A Na⁺/Cl⁻-coupled GABA Transporter, GAT-1, from Caenorhabditis elegans

STRUCTURAL AND FUNCTIONAL FEATURES, SPECIFIC EXPRESSION IN GABA-ERGIC NEURONS, AND INVOLVEMENT IN MUSCLE FUNCTION

Received for publication, July 26, 2004, and in revised form, November 8, 2004
Published, JBC Papers in Press, November 12, 2004, DOI 10.1074/jbc.M408470200

Guoliang Jiang‡, Lina Zhuang‡, Seiji Miyauchi¶, Katsuya Miyake§, You-Jun Fei‡, and Vadivel Ganapathy††

From the ‡Department of Biochemistry and Molecular Biology and ¶Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, Georgia 30912

GABA functions as an inhibitory neurotransmitter in body muscles and as an excitatory neurotransmitter in enteric muscles in Caenorhabditis elegans. Whereas many of the components of the GABA-ergic neurotransmission in this organism have been identified at the molecular and functional levels, no transporter specific for this neurotransmitter had been identified to date. Here we report on the cloning and functional characterization of a GABA transporter from C. elegans (ceGAT-1) and on the functional relevance of the transporter to the biology of body muscles and enteric muscles. ceGAT-1 is encoded by snf-11 gene, a member of the sodium-dependent neurotransmitter symporter gene family in C. elegans. The cloned ceGAT-1 functions as a Na⁺/Cl⁻-coupled high-affinity transporter selective for GABA with a Kᵣ of ~15 μM. The Na⁺:Cl⁻:GABA stoichiometry for ceGAT-1-mediated transport process is 2:1:1. The transport process is electrogenic as evidenced from GABA-induced inward currents in Xenopus laevis oocytes that express ceGAT-1 heterologously. The transporter is expressed exclusively in GABA-ergic neurons and in two other additional neurons. We also investigated the functional relevance of ceGAT-1 to the biology of body muscles and enteric muscles by ceGAT-1-specific RNA interference (RNAi) in rrf-3 mutant, a strain of C. elegans in which neurons are not refractory to RNAi as in the wild type strain. The down-regulation of ceGAT-1 by RNAi leads to an interesting phenotype associated with altered function of body muscles (as evident from changes in thrashing frequency) and enteric muscles (as evident from the rates of defecation failure) and also with altered sensitivity to aldicarb-induced paralysis. These findings provide unequivocal evidence for a modulatory role of GABA and ceGAT-1 in the biology of cholinergic neurons and in the function of body muscles and enteric muscles in this organism.

γ-Aminobutyric acid (GABA)1 is the most abundant inhibitory neurotransmitter in vertebrates and invertebrates. GABA-ergic neurotransmission is used clinically as the target of antiepileptic, anxiolytic, and antispasmodic drugs (1–5). Several molecular components are involved in GABA-ergic neurotransmission. These include glutamic acid decarboxylase (the enzyme needed for the production of GABA from glutamate), vesicular GABA transporter (the transporter in presynaptic neurons that transports GABA into synaptic vesicles), GABA receptors (the receptors present on the postsynaptic membrane to transmit the GABAergic signals), and GABA transporters (the transporters present in presynaptic neurons and glial cells that participate in the clearance of GABA from the synapse). Mammals, including humans, express four different GABA transporters (GATs) of which only two are present specifically in GABA-ergic neurons. These are GAT-1 and GAT-3 (6, 7). GAT-1 is the predominant GABA transporter in the brain, co-localizes with markers of GABAergic neurons, and is expressed in the axons and presynaptic membranes (8–10). GAT-3 is also expressed exclusively in the brain where it is found in GABA-ergic nerve terminals as well as in glial cells (11, 12). The other two GABA transporters (GAT-2 and BGT-1/GAT-4, also known as betaine/GABA transporter) are not related to GABA-ergic neurotransmission. Antiepileptic, anxiolytic, and antispasmodic drugs produce their therapeutic effects by enhancing GABAergic neurotransmission (1–5). The activation of GABAergic neurotransmission can be accomplished by drugs that function either as agonists of GABA receptors or inhibitors of GABA transporters. The latter class of drugs such as titanium (an antiepileptic drug) increases the levels of GABA in the synapse via the ability to block the clearance of the neurotransmitter from the synapse (5). Even though GAT-1 and GAT-3 have been identified at the molecular level more than a decade ago and are known to be linked to diseases such as epilepsy and schizophrenia (6, 7), there are no knock-out animal models reported in the literature describing the phenotypic changes that occur as a consequence of specific deletion of either GAT-1 or GAT-3.

GABA-ergic neurotransmission plays a critical role in the function of body muscles and enteric muscles in the nematode Caenorhabditis elegans (13). There are 26 GABA-ergic neurons in this organism as identified by immunocytochemistry using a polyclonal antibody specific for GABA (14). These consist of the 6 DD neurons, 13VD neurons, 4 RME neurons, 1 AVL neuron, transcription; GFP, green fluorescent protein; HRPE, human retinal pigment epithelial; NMDG, N-methyl-D-glucamine; dsRNA, double-stranded RNA; cRNA, complementary RNA; snf, sodium-dependent neurotransmitter symporter family; BGT, betaine/GABA transporter; unc, uncoordinated.

* This work was supported in part by the National Institutes of Health Grants HD44404, HL64196, AI49849 (to V. G.), and AG22468 (to Y.-J. F.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY529124.

† To whom correspondence should be addressed. E-mail: vganapat@mail.mcg.edu.

¶ The abbreviations used are: GABA, γ-aminobutyric acid; ceGAT-1, C. elegans GABA transporter-1; RNAi, RNA interference; RT, reverse

From the Department of Biochemistry and Molecular Biology and Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, Georgia 30912.
1 DVB neuron, and 1 RIS neuron. The connectivity of all of these neurons that use GABA as the neurotransmitter has been well established (14). The DD and VD neurons control the function of body muscles that are involved in locomotion. The RME neurons innervate the muscle in the head that participates in foraging. The AVL and DVB neurons control the function of enteric muscles that participate, together with body muscles, in the defecation cycle. The function of the RIS neuron is not well understood. The molecular components of GABA-ergic neurotransmission in C. elegans are similar to those in mammals. These include glutamate acid decarboxylase (known as unc-25), vesicular GABA transporter (known as unc-47), and a GABA receptor (known as unc-49) (13, 15). Two other proteins have also been shown to be essential for normal GABA-ergic neurotransmission in C. elegans (i.e. unc-30 that participates in the differentiation of GABA-ergic neurons and unc-46 that is essential for synaptic release of GABA) (13, 15). unc-25, unc-30, unc-46, unc-47, and unc-49 are members of the unc-ordinated gene class. All of the members of this gene class, when made nonfunctional, lead to a loss of coordination of muscle function in the organism. The C. elegans counterpart(s) of mammalian GABA transporters has/have not yet been identified at the molecular level.

GABA transporters in mammals belong to the SLC6 gene family, which consists of at least 19 members (7). Most of the transporters in this gene family recognize neurotransmitters/neuromodulators (e.g., GABA, serotonin, norepinephrine, dopamine, glycine, proline, and taurine) as substrates and are energetically coupled to the transmembrane gradients of Na+ and Cl−. Therefore, this family is called Na+/Cl−-dependent neurotransmitter transporter gene family. C. elegans also contains a sodium-dependent neurotransmitter symporter family (snf). A search in the WormBase (Release WS93 at www.wormbase.org) using the mammalian GABA transporter protein sequence as a query revealed that there are 14 members in this snf gene family (snf-1, snf-2, and so forth) in the worm genome. Only two of them have so far been identified at the functional level. dat-1 (T23G5.5) encodes a dopamine transporter (16) and mod-5 (Y54E10BR.7) encodes a serotonin transporter (17). Recently, we undertook a study to identify the synergic transporter in C. elegans. When we searched the database for the snf gene family with the mammalian synergetic transporter as a query to identify the most likely candidate for the C. elegans symporter transporter, the search yielded snf-11 as the likely candidate based on the predicted amino acid sequence. Therefore, we isolated a full-length snf-11 cDNA clone from a C. elegans cDNA library and characterized its transport function, hoping that this clone would function as a Na+/Cl−-coupled transporter for taurine. Quite unexpectedly, these studies showed that snf-11 is not a taurine transporter but a GABA transporter. This represents the first GABA transporter to be identified at the functional level in C. elegans. In this paper, we describe the structural and functional aspects of this GABA transporter (ceGAT-1). In addition, we show here that ceGAT-1 is expressed in all of the GABA-ergic neurons (plus in two non-GABAergic neurons) and that down-regulation of GAT-1 expression by gene-specific RNA interference (RNAi) leads to changes in the function of body muscles and enteric muscles. Furthermore, RNAi-induced down-regulation of GAT-1 also leads to an interesting pharmacologic phenotype with altered cholinergic neurotransmission as evident from enhanced sensitivity to aldicarb (an inhibitor of acetylcholineesterase)-induced paralysis. To our knowledge, this represents the first report on the consequences of down-regulation of a GABA transporter in an animal model.

EXPERIMENTAL PROCEDURES

Materials—[3H]GABA (specific activity, 70 Ci/mmol) was purchased from PerkinElmer Life Sciences. The human retinal pigment epithelial (HRPE) cell line, used routinely in our laboratory for heterologous expression of cloned transporters, was maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Lipofectin was purchased from Invitrogen. Restriction enzymes were obtained from New England Biolabs (Beverly, MA). Magna monolayer transfer membranes used in library screening were purchased from Micron Separations (Westboro, MA). Unlabeled GABA and its analogs and other amino acids were obtained from Sigma. A wild type nematode strain, C. elegans N2 ( Bristol), was used as the template in the restriction protocol. The nematodes were cleaned by sedimentation through 15% (w/v) Ficoll 400 in 0.1 x NaCl. The pellet was then used for total RNA preparation. Total RNA was isolated using the TRIzol reagent (Invitrogen). Poly(A)+ mRNA was purified by affinity chromatography using oligo(dT)-cellulose.

Reverse Transcription (RT)-PCR and Hybridization Probe Preparation—Our original aim was to clone the C. elegans taurine transporter. A search in the WormBase using the mammalian taurine transporter protein sequences as queries revealed that the gene T03F7.1 (GenBankTM accession number Z74041) located on chromosome IV encodes a candidate plasma membrane transporter that may function as the sodium/chloride-coupled taurine transporter. Therefore, a pair of PCR primers specific for this gene was designed based on the sequence of the cosmid T03F7.1, 5′-GCC CTT TCC CTT CTC GTG-3′ (forward primer) and 5′-GCA AAA ACC CAG CAA AAC TTC-3′ (reverse primer). These primers yielded a single DNA fragment in RT-PCR using the total RNA isolated from C. elegans as the template in the reaction. The size of the product was ~0.9 kb, which agreed with the predicted size based on the distance between the two primers in the theoretically derived gene transcript. The RT-PCR product was gel-purified and subcloned into pGEM-Teasy vector (Promega Madison, WI). The molecular identity of the insert was established by DNA sequencing. This DNA fragment was used as a probe to screen a C. elegans cDNA library.

Construction of a Directional C. elegans cDNA Library—SuperScript plasmid system from Invitrogen was used to establish the cDNA library using the poly(A)+ RNA from C. elegans. The transformation of the ligated cDNA into Escherichia coli was performed by electroporation using ElectroMAX DH10B competent cells. The bacteria plating, the filter lifting, the DNA fragment labeling, and the hybridization methods (Southern blotting, routine procedures) were carried out as described previously (19–24). Total RNA isolated from the muscle strain, C. elegans (e156), and the Caenorhabditis Genetics Center (St. Paul, MN). Nematode Culture and RNA Preparation—Nematode culture was carried out using a standard procedure with a large-scale liquid culti-
In Vitro Transcription, Oocyte Expression, and Electrophysiological Studies—ceGAT-1 was expressed heterologously in Xenopus laevis oocytes by microinjection of ceGAT-1 cRNA. The procedures for in vitro transcription, oocyte isolation, and microinjection have been described previously (19–22). The plasmid ceGAT-1 cDNA (constructed in pSPORT vector) was linearized with NotI and transcribed in vitro to cRNA using the mMESSAGE mACHINE RNA transcription kit (Ambion, Austin, TX). The expression of ceGAT-1 was initially detected by comparing the uptake of [%3H]GABA in uninjected oocytes and ceGAT-1 cRNA-injected oocytes. The electrophysiological characteristics of the heterologously expressed ceGAT-1 were then studied as described previously (19–22).

Analysis of Tissue-specific Expression Pattern of ceGAT-1—To study the tissue-specific expression pattern of the cegat-1 gene in C. elegans, a transcriptional cegat-1::gfp fusion gene was constructed and transgenic animals expressing these transgenes were established as described previously (19–22). The GFP expression vector pPD117.01 was kindly provided by Dr. A. Fire (Carnegie Institute, Baltimore, MD). The cosmid T03F7.1 containing the cegat-1 gene and its promoter was obtained from the Sanger Center (Cambridge, United Kingdom). A DNA fragment containing the cegat-1 gene promoter region was generated by PCR using this cosmid DNA as the template. The sense primer (coordinates in cosmid T03F7.1 are 3,896–5,925) with an XbaI adapter attached was 5′-TCT ACT TGC GAT CCA GAG GAG 3′, and the reverse primer (coordinates in cosmid T03F7.1 are 5,609–5,637) with a BamHI adapter attached to the 5′-end was 5′-GGA TCC CCT GCA GGA GAT TCC TGT ATA CAA-3′. A recombinant KlenTag DNA polymerase (AB Peptides, St. Louis, MO) was used for the long-range PCR. The size of the expected PCR product is ~2.0 kb. The amplified region contains the putative promoter for the cegat-1 gene and was digested with XbaI and BamHI, and inserted into the expression vector for transgenic animals by microinjection, leading to their characteristic rolling behavior and cultured individually to establish transformed lines. GFP expression pattern was determined by fluorescence microscopy (19–22). F2 rollers with extrachromosome arrays were selected for fluorescence microscopy to determine the GFP expression pattern. Cell identification was validated by their morphological characteristics in combination with relative positions of the GFP-positive cells in the same animals using differential interference contrast image.

Bacteria-mediated RNAi—A fragment of the coding region of ceGAT-1 cDNA was generated by EcoRI digestion and subcloned into a “double T7” plasmid pPD129.36 (kindly provided by Dr. A. Fire, Carnegie Institute), which was subsequently transformed into HT115(DE3) cells. The induction of HT115 cells harboring the double T7 plasmid to express the bacterial RNAi machinery was carried out as previously described (21, 22). To serve as a negative control for the bacteria-mediated RNAi in assessment of the influence of ceGAT-1 on animal behavior, we included an HT115 strain containing the empty vector alone. RNAi experiments were carried out using the normal strain N2 as well as the RNAi-hypersensitive rrf-3 strain. Three mutant strains, unc-23 (e156), unc-47 (e507), and unc-2 (e55) (15, 27–29) were also used as references for animal phenotypes associated with defective GABA-ergic neurotransmission.

Assay of Aldicarb-induced Paralysis—This was done according to the procedure described by Mathews et al. (30). Control and experimental (RNAi) worms (N2 and rrf-3 strains) (20–25 worms/experiment) were exposed to 0.5 mM aldicarb. Paralyzed worms were identified by a lack of response in terms of movement upon poking with a metal wire. The number of paralyzed worms was counted at different time periods following exposure to aldicarb. The experiment was repeated three times, and the data were analyzed as the percent of animals that were paralyzed at each time point. In the experiment in which the relevance of the abolition of GABA-ergic neurotransmission to aldicarb-induced paralysis was investigated using mutant strains, the worms (control strain and cegat-1 transgenic strain) were exposed to 0.5 mM aldicarb without the bacteria-mediated RNAi and the percent of paralyzed worms was determined at different time points following the exposure to aldicarb. This experiment was repeated twice.

Thrashing Assay—This assay was used to investigate the frequency of contraction of body muscles in worms subjected to RNAi. These experiments were done using the RNAi-hypersensitive rrf-3 strain. Control and experimental (RNAi) worms were synchronously grown to the L4 stage. Individual worms were transferred into a microtiter well containing 60 μL of M9 buffer on top of agar. After a 2-min recovery period from the picking procedure, thrashes were counted for 2 min. A thresh is defined as a change in the direction of bending in the middle of the body (26, 30, 31). Eight worms were examined in each group. The experiment was repeated twice. Data are given as frequency of thrashing/2 min.

Defecation Failure Assay—This assay was used to investigate the function of enteric muscles. These experiments were also done using the RNAi-hypersensitive rrf-3 strain. For the analysis of expulsion of intestinal contents during the defecation cycle, synchronously grown control and experimental (RNAi) worms were examined with a dissecting microscope (×50 magnification) for the presence or absence of an expulsion of intestinal contents through the anus after each contraction of the posterior muscles of the body (26, 30–32). 10 worms were examined in each group, and each animal was observed for 12 consecutive defecation cycles. The experiment was repeated twice. Data are given as the percent of events in which there was no discharge of intestinal contents, but defecation cycle occurred as determined by posterior muscle contraction.

Analysis of Steady-state Levels of ceGAT-1 mRNA in Control and RNAi Worms—The efficiency of RNAi-induced down-regulation of ceGAT-1 expression was evaluated by monitoring the steady-state levels of ceGAT-1 mRNA in control and RNAi worms. Experiments were done using the RNAi-hypersensitive rrf-3 strain. The RNAi-hypersensitive rrf-3 strain was used in these experiments. Total RNA was isolated from control and experimental (RNAi) worms, and ceGAT-1 mRNA levels were measured by semiquantitative RT-PCR. Because RNAi was induced with a dsRNA representing the 5′-half of ceGAT-1 cDNA, RT-PCR primers were designed based on the sequence in the 3′-half of the cDNA. The sense and antisense primers were 5′-GAA GCC GTA GGC ATC CTA TCT C-3′ and 5′-GCAGATAAAGTGGCCCATC-3′, respectively. Semiquantitative RT-PCR was carried out as described previously (33) using a commercially available kit (Ambion) following manufacturer’s protocol. Universal 18 S RNA primers were used as an internal control. The RT-PCR products were separated on an agarose gel, and the bands were visualized with ethidium bromide. The signals were quantified using STORM phosphorimager (Molecular Dynamics) or were processed using ImageQuant (version 4.2a) software application. The experiment was repeated twice with separate sets of control and RNAi worms, and RT-PCR experiments were repeated three times with each set of RNA samples. Data are expressed as ceGAT-1 mRNA/18 S RNA ratio.

RESULTS

Molecular Cloning and Structural Characterization of ceGAT-1—We isolated a full-length cDNA clone coded by the gene T03F7.1, which we thought of as a likely candidate for the Na+/Cl− coupled taurine transporter in C. elegans based on the similarity in amino acid sequence. However, functional studies with this cDNA clone in heterologous expression systems revealed that it was a Na+/Cl− cotransporter for GABA rather than taurine (see below). Therefore, we named this transporter cegat-1 because it represented the first GABA transporter to be identified at the molecular and functional levels in C. elegans. The gene coding for this transporter is localized on chromosome V. The cegat-1 gene was originally designated as snf-11 (sodium-dependent neurotransmitter sympporter family member 11). Its size is at least ~3.1 kb, excluding the putative promoter region. The gene consists of six exons as deduced by a comparison between the sequence of the cloned cDNA and that of the GenBank accession number Z74041 for T03F7.1 from the nematode genome sequence project (WormBase release WS93). The structural organization of the gene is shown in Fig. 1A. The ceGAT-1 cDNA is 2,162 bp long and contains a poly(A) tail. The 5′- and the 3′-untranslated regions are 27- and 302-bp long, respectively. The ceGAT-1 protein deduced from the cDNA sequence contains 610 amino acids with an estimated molecular size of 69.3 kDa and an isoelectric point of 6.38 (Fig. 1B). According to the Kyte-Doolittle hydrophathy plot, the ceGAT-1 protein possesses 12 putative transmembrane domains. The sequences of ceGAT-1 cDNA and its encoded protein have been deposited into the GenBank (accession number AY529124). A pairwise compar-
Relevance of GABA Transporter to Muscle Function in C. elegans

FIG. 1. Organization of cegat-1 gene (A) and primary structure of cegat-1 (B). A, exons are indicated by filled boxes and numbered, and introns are indicated by solid lines. The untranslated regions in exons 1 and 6 are indicated by blank boxes. The consensus polyadenylation signal AATAAA is also shown. Sizes and positions of the exons and the introns are drawn to the exact scale. B, amino acid sequence of cegat-1. Putative transmembrane domains are underlined and numbered.
with the transporter. β-Alanine, glutamate, and carnitine showed very little but significant ability to compete with GABA. Other compounds such as taurine, creatine, choline, betaine, and glycine had no effect on GABA uptake. It is of interest to note that betaine, a substrate for mammalian BGT-1/GAT-4, was not recognized by ceGAT-1. The ceGAT-1-mediated GABA uptake was saturable with a Michaelis-Menten constant ($K_m = 17 \mu M$) (Fig. 4B). These data show that...
ceGAT-1 is a Na⁺/Cl⁻ coupled high-affinity GABA transporter. The effect of Na⁺ or Cl⁻ on the ceGAT-1-mediated uptake of GABA was then investigated by measuring the uptake in the presence of varying concentrations of extracellular Na⁺ (with a fixed concentration of Cl⁻) or Cl⁻ (with a fixed concentration of Na⁺). These experiments were done only with cells transfected with ceGAT-1 cDNA. The uptake measured in the absence of either Na⁺ or Cl⁻ was subtracted from the uptake measured in the presence of varying concentrations of Na⁺ or Cl⁻ to calculate cDNA-specific uptake. For Na⁺ activation kinetics, the concentration of Na⁺ in the uptake medium was varied from 0 to 140 mM. The osmolality of the medium was maintained by adding the appropriate concentrations of NMDG chloride as a substitute for NaCl. The relationship between the cDNA-specific uptake and Na⁺ concentration was sigmoidal with a Hill coefficient of 1.7 ± 0.2, suggesting the involvement of more than one Na⁺ per GABA molecule transported (Fig. 5B). Thus, the Na⁺:Cl⁻:GABA stoichiometry for ceGAT-1-mediated uptake is 2:1:1.

Functional Characterization of ceGAT-1 Using the X. laevis Oocyte Expression System—GABA exists as a zwitterion with one positive charge and one negative charge on the molecule under the experimental conditions. Therefore, the predicted Na⁺:Cl⁻:GABA stoichiometry of 2:1:1 would suggest that the transport process mediated by ceGAT-1 should be electrogenic. To determine whether this is true, we used the X. laevis oocyte expression system to express ceGAT-1 heterologously. First, we tested whether the transporter is expressed in oocytes injected with ceGAT-1 cRNA by measuring the uptake of [3H]GABA (1 μM) in the presence of NaCl in cRNA-injected oocytes and in uninjected control oocytes. The uptake in cRNA-injected oocytes was 1.45 ± 0.04 pmol/oocyte/h (Fig. 6A). This value was 4-fold higher than the uptake measured under identical conditions in control oocytes (0.38 ± 0.02 pmol/oocyte/h). Second, we examined the electrogenic nature of the ceGAT-1-mediated transport process by using the two-microelectrode voltage-clamp technique. Perfusion of cRNA-injected oocytes with GABA (1 mM) in the presence of NaCl induced inward currents (~15 nA) at a holding membrane potential of −50 mV (Fig. 6B).
These data show unequivocally that ceGAT-1-mediated GABA transport is electrogenic, associated with transfer of positive charge into the oocytes. The GABA-induced current was obligatorily dependent on the presence of both Na\(^+/\)H\(^-\) and Cl\(^-/\)H\(^-\). In the absence of Na\(^+/\)H\(^-\) or Cl\(^-/\)H\(^-\) (NaCl was substituted by NMDG chloride or by sodium gluconate, respectively) in the perfusion buffer, GABA did not induce any detectable current in cRNA-injected oocytes (Fig. 6B). The GABA-induced currents of ceGAT-1 were further analyzed in terms of their dependence on membrane potential. Steady-state currents induced by 1 mM GABA were determined at different testing membrane potentials (from +10 to −150 mV) using a jumping protocol (Software Clampex, version 10.0, Axon Instruments, Inc., Union City, CA). The GABA-induced currents increased with rising testing membrane potentials (i.e. more negative membrane potentials) (Fig. 6C). These data show that the transport rate increases with increasing membrane potentials, suggesting a role for membrane potential as a driving force for ceGAT-1. Thus, the transport process mediated by ceGAT-1 derives its driving force from the electrochemical Na\(^+/\)H\(^-\) and Cl\(^-/\)H\(^-\) gradients as well as from membrane potential. We did not attempt to investigate the kinetics of the transport process (substrate saturation kinetics, Na\(^+/\)activation kinetics, and Cl\(^-/\)activation kinetics) using the oocyte expression system because of the small magnitude of the currents induced by the substrate.

**Analysis of Tissue Expression Pattern of the cegat-1 Gene**—We investigated the tissue expression pattern of the cegat-1 gene in *C. elegans* using transgenic GFP fusion technique in which the transgene consisted of cegat-1 gene-specific promoter fused with GFP cDNA. The expression of GFP in this fusion gene is controlled by the cegat-1 gene-specific promoter. Therefore, the expression pattern of GFP in transgenic C. elegans expressing ceGAT-1 mediated GABA transport in HRPE cells.
the fusion gene is likely to match the expression pattern of the native cegat-1 gene. With this technique, we determined that ceGAT-1 is expressed in all of the GABA-ergic neurons whose identity has been previously established by immunocytochemistry by McIntire et al. (14) using polyclonal antibody against GABA. These GFP-positive neurons include the VD and DD neurons in the ventral cord, the RMED, RMEV, RMER, AVL, and RIS neurons in the head area and the DVB neuron in the tail region (Fig. 7). There are two additional GFP-positive neurons in the tail region that have not been classified as GABA-ergic neurons based on lack of staining for GABA by immunocytochemistry (14). These two neurons are PVQR and PVQL, which are the anterior interneurons in the right and left lumbar ganglia. These two neurons innervate the ventral cord motor neurons and may also participate in the regulation of the coordinated body muscle movement (www.wormatlas.org) (34). We confirmed the identity of these GFP-positive neurons by epifluorescence microscopy and by the location of the neurons as revealed by a combination of Nomarski-differential interference contrast microscopic observation and 4',6-diamidino-2-phenylindole staining method. This expression pattern is evident from the early larva stage through the adult stage (data not shown). We observed an identical expression pattern with at least 10 transgenic animals.

Consequences of RNAi-mediated Down-regulation of cegat-1 in C. elegans—GABA-ergic neurons are involved in the function of body muscles and enteric muscles, and ablation of specific GABA-ergic neurons by laser microsurgery leads to specific phenotypes as a consequence of a loss of function of body muscles and enteric muscles (13, 14). These phenotypes include three distinct types: 1) a “shrinker” phenotype related to body muscles; 2) a “loopy” phenotype related to muscles in the head involved in foraging; and 3) “defecation defect” phenotype related to enteric muscles (14). Because ceGAT-1 is expressed specifically in GABA-ergic neurons, we hypothesized that RNAi-mediated down-regulation of this transporter would influence the function of body muscles and enteric muscles and hence produce specific phenotypes. First, we evaluated the function of body muscles by analyzing the influence of RNAi-mediated down-regulation of ceGAT-1 on aldicarb-induced pa-
that the expression of GFP was driven by the GFP reporter fusion construct. The construct was designed such that the expression of GFP was driven by the promoter. This construct was then used to generate GFP-expressing transgenic worms. The middle panel identifies the GFP-positive DD andVD neurons in the ventral cord (20X), and the bottom panel identifies the GFP-positive neurons in the head region (40X).

Aldicarb is an inhibitor of acetylcholinesterase, and exposure of the worms to this compound leads to paralysis due to enhanced cholinergic signals at the motor endplate (30). When we used the normal strain N2, exposure the worms to aldicarb (0.5 mM) induced paralysis in a time-dependent manner; however, this phenotype was not altered by feeding the worms with bacteria harboring dsRNA specific for ceGAT-1. It is known that neurons are refractory to RNAi effects compared with other cell types in C. elegans (35, 36). Because ceGAT-1 is expressed exclusively in neurons, it is likely that the apparent lack of any effect on aldicarb-induced paralysis by RNAi in N2 worms is due to the refractory nature of the neurons. Therefore, we repeated the RNAi experiments using the RNAi-hypersensitive strain rrf-3. In this strain, even neurons are sensitive to RNAi (35, 36). With this strain, we found that RNAi-mediated down-regulation of ceGAT-1 enhanced the sensitivity of the worms to aldicarb-induced paralysis (Fig. 8). The time needed for aldicarb exposure to induce paralysis in 50% of the worms doubled in worms subjected to RNAi compared with controls. Both with normal strain and rrf-3 strain, control experiments consisted of feeding the worms with bacteria harboring the pPD129 vector alone without the ceGAT-1-specific cDNA insert. These data show that ceGAT-1-specific RNAi renders the rrf-3 worms, but not the N2 worms, hypersensitive to aldicarb-induced paralysis. Thus, there is clearly an interaction between cholinergic neurotransmission and GABA-ergic neurotransmission.

We also evaluated the role of GABA-ergic neurotransmission in cholinergic signaling by using unc-25 (a mutant lacking glutamic acid decarboxylase) and unc-47 (a mutant lacking the vesicular GABA transporter). We compared the rate of aldicarb-induced paralysis between control N2 worms and the two mutant worms (Fig. 9). These experiments did not involve RNAi. Control N2 worms exhibited normal GABAergic neurotransmission, whereas unc-25 and unc-47 exhibited a loss of GABA-ergic neurotransmission. Therefore, a comparison among these worms in terms of their response to aldicarb-induced paralysis would directly provide information on the role of GABA-ergic neurotransmission in cholinergic signaling. These studies showed that both mutants that lack GABA-ergic neurotransmission exhibited markedly enhanced sensitivity to aldicarb-induced paralysis. As an internal control for these experiments, we used the unc-2 mutant that lacks a major calcium channel and hence refractory to cholinergic signaling (29). As predicted, exposure to aldicarb, which is expected to enhance cholinergic signaling by increasing the levels of acetylcholine at the motor endplates, did not have any effect on the worms (Fig. 9). In other words, there was no paralysis in CD55 mutant worms in response to aldicarb exposure.

Aldicarb functions similar to nerve gases such as sarin and tabun, which increase the levels of acetylcholine at nerve terminals and at motor endplates and induce paralysis. The experiments described thus far show that down-regulation of ceGAT-1 in the presence of aldicarb sensitizes the worms to aldicarb-induced paralysis. What happens to the function of body muscles and enteric muscles when ceGAT-1 is down-regulated in the absence of aldicarb? To answer this question, we subjected the RNAi-hypersensitive rrf-3 worms to ceGAT-1-specific RNAi and quantified the changes in the function of body muscles and enteric muscles by the thrashing assay and the defection failure assay, respectively. GABA acts as an inhibitory neurotransmitter in body muscles (13, 14), and congruent with this function of GABA-ergic neurons, the rates of body movement as assessed by the thrashing assay were significantly increased by RNAi-induced down-regulation of ceGAT-1 (thrashing rate: 152 ± 8 times/2 min in control worms and 188 ± 9 times/2 min in RNAI worms; p < 0.005) (Fig. 10A). In contrast, GABA acts as an excitatory neurotransmitter in enteric muscles (13, 14) and hence GABA-ergic neurons are involved in the maintenance of normal defection cycle. In agreement with this function, the rates of defection failure increased significantly in worms subjected to RNAi-induced down-regulation of ceGAT-1 (Fig. 10B).

To determine the effectiveness of RNAi-induced down-regulation of ceGAT-1 expression, we compared the steady-state levels of ceGAT-1 mRNA in control rrf-3 strain worms and in rrf-3 worms subjected to RNAi. Semiquantitative RT-PCR was used to monitor the levels of ceGAT-1 mRNA. The levels of 18 S RNA were measured simultaneously as an internal control. These studies showed that RNAi led to a 40% decrease in ceGAT-1 mRNA levels (Fig. 11).

Taken collectively, these functional studies show that GABA-ergic neurotransmission is affected by ceGAT-1-specific RNAi and that GABA plays a critical role in the function of body muscles and enteric muscles through interaction with cholinergic signaling.
DISCUSSION

We have cloned and functionally identified a GABA transporter from *C. elegans*. The transporter is a member of the snf family identified in this organism. The cloned transporter is snf-11 and functions as a Na\(^+\)/H\(^+\)/Cl\(^-\)/H\(^+\) -coupled transporter for the neurotransmitter GABA. It exhibits high-affinity for GABA (K\(_T\) \(\approx 15\) \(\mu M\)) and is highly selective for GABA. The only other structurally related compound with the ability to interact with the transporter to a significant extent is \(-\)aminobutyric acid. Other neurotransmitters/neuromodulators such as taurine, glycine, and glutamate do not interact with the transporter. The function of this transporter is demonstrable in two different heterologous expression systems: 1) mammalian cells and 2) *X. laevis* oocytes. The transport process exhibits a Na\(^+\)/H\(^+\)/Cl\(^-\)/H\(^+\) stoichiometry of 2:1:1 and is electrogenic. Because this is the first GABA transporter identified at the molecular and functional levels in *C. elegans*, we named this transporter ceGAT-1.

Mammals express four different GABA transporters known as GAT-1, GAT-2, GAT-3, and BGT-1/GAT-4 (6, 7). Based on the functional characteristics and expression pattern, the cloned ceGAT-1 is distinct from BGT-1/GAT-4 because ceGAT-1 does not interact with betaine. Whereas ceGAT-1 is expressed exclusively in GABA-ergic neurons, there is no overlap between the markers of GABAergic neurons and BGT-1/GAT-4 in mammalian brain (6, 7). Furthermore, we have cloned snf-3, another member of snf family from *C. elegans*, which we have shown to function as a Na\(^+\)/Cl\(^-\) -coupled transporter for betaine.\(^2\) Whether *C. elegans* expresses additional GABA transporters is not known at present. We have cloned three other members of the *C. elegans* snf family (snf-5, snf-6, and snf-12), and functional analysis with heterologous expression in mammalian cells has shown that none of these cells possesses the ability to

---

\(^2\) G. Jiang, Y. J. Fei, and V. Ganapathy, unpublished data.
transport GABA. Nonetheless, because ceGAT-1 is selectively associated with GABA-ergic neurons, we postulate that this transporter is likely to be the C. elegans ortholog of either GAT-1 or GAT-3 in mammals. GAT-1 and GAT-3 are closely associated with GABA-ergic neurons in mammalian brain. The identification of GAT-1 in C. elegans suggests that the role of the transporter in the modulation of GABA-ergic neurotransmission is likely to be similar to that of GAT-1 and/or GAT-3 in mammalian GABA-ergic neurons. The functional characteristics of ceGAT-1 identifying it as a Na⁺/Cl⁻/H⁺ coupled, high-affinity, and selective transporter for GABA support the hypothesis that the transporter functions in the clearance of GABA from the synaptic cleft or motor endplate. These findings are of significance with respect to the biology of GABA-ergic neurotransmission in C. elegans. Previous studies have identified at the molecular and functional levels the enzyme responsible for GABA synthesis (unc-25), the transporter responsible for packaging GABA in synaptic vesicles (unc-47), and the receptor responsible for the transmission of GABA signals (unc-49) (15), but there are no reports in the literature on the molecular and functional identity of any transporter that is responsible for the clearance of GABA from the synaptic cleft or motor endplate. Thus, the findings presented in this paper fill an important gap in our knowledge in terms of GABA-ergic neurotransmission in this organism.

Even though all of the currently known GABA transporters in mammals have been cloned and functionally characterized several years ago, there are no reports to date in the literature on the generation of knock-out animal models for any of these transporters. There is strong evidence from clinical and pharmacological studies that GABA-ergic neurons are involved in muscle function as evidenced from a convincing relationship between epilepsy and GABA-ergic neuronal dysfunction (37–39). The data presented in this paper provide evidence for the first time for the involvement of a GABA transporter in the maintenance of muscle function in an animal model by characterizing the phenotype associated with the down-regulation of the transporter.

The phenotype resulting from the RNAi-mediated down-regulation of ceGAT-1 is seen only with the RNAi-hypersensitive rrf-3 strain but not with the normal N2 strain. This is expected because neurons in normal worms are comparatively more refractive to RNAi than non-neuronal cells (35, 36). The rrf-3 strain exhibits hypersensitivity to RNAi, and neuron-specific genes can be down-regulated by RNAi in this strain. RNAi is made possible in C. elegans because of the expression of Sid-1, a transporter for dsRNA in cells (40, 41). However, Sid-1 is expressed at very low levels in neurons in this organism (40) and that is why neurons in C. elegans are resistant to RNAi-induced effects. Recently, this problem was solved by the iden-
tification of a *C. elegans* strain (rrf-3) in which neurons became sensitive to RNAi (35, 36). The hypersensitivity of the rrf-3 strain to RNAi despite the low levels of expression of Sid-1 is attributed to the loss of function of a putative RNA-directed RNA polymerase. The neurons that are generally refractory to RNAi in the wild type strain respond effectively to RNAi in the rrf-3 mutant strain. It was necessary to use the rrf-3 strain for our studies because ceGAT-1 is expressed exclusively in neurons.

The RNAi-mediated down-regulation of ceGAT-1 in the rrf-3 mutant strain produces a specific phenotype associated with changes in the function of body muscles and enteric muscles. The phenotype includes increased rates of body movement that is detectable in the frequency of thrashing, decreased function of enteric muscles that is detectable in the frequency of defecation failure, and increased sensitivity to aldicarb-induced paralysis. GABA has a differential function in body muscles and in enteric muscles. In the control of the function of body muscles, GABA serves as an inhibitory neurotransmitter, whereas in the control of the function of enteric muscles, it serves as an excitatory neurotransmitter (13, 14). This differential function is made possible because of the distinct GABA-related receptors associated with these functions. The unc-49 region contains three genes coding for different isoforms of GABA receptors, which function as anion channels (42–44). GABA-induced influx of chloride via these receptors leads to hyperpolarization of muscle membrane and thus causes relaxation of the muscles. The body muscles are associated with unc-49 gene products, and thus GABA functions as an inhibitory neurotransmitter in these muscles. In contrast, enteric muscles are associated with exp-1 gene product that codes for a GABA-gated cation-selective channel (45). Therefore, GABA-induced influx of cations via this receptor in enteric muscles leads to depolarization and hence contraction of the muscles. There is precedence for such a differential function of GABA. Whereas GABA acts predominantly as an inhibitory neurotransmitter in adult mammalian brain, it serves as an excitatory neurotransmitter in immature mammalian brain (46). In contrast to *C. elegans*, however, the differential functions of GABA in the mammalian brain are not mediated by different GABA receptors but rather by the varied functions of the same receptors (chloride influx versus cation influx due to changes in transmembrane chloride gradients in immature brain versus adult brain). In the rrf-3 mutant strain, ceGAT-1-specific RNAi leads to increased frequency of thrashing, which suggests that the body muscles contract at a higher frequency. This was an unexpected result. Because ceGAT-1 is expected to function in the clearance of GABA from the synaptic cleft or motor endplates, we thought that RNAi-mediated down-regulation of ceGAT-1 would lead to increased levels of GABA signaling. GABA is an inhibitory neurotransmitter in the function of body muscles, and therefore, increased GABA signaling resulting from the down-regulation of ceGAT-1 is expected to increase the inhibitory tone in the function of body muscles. Instead, what we found was that the down-regulation of ceGAT-1 led to a decrease in the inhibitory tone of these muscles as evidenced from increased body movement. We speculate that chronic up-regulation of GABA signaling due to continued loss of the GABA clearance mechanism leads to either desensitization or down-regulation of GABA receptors. Such an effect would explain the increased frequency of body movement in the rrf-3 mutant strain subjected to ceGAT-1-specific RNAi. This would also provide the molecular basis for the increased defecation failure in these worms. Because GABA normally functions as an excitatory neurotransmitter in the function of enteric muscles, down-regulation of GABA signaling would decrease the contraction frequency of enteric muscles that are intimately involved in the defecation process. This would result in increased defecation failure, as it has been indeed observed in our studies.

The phenotype associated with increased sensitivity to aldicarb-induced paralysis in the rrf-3 mutant strain subjected to RNAi-mediated down-regulation of ceGAT-1 is also interesting. Cholinergic neurons function in the contraction of body muscles, and aldicarb, an inhibitor of acetylcholinesterase, is expected to increase cholinergic neurotransmission by interfering with the breakdown of acetylcholine. However, aldicarb exposure does not result in increased muscle function but rather causes paralysis. This is again most likely due to desensitization or down-regulation of cholinergic receptors following continued elevation of acetylcholine in the synaptic cleft or motor endplates. Alternatively, aldicarb may lead to hypercontractile paralysis. A loss of GABA-ergic neurotransmission in unc-25 and unc-47 mutants enhances the sensitivity of the worms to aldicarb-induced paralysis. The molecular mechanisms responsible for these effects are not clear, but the findings show convincingly that GABA-ergic neurons modulate the function of cholinergic neurotransmission. RNAi-mediated down-regulation of ceGAT-1 in rrf-3 mutant strain also enhances the sensitivity of the organism to aldicarb-induced paralysis, an effect similar to that resulting from the loss of GABA-ergic neurotransmission in unc-25 and unc-47 mutants. These findings again support our hypothesis that chronic RNAi-mediated down-regulation of ceGAT-1 actually leads to a loss of GABA-ergic neurotransmission rather than an increase in GABA signaling.

In summary, we have shown here that snf-11, a member of the sodium-dependent neurotransmitter symporter gene family in *C. elegans*, codes for a Na+/Cl- coupled high-affinity GABA transporter (ceGAT-1) and that this transporter plays a critical role in GABA-ergic neurotransmission. Down-regulation of ceGAT-1 by RNAi leads to an interesting phenotype associated with altered function of body muscles and enteric muscles and also with altered sensitivity to aldicarb-induced paralysis. These findings provide unequivocal evidence for a modulatory role of GABA in the biology of cholinergic neurons and in the function of body muscles and enteric muscles in this organism.

Acknowledgments—We thank Dr. Michael Krause (Laboratory of Molecular Biology/NIDDK, National Institutes of Health, Bethesda, MD) for assistance in confirming, by fluorescence microscopy, the identity of the GFP-positive neurons in transgenic *C. elegans* expressing the transcriptional cegat-1:gfp fusion constructs.

REFERENCES

1. Bowery, N. G., and Nistico, G. (1989) GABA, Basic Research and Clinical Applications, pp. 1–426, Pythagora, Rome, Italy
2. Brambilla, P., Perez, J., Barale, F., Schettini, G., and Soares, J. C. (2003) Mol. Psychiatry 8, 721–737
3. Lydiard, R. B. (2003) J. Clin. Psychiatry 64, Suppl. 3, 21–27
4. Ashton, H., and Young, A. H. (2003) J. Psychopharmacol. 17, 174–178
5. Sarup, A., Larsson, O. M., and Schoosboue, A. (2003) Curr. Drug Targets CNS Neurol. Disord. 2, 269–277
6. Dalley, N. O. (2003) Eur. J. Pharmacol. 475, 127–137
7. Chen, N. H., Reith, M. E. A., and Quick, M. W. (2004) Pflugers Arch. Eur. J. Physiol. 447, 519–531
8. Radian, R., Ottersen, O. P., Storm-Mathisen, J., Castel, M., and Kanner, B. I. (1990) J. Neurosci. 10, 1319–1330
9. Minelli, A., Breecha, N. C., Karschin, C., DeBisi, S., and Conti, F. (1995) J. Neurosci. 15, 7734–7746
10. Ibegaki, N., Saito, N., Hashima, M., and Tanaka, C. (1994) Brain Res. Mol. Brain Res. 26, 47–54
11. Minelli, A., DeBisi, S., Breecha, N. C., Zuccarello, L. V., and Conti, F. (1996) J. Neurosci. 16, 6255–6264
12. DeBisi, S., Vitillaro-Zuccarello, L., and Breecha, N. C. (1998) Neuroscience 83, 815–828
13. Schous, K., Beg, A. A., and Jorgensen, E. M. (2004) Trends Neurosci. 27, 407–414
14. McIntire, S. L., Jorgensen, E., Kaplan, J., and Horvitz, H. R. (1993) Nature 364, 337–341
15. McIntire, S. L., Jorgensen, E., and Horvitz, H. R. (1993) Nature 364, 334–337
16. Jayanthi, L. D., Apparsundaram, S., Malene, M. D., Ward, E., Miller, D. M., Eppler, M., and Blakeley, R. D. (1998) Mol. Pharmacol. 54, 601–609
17. Ranganathan, R., Sawin, E. R., Trent, C., and Horvitz, H. R. (2001) J. Neurosci. 21, 5871–5884
18. Lewis, J. A., and Fleming, J. T. (1995) Methods Cell Biol. 48, 3–29
19. Fei, Y. J., Fujita, T., Lapp, D. F., Ganapathy, V., and Leibach, F. H. (1998) Biochem. J. 332, 565–572
20. Fei, Y. J., Romero, M. F., Krause, M., Liu, J. C., Huang, W., Ganapathy, V., and Leibach, F. H. (2000) J. Biol. Chem. 275, 9063–9071
21. Jin, Y., Jorgensen, E., Hartwieg, E., and Horvitz, H. R. (1999) J. Neurosci. 19, 539–548
22. McIntire, S., Reimer, R., Schuske, K., Edwards, R., and Jorgensen, E. (1997) Nature 389, 870–876
23. Schafer, W. R., and Kenyon, S. J. (1995) Nature 375, 73–78
24. Mathews, E. A., Garcia, F., Santi, C. M., Mullen, G. P., Thacker, C., Moerman, D. G., and Snutch, T. P. (2003) J. Neurosci. 23, 6537–6545
25. Lewis, J. A., and Fleming, J. T. (1995) Methods Cell Biol. 48, 3–29
26. Sulston, J. E., and Hodgkin, J. (1988) in The Nematode Caenorhabditis elegans (Wood, W., ed) pp. 587–606, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
27. Jin, Y., Jorgensen, E., Hartwieg, E., and Horvitz, H. R. (1999) J. Neurosci. 19, 539–548
28. McIntire, S., Reimer, R., Schuske, K., Edwards, R., and Jorgensen, E. (1997) Nature 389, 870–876
29. Schafer, W. R., and Kenyon, S. J. (1995) Nature 375, 73–78
30. Mathews, E. A., Garcia, F., Santi, C. M., Mullen, G. P., Thacker, C., Moerman, D. G., and Snutch, T. P. (2003) J. Neurosci. 23, 6537–6545
31. Liu, D. W., and Thomas, J. H. (1994) J. Neurosci. 14, 1953–1962
32. Thomas, J. H. (1990) Genetics 124, 855–872
33. Bridges, C. C., Hu, H., Miyauachi, S., Siddaramappa, U. N., Ganapathy, M. E., Ignatowicz, L., Maddox, D. M., Smith, S. B., and Ganapathy, V. (2004) Invest. Ophthalmo. Vis. Sci. 45, 2906–2914
34. Deleted in proof
35. Simmer, F., Tijsterman, M., Parrish, S., Koushika, S. P., Nonet, M. L., Fire, A., Abringer, J., and Plasterk, R. H. (2002) Curr. Biol. 12, 1317–1319
36. Simmer, F., Moorman, C., Van Der Linden, A. M., Kuijk, E., Van Den Berghe, P. V., Kamath, R., Fraser, A. G., Abringer, J., and Plasterk, R. H. (2003) PLoS Biol. 1, E12
37. Scheffer, I. E., and Berkovic, S. F. (2003) Trends Pharmacol. Sci. 24, 428–433
38. Gupta, M., Gogna, H. M., Grouse, M., Bottiglieri, T. G., Crecas, A., Biggo, G., Sogliano, C., Rigamonti, A. E., Pearl, P. L., Sneed, O. C., Jakobs, C., and Gibson, K. M. (2003) Ann. Neurol. 54, Suppl. 6, S81–S90
39. Weng, C. G. T., Bottiglieri, T., and Sneed, O. C. (2003) Ann. Neurol. 54, Suppl. 6, S3–S12
40. Winton, W. M., Molodowitch, C., and Hunter, C. P. (2002) Science 295, 2456–2459
41. Feinberg, E. H., and Hunter, C. P. (2003) Science 301, 1545–1547
42. Bamber, B. A., Beg, A. A., Twyman, R. E., and Jorgensen, E. M. (1999) J. Neurosci. 19, 5348–5359
43. Richmond, J. E., and Jorgensen, E. (1999) Nat. Neurosci. 2, 791–797
44. Bamber, B. A., Twyman, R. E., and Jorgensen, E. M. (2003) Br. J. Pharmacol. 138, 883–893
45. Beg, A. A., and Jorgensen, E. (2003) Nat. Neurosci. 6, 1145–1152
46. Haver, S. C., Mount, D. B., and Gamba, G. (2004) Pfluegers Arch. Eur. J. Physiol. 447, 580–593
A Na⁺/Cl⁻-coupled GABA Transporter, GAT-1, from Caenorhabditis elegans: STRUCTURAL AND FUNCTIONAL FEATURES, SPECIFIC EXPRESSION IN GABA-ERGIC NEURONS, AND INVOLVEMENT IN MUSCLE FUNCTION

Guoliang Jiang, Lina Zhuang, Seiji Miyauchi, Katsuya Miyake, You-Jun Fei and Vadivel Ganapathy

J. Biol. Chem. 2005, 280:2065-2077.
doi: 10.1074/jbc.M408470200 originally published online November 12, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M408470200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 43 references, 17 of which can be accessed free at http://www.jbc.org/content/280/3/2065.full.html#ref-list-1