the 11 levamisole-resistant loci, it has been shown that *lev-1* and *unc-29* both encode non-α nAChR subunits, whereas *unc-38* encodes a nAChR α subunit (10). Expression in *Xenopus laevis* oocytes of combinations of these subunits that include UNC-38 resulted in small amplitude, dose-dependent, inward currents in response to ACh and levamisole that were suppressed by several nAChR antagonists (10). More recently, electrophysiological studies have shown that UNC-29 and UNC-38 are essential components of the native levamisole-sensitive nAChR at the nematode neuromuscular junction (11).
Four other genes mediating levamisole resistance have been identified. These are: \textit{unc-22}, the product of which is known as twitchin (12); \textit{unc-50} which encodes for a novel transmembrane protein (13); \textit{unc-68} which encodes for a ryanodine receptor (14); \textit{lev-11} which encodes for tropomyosin (15). The characterization of the remaining levamisole resistance loci (\textit{unc-63, unc-74, lev-9} and \textit{lev-10}) have yet to be reported. Here we describe the cloning of a new \textit{nAChR} $\alpha$ subunit and show that it corresponds to the \textit{unc-63} locus (7,8). GFP reporter constructs reveal that UNC-63 is expressed in body wall muscles as well as neurons. Using electrophysiology on muscle preparations, we also show that \textit{unc-63} is necessary for the function of the levamisole-sensitive \textit{nAChR} at the neuromuscular junction.
EXPERIMENTAL PROCEDURES

C. elegans strains and general methods. The handling of C. elegans was performed as described by Sulston and Hodgkin (16). The following strains were used: N2 wild type C. elegans (Bristol variety), ZZ13 unc-63(x13) I, ZZ26 unc-63(x26) I, DH404 unc-63(b404), ZZ37 unc-63(x37) I, ZZ1004 unc-63(x18) dpy-5(e61) I and CB211 lev-1 (e211) IV.

Tests for levamisole sensitivity and locomotor function. The sensitivity to levamisole was assessed on NGM plates containing 1 mM levamisole. Locomotion was assessed by transferring adult hermaphrodites to NGM (nematode growth media) plates without levamisole and after 1 hour counting the number of body bends per minute.

DNA extraction and sequencing. Cloning was carried out using standard methods (17). The C. elegans genomic DNA extraction protocol was obtained from http://www.dartmouth.edu/artsci/bio/ambros/protocols/worm_protocols.html. Plasmid DNA for micro-injection into nematodes was purified using a Qiagen plasmid mini kit (Qiagen, UK). Sequencing was performed at the Babraham Institute or at the Oxford University Biochemistry Department Sequencing Facility.

Cloning jtf#38 cDNA. A mixed-stage N2 cDNA library in λgt10 (provided by S. Kim) was hybridized with an unc-38 probe at 50°C as previously described (10). The final washes were carried out at moderate stringency (65°C in 2xSSC, 0.1 % SDS). Sequence analysis revealed that
one of the positive clones, \textit{jtf\#38}, contained a partial cDNA homologous to nAChR subunits. The 5’ and 3’ ends of the \textit{jtf\#38} coding region was determined using the 5’/3’ RACE kit (Roche).

\textit{Sequence analysis.} Sequence alignment and analysis were performed using the GCG packaging, CLUSTALW. The BLAST alignment tool (18) was used to search the genome database.

\textit{Genetic localization of the \textit{jtf\#38} gene.} The \textit{jtf\#38} cDNA was used to probe an ordered grid of yeast artificial chromosomes (YACs) following the protocol described by Coulson et al. (19).

\textit{Sequencing of mutant alleles.} RT-PCR was performed to amplify \textit{unc-63} and \textit{lev-1} from mutant alleles and the PCR products were sequenced. After a putative mutation was found in the cDNA, the corresponding genomic fragment from the mutants was amplified using single worm PCR (20) and sequenced.

\textit{Germline transformation.} Germ line transformation was performed according to the method of Mello et al. (21).

\textit{Mutant rescue experiment.} We were unable to make a GFP construct consisting of the entire \textit{unc-63} coding region for both rescue of \textit{unc-63} mutants and localization of the UNC-63 subunit. Thus, for mutant rescue studies, we used a construct (\textit{punc-63.1}) that was generated by PCR on wild type genomic DNA using the Expand Long Template System (Roche) and the primers TATTTGGCGGCCGCTCTGACTGCCTATGG and GGAAGAGGTACCATGGCAGAACACGTGATG (engineered restriction sites are underlined).
The PCR product was cloned into the pGEM-T plasmid (Promega) and clones were subsequently checked using restriction analysis and sequencing. The *punc-63.1* construct contained a 12.5 kb insert comprising 4.5 kb of 5’ upstream region, all the genomic coding region and 1kb of downstream 3’ sequence. Germ line transformation was performed by co-injecting the test DNA (80 µg ml⁻¹) and the marker plasmid pPD93.65 (100 µg ml⁻¹). Injection of pPD93.65, which contains the GFP gene under the control of the myosin heavy chain *unc-54* gene promoter, resulted in GFP expression in all body wall muscle cells. Transgenic animals with GFP fluorescence in body wall muscle cells were used for further studies on the phenotype of rescued worms. Three stable lines were obtained.

*Generation of UNC-63::GFP and LEV-1::GFP constructs.* As indicated in Fig. 4A, the UNC-63::GFP fusion construct was made using the expression vector pPD95.70 and a PCR product containing 4.5 kb upstream of the *unc-63* start codon to part of exon 7 (encoding part of the large cytoplasmic loop). The primers used to amplify the *unc-63* genomic fragment were TTTTGGCCCGGGATGTGTGTGTGGGATCG and TATTGGGCTGCTCTGTGACTGCCTATGG. For the LEV-1::GFP construct, 9 kb of genomic DNA including 4 kb upstream of the *lev-1* start codon was amplified using the primers AGCTCCTCTTCCGCACTCG and TTCAGAAAATACCAAGAACTGTGGTTG then cloned into vector pPD95.79. The GFP was fused in-frame with the C-terminus of LEV-1 (Fig. 4A), removing the two C-terminal amino acids of LEV-1. The UNC-63::GFP fusion construct (80 µg ml⁻¹) was co-injected with plasmid pRF4 (100 µg ml⁻¹) into wild type animals. Transgenic animals were selected by their roller phenotype (21). The LEV-1::GFP fusion construct (50-80 µg ml⁻¹) was injected into the *lev-1 (e211)* mutant. Transgenic worms with GFP fluorescence
were selected and animals were viewed by fluorescence microscopy on a Leica DMRE attached to a Leica confocal system TCS.

Electrophysiology. Whole-cell, voltage clamp recordings were obtained from ventral, medial body wall muscles of dissected of *C. elegans* adults, as previously described (11). An EPC-9 patch-clamp amplifier (HEKA, Lambrecht Germany) was deployed to record currents that were digitized at 2.9 kHz using an IT6 interface (Instrutech, Great Neck, New York). Data acquisition was controlled by Pulse software (HEKA) run on a Power Mac 6500/255 computer. Recordings were made at a holding potential of –60 mV. Extracellular saline of the following composition (in mM) was used: 150 NaCl; 5 KCl; 5 CaCl$_2$; 4 MgCl$_2$; 10 glucose; 5 sucrose; 15 HEPES (pH 7.3, ~330 mOsm). The patch pipette was filled with the following solution (in mM): 120 KCl; 20 KOH; 4 MgCl$_2$; 5 (N-tris[Hydroxymethyl] methyl-2-aminoethane-sulfonic acid); 0.25 CaCl$_2$; 4 NaATP; 36 sucrose; 5 EGTA (pH 7.2, ~315 mOsm). Subsequent analysis and graphing were carried out using Pulsefit (HEKA) and Igor Pro (Wavemetrics, Lake Oswego, Oregon). Statistical analysis was performed using the unpaired t-test (for ACh responses) or the Mann Whitney test (for levamisole responses and dihydro-β-erythroidine (DHβE) block). All statistically-derived values are given as mean ± S.E.M.

Chemicals. Unless otherwise indicated all chemicals were obtained from Sigma (UK).
Results

Cloning a novel \textit{C. elegans} nAChR subunit

We employed a cross-hybridization strategy to identify new nAChRs in \textit{C. elegans}. A \textit{C. elegans} cDNA phage library was screened at low stringency using the \textit{unc-38} and \textit{unc-29} cDNAs as probes. Several positive clones were obtained, including one which hybridized specifically at moderate stringency to the \textit{unc-38} cDNA probe. The clone was a partial cDNA whose amino acid sequence showed significant identity with previously characterized nAChR subunits. We utilized RACE PCR to complete the cDNA sequence and the RNA splice leader SL1 (22) was observed at the 5’ end. The full-length cDNA clone was designated \textit{jtf#38} (GenBank accession number AAK83056).

Location of the \textit{jtf#38} clone on the \textit{C. elegans} physical and genetic maps

We mapped the location of \textit{jtf#38} to YACs Y55F5 and Y72D6 on the physical map by hybridizing the cloned cDNA to a YAC grid (Fig. 1A). Y55F5 and Y72D6 are located near the center of chromosome I. During the course of this study, the genomic sequence of the region containing \textit{jtf#38} was completed by the \textit{C. elegans} sequencing consortium (23). We therefore compared the genomic region and \textit{jtf#38} cDNA sequences, and found that \textit{jtf#38} corresponds to the predicted gene Y110A7A.3 which encodes a putative nAChR subunit. The gene is composed of 10 exons spanning 7.5 kb (Fig. 1B). Because Y110A7A.3 lies close to the levamisole-resistant locus \textit{unc-63}, we investigated whether Y110A7A.3 corresponds to \textit{unc-63}.
Y110A7A.3 is the unc-63 locus.

To test whether Y110A7A.3 corresponds to unc-63, we first sequenced Y110A7A.3 genomic DNA in five unc-63 mutant alleles. All mutant alleles were isolated using EMS mutagenesis (7). The unc-63 mutants exhibit a range of phenotypes from strongly uncoordinated locomotion with strong levamisole resistance (alleles x37, x13, x18) to almost wild type locomotion with mild levamisole resistance (alleles b404, x26). We found 1 missense mutation (x26), one nonsense mutation (x13), one deletion (b404) and 2 mutations (x37 and x18) that disrupt splice junction consensus sites (Fig. 1B). These results provide strong evidence that unc-63 corresponds to the nAChR subunit encoded by Y110A7A.3.

In addition, we rescued the unc-63 uncoordinated phenotype by injecting the construct punc-63.1 into the syncitial gonad of young adult unc-63(x37) animals. Three independently transformed lines carrying the extrachromosomal array of unc-63 (LM200, unc-63(x37);Ex unc-63) were obtained, each of which showed restored sensitivity to levamisole and phenotypically wild-type locomotion. We analyzed the phenotype of one rescued mutant by comparing speed of locomotion of strains N2 (wild-type), unc-63(x37);Ex unc-63 and unc-63(x37). The locomotion characteristics were as follows (bends/min ± standard deviation, n = number of animals): wild-type (24 ± 2, n = 30), unc-63(x37);Ex unc-63 (21 ± 2, n = 49) and unc-63(x37) (10 ± 1, n = 20). Together with the sequences of 5 unc-63 mutant alleles, these results show that the jtf#38 cDNA clone we isolated corresponds to the unc-63 gene.

Fig. 2 here

Features of the UNC-63 polypeptide

As shown in Fig. 2, UNC-63 consists of 502 amino acids and possesses motifs common to Cys-loop LGICs, including an N-terminal signal peptide of 23 amino acids (24), 4
transmembrane regions and the Cys-loop (1). Also present are conserved stretches of amino acids in loops A-F which are involved in ligand binding. In loop C, there are two adjacent cysteines, defining UNC-63 as a nAChR α subunit. Using the GCG MOTIFS program, we identified one putative glycosylation site at position N136 and potential phosphorylation sites within the large M3-M4 intracellular loop (Fig. 2). A phylogenetic tree of C. elegans nAChR subunits based on derived sequence identity is shown in Figure 3. As indicated in the tree, UNC-63 is most similar to UNC-38 sharing 49 % identity. However, it is interesting to note that in loop C of UNC-63 the typical YxCC motif is present rather than the unusual YxxCC motif found in loop C of UNC-38 (6).

The unc-63 gene is expressed in both muscle cells and neurons

To determine the tissue-specific localization of unc-63, we injected N2 animals with a genomic fragment of unc-63 fused to DNA encoding GFP (Green Fluorescent Protein (25)) as shown in Fig. 4A. In transgenic animals expressing the UNC-63::GFP fusion construct, fluorescence was observed in all body wall muscles and in vulval muscles (Fig. 4). We also detected expression in many cells of the nervous system, including motor neurons in the ventral nerve cord (AS, DA, DB, VC, VA, VB and VD types) and neurons in head, posterior lateral, pre-anal and lumbar ganglia. The expression of unc-63 in both body wall muscles and motor neurons is consistent with locomotion defects observed for unc-63 mutants.

LEV-1 is a possible partner for the UNC-63 α subunit in a native nAChR

The subunits, UNC-38, UNC-29 and LEV-1, are also associated with levamisole resistance in C. elegans. Both UNC-29 (10) and UNC-38 (11) are expressed in the body wall muscles of C.
Culetto et al.

elegans, overlapping at least in part with the UNC-63 expression pattern. This raises the possibility that UNC-63, UNC-38 and UNC-29 subunits may co-assemble to form a native levamisole-sensitive receptor. To test whether LEV-1 might also be a component of this body wall muscle receptor, we determined the expression pattern of LEV-1. We made a gene fusion construct (LEV-1::GFP) with 4 kb of the lev-1 5’ genomic sequence and the entire lev-1 genomic coding sequence fused at the C-terminus to GFP (Fig. 4A). The transgene was then injected into the recessive lev-1 (e211) mutant. The lev-1(e211) allele contains a missense mutation (G461E) located in the M4 region and exhibits normal locomotion in the absence of levamisole, becoming uncoordinated (but not hypercontracted as with wild-type) in the presence of 1 mM levamisole. Injected LEV-1::GFP restored levamisole sensitivity to the lev-1 mutant, demonstrating that the transgene rescued lev-1(e211) and, as shown in Fig. 4 (I-J), GFP expression was observed in all body wall muscle cells and in a subset of motor neurons in the ventral nerve cord. Thus LEV-1 has overlapping expression with UNC-63 as well as UNC-38 and UNC-29.

**UNC-63 and LEV-1 are part of the levamisole-sensitive acetylcholine receptor**

Two pharmacological classes of acetylcholine receptors function on muscles at *C. elegans* neuromuscular junctions. One is specifically activated by levamisole, whereas the other is activated by nicotine and inhibited by DHβE (11). The levamisole-sensitive current recorded from body wall muscle requires the function of unc-38 and unc-29 (11). We wanted to test whether unc-63 and lev-1 are also required for functional levamisole-sensitive, muscle nAChRs. Therefore the electrophysiological properties of the *C. elegans* body wall muscle nAChRs were examined in wild type, unc-63 and lev-1 mutant animals. As observed previously (11), muscles of wild type worms responded to 100 µM ACh by generating inward currents with rapid onset
and decay, and the same cells responded to 100 μM levamisole with similar currents but with slower onset and decay. As shown in Fig. 5A, the response to 100 μM levamisole was almost completely abolished for *unc-63(x37)* and dramatically reduced to only 14% of that observed in the wild type in *lev-1(e211)*. We then tested whether the second nAChR type was still present in both mutants by pressure-application of ACh onto voltage-clamped body wall muscle cells of *unc-63(x37)* and *lev-1(e211)* mutants (Fig. 5B). In both mutants, ACh elicited a robust inward current although the responses were smaller than the wild type. This reduction in response to ACh is consistent with loss of levamisole receptor contribution to the total ACh response in *unc-63(x37)* and *lev-1(e211)*. We also measured the effects of DHβE on the ACh-elicited responses (Fig. 5B). In wild type worms the inward current was blocked by 84%, whereas in *lev-1(e211)* there was an 89% block and an almost complete block of 95% was observed in *unc-63(x37)*, consistent with loss of the second muscle nAChR type.

Overall, these data suggest that levamisole-sensitive receptors in body wall muscle require the functional expression of UNC-63 and LEV-1 in addition to UNC-29 and UNC-38.
DISCUSSION

Levamisole is a potent anthelmintic drug, which is used to eradicate nematode infestations in a number of domestic animals (26). Application of levamisole to wild-type *C. elegans* causes muscle hypercontraction, paralysis and ultimately death, although the exact cause of death is unknown. Through the analysis of levamisole-resistant mutants we can begin to understand the actions of levamisole by identifying targets within the worm that are required for levamisole sensitivity. Previous studies suggest that the molecular targets of levamisole include nAChRs at the *C. elegans* neuromuscular junction (8,9,11). Application of levamisole directly onto voltage-clamped *C. elegans* body wall muscles produces inward currents that are abolished in mutants encoding the nAChR subunits UNC-38 and UNC-29 (11). This observation is consistent with the muscle expression of these two subunits and can explain the observed muscle hypercontraction of worms exposed to levamisole.

Here we report the identification of a novel nAChR subunit in *C. elegans* which shows greatest sequence similarity with UNC-38 and physically maps in the vicinity of *unc-63*, one of 11 loci identified in genetic screens for mutants that confer levamisole resistance (7). Sequence analysis of 5 *unc-63* alleles revealed mutations in the predicted open reading frame of the nAChR clone, indicating that *unc-63* encodes the novel nAChR. This was confirmed by demonstrating that the strong levamisole resistance allele, *unc-63(x37)*, could be behaviorally rescued by expressing an extrachromosomal array of the wild type nAChR cDNA clone. Using an UNC-63::GFP fusion construct, we show that UNC-63 is present in muscles. Patch-clamp electrophysiology was deployed to show that muscle responses to direct applications of levamisole are virtually eliminated in the *unc-63(x37)* allele, indicating that UNC-63 is an essential component of a levamisole receptor.
We have also demonstrated that LEV-1, in addition to UNC-63, UNC-38 and UNC-29, is expressed in body wall muscles of *C. elegans*. The *lev-1(e211)* mutant worms are resistant to levamisole although their locomotion appears normal. Electrophysiological analysis of the *lev-1(e211)* mutant animals indicated that 86% of the levamisole-sensitive current was abolished (Fig. 5A). Since the *lev-1(e211)* produces a missense mutation, the residual levamisole response may reflect only a partial loss-of-function of the LEV-1 subunit or the presence of a poison subunit that reduces the efficacy of the heteromeric receptor. Alternatively, the LEV-1 subunit may be less essential, possibly being replaced by other non-α subunits such as UNC-29.

We also show that UNC-63 and LEV-1 subunits, like UNC-29 and UNC-38, are expressed in a subset of *C. elegans* neurons. This suggests that these subunits participate in neuronal excitability and these may represent additional targets for levamisole. Other nAChR subunits have overlapping expression with UNC-63 in several identified neurons such as ACR-5 (DB, VB) (27) and ACR-2 (DA, DB, VA, VB) (28). The composition of the nAChR receptors in these neuronal locations and their potential roles in levamisole sensitivity will require further analysis.

Thus, we have identified a fourth nAChR subunit that is a constituent of nematode levamisole-sensitive receptors and we have established that LEV-1 contributes to the normal functioning of this receptor. It remains to be determined whether there is either a muscle levamisole-sensitive nAChR composed of UNC-38, UNC-63, UNC-29 and LEV-1, or perhaps more than one receptor each made from various combinations of the four subunits. It is clear however, that UNC-38, UNC-63 and UNC-29 are required subunits of all functional levamisole receptors on the medial body muscles. It is worth noting that, in terms of sequence identity, UNC-38 and UNC-63 are most closely related with the α subunit ACR-6 whilst UNC-29 and
LEV-1 are most similar to the non-α subunits ACR-2 and ACR-3 (Fig. 3). Whereas ACR-6 has yet to be characterized, ACR-2 or ACR-3 coexpress with UNC-38 in *Xenopus* oocytes to form functional ion channels upon which levamisole acts as an agonist (29,30). Indeed, ACR-2 was shown to be expressed in a number of ventral cholinergic motor neurons (28), overlapping with the neuronal expression of UNC-63. It would thus be of interest to determine the expression patterns of ACR-3 and ACR-6 as a first step in evaluating their potential as further components of neuronal levamisole-sensitive nAChRs. Studies of these subunits as well as other levamisole-resistant loci may prove instructive in understanding the molecular mechanisms of drug action and developing improved parasite-control agents as well as investigating mechanisms of drug resistance.
ACKNOWLEDGMENTS

The authors acknowledge the support of The Medical Research Council (EC, HAB and DBS), the Biotechnology and Biosciences Research Council (BBSRC) (EC, DBS). Nematode strains used in this work were provided by the *Caenorhabditis* Genetic Center (University of Minnesota), which is funded by the NIH National Center for research resources (NCRR). We acknowledge with thanks Dr A. Fire for providing pPD plasmids. We thank Dr A. Coulson (Sanger Centre UK) for providing the YAC grid. We also thank B. Esmaeili, E. B. Maryon and D. E. Featherstone for critically reading the manuscript.
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Figure Legends

Figure 1.
Cloning of unc-63. (A) Chromosomal localization of the *C. elegans* jtf#38 cDNA. Jtf#38 was mapped on a YAC grid to YAC Y55F5 and Y72D6, spanning the unc-63 locus on chromosome I. (B) The genomic organization of the unc-63 gene is shown with location of identified changes found in unc-63 mutant alleles.

Figure 2.
Deduced amino acid sequence of UNC-63. The protein alignment, also including UNC-38, LEV-1, UNC-29 and the human muscle $\alpha$ nAChR subunit, was constructed using the ClustalX algorithm (31) and shown using the GeneDoc program (http://www.psc.edu/biomed/genedoc). Amino acids are numbered beginning at the first methionine. Amino acids of loops A-F contributing to the ACh binding domain are indicated as well as the four transmembrane regions (M1-M4). The potential N-glycosylation site of UNC-63 is indicated by # and putative phosphorylation sites within the large intracellular loop of UNC-63 are shown by * which include calmodulin-dependent protein kinase (CaMKII) sites (T352, T396 and S432) and a protein kinase C site (S418).

Figure 3.
Phylogenetic tree showing the relationship of UNC-63 with other members of the *C. elegans* nAChR subunit family. The tree was constructed using Clustal X (31) and displayed using the TreeView application (32). Numbers at each fork show bootstrap values with 1000 replicates and
the scale bar represents substitutions per site. The *C. elegans* ionotropic GABA receptor subunit, UNC-49B (33), was chosen as the outgroup. The *C. elegans* nAChR subunits are classified in five major subgroups; DEG-3-like, ACR-16-like, UNC-38-like, ACR-8-like and UNC-29-like (34). UNC-38, UNC-63, UNC-29 and LEV-1 are highlighted in bold.

**Figure 4.**

Expression pattern of the UNC-63::GFP and the LEV-1::GFP constructs. (A) Structure of the injected GFP constructs. (B) Composite image of L3/L4 transgenic larvae stably transformed with UNC-63::GFP. The GFP signal is observed in a large number of neurons in the lumbar ganglia (lg), pre-anal ganglia (pag), head ganglia (hg) and ventral nerve cord (arrows indicate motor neuron cell bodies), scale bar 50 µm. (C) UNC-63::GFP signal is shown in a subset of neurons in the anal ganglia. No expression is observed either in the sphincter muscle cell or in the anal depressor muscle, scale bar 5 µm. (D) Dorsal view of a young adult animal. All the body wall muscle cells, visible as large trapezoidal cells (example indicated by arrow), express UNC-63::GFP. A dark strip separates the left and right quadrants, scale bar 50 µm. (E) Four neurons of the posterior lateral ganglion express UNC-63::GFP, bar represents 5 µm. (F) The UNC-63::GFP signal is expressed in some of the vulval muscle cells indicated by arrows, scale bar 10 µm. (G) The LEV-1::GFP construct was injected into the *lev-1* mutant (allele e211). GFP expression is observed from embryo to the adult stage where signal is found in a subset of neurons in the ventral nerve cord indicated by arrows. (H) LEV-1::GFP expression in body wall muscles, scale bar 50 µm.
Figure 5.

unc-63(x37) and lev-1(e211) mutants showed reduced amplitude levamisole-induced currents in body wall muscles of *C. elegans*. (A) Body wall muscles produce an inward current in response to a 100 ms pressure-ejected pulse of levamisole. The levamisole response is almost completely abolished in the unc-63 mutant and is largely reduced in the lev-1 mutant (wild type 279 ± 28 pA n=5; unc-63(x37) 2.7 ± 5.8 pA n=3; lev-1(e211) 39 ± 7.4 pA n=4) as shown in typical traces and a histogram with averaged amplitudes. (B) Pressure-ejection of ACh (100ms pulses) produces a robust inward current in wild-type body wall muscles. This current represents the activation of both the levamisole-sensitive current and the levamisole-insensitive current. The ACh response was significantly reduced in unc-63 and lev-1 mutants as reflected in the example traces and histograms (wild type 1963 ± 156 pA n=7, unc-63(x37); 1356 ± 142 pA n=7, lev-1 (e211); 1559 ± 156 pA n=8). Blocking the levamisole-insensitive current with DHβE almost completely abolished the ACh response in unc-63 mutants and markedly reduced the response in lev-1 mutants and wild type worms (wild type 319 ± 45 pA n=9, unc-63(x37); 66 ± 19 pA n=6, lev-1 (e211); 154 ± 48 pA n=6). Values in histograms statistically different from wild type are marked with an asterisk.
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*J. Biol. Chem.* published online July 27, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M404370200

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