Review TRENDS in Neurosciences Vol.27 No.7 July 2004

The GABA nervous system in *C. elegans*

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GABA neurotransmission requires a specialized set of proteins to synthesize, transport or respond to GABA. This article reviews results from a genetic strategy in the nematode *Caenorhabditis elegans* designed to identify the genes responsible for these activities. These studies identified mutations in genes encoding five different proteins: the biosynthetic enzyme for GABA, the vesicular GABA transporter, a transcription factor that determines GABA neuron identity, a classic inhibitory GABA receptor and a novel excitatory GABA receptor. This review discusses the strategy employed to identify these genes as well as the conclusions about GABA transmission derived from study of the mutant phenotypes.

Activity in the vertebrate brain depends on a yin and yang balance between excitatory and inhibitory neurotransmission. A deficit of excitatory neurotransmission will lead to unconsciousness; a deficit of inhibitory neurotransmission will lead to epileptic seizures. The most abundant inhibitory neurotransmitter in the brain is GABA. Normal GABA function requires specialized proteins such as biosynthetic enzymes, transporters and receptors. Defects in these proteins can lead to a specific imbalance of GABA neurotransmission and lead to diseases. For example, mutations in the α1 and γ2 GABA receptor subunits can cause familial forms of epilepsy [1–3].

Over ten years ago, a strategy was devised to identify the proteins specialized for GABA function using a genetic approach in the nematode *Caenorhabditis elegans* [4,5]. These studies have lead to some important discoveries. Specifically, the vesicular GABA transporter (VGAT) was first discovered in *C. elegans* and this sequence was used to identify the mammalian homolog [6]. The homeodomain protein UNC-30 identified a class of transcription factors required for multiple aspects of GABA neuron identity [7,8]. These studies also revealed the first cation-selective GABA receptor [9]. This review will discuss first the strategy used to identify proteins required for GABA neurotransmission in *C. elegans* and then the impact that their discovery has had on our understanding of GABA function.

Strategy

To define the proteins required for GABA function, McIntire and colleagues looked for mutations in the nematode *C. elegans* that specifically disrupted GABA-mediated behaviors [4,5]. To identify the GABA-containing cells in *C. elegans*, animals were stained using antibodies against the neurotransmitter. Antibody staining revealed that 26 of the 302 neurons present in *C. elegans* express the neurotransmitter GABA (Figure 1a). These 26 GABA-positive neurons comprise six DD neurons, 13 VD neurons, four RME neurons, an RIS neuron, an AVL neuron and a DVB neuron (Figure 1b). These neurons fall into different classes based on their synaptic outputs: the D-type neurons – that is, the DD and VD motor neurons – innervate the dorsal and ventral body muscles, respectively; the RME motor neurons innervate the head muscles; the AVL and DVB motor neurons innervate the enteric muscles; and RIS is an interneuron [10]. There are two surprises here. First, GABA is primarily acting at neuromuscular junctions; in vertebrates, GABA acts in the CNS. Second, GABA neurons comprise $<10\%$ of the nervous system; in vertebrates, 30–40% of synapses in the brain are GABAergic [11]. The explanation for these two observations is that glutamate can act as an inhibitory neurotransmitter in the nematode. In contrast to GABA, which primarily acts at neuromuscular junctions, glutamate activates Cl^- channels at both neuronal synapses and neuromuscular junctions in the nematode [12]. Thus, fast inhibitory neurotransmission is divided between GABA and glutamate in the nematode. In vertebrates, fast inhibitory neurotransmission is divided between GABA and glycine. Interestingly, the glycine-gated and

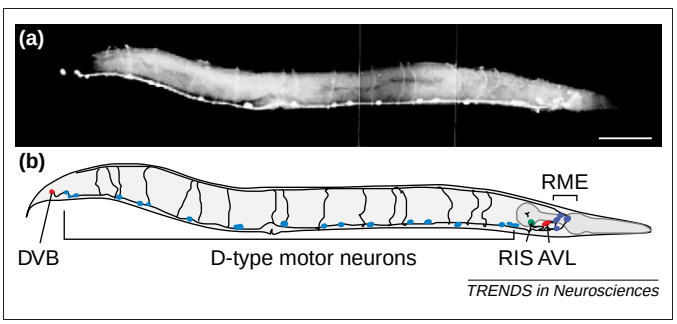

Figure 1. The GABA nervous system. **(a)** Fluorescent micrograph of an adult wild-type *Caenorhabditis elegans* hermaphrodite stained with antiserum raised against GABA. There are 26 neurons that stain for GABA. Anterior is to the right and the right side of the body is shown. Scale bar, 0.1 mm. **(b)** Schematic drawing of the positions of the 26 GABA-containing neurons in the animal above. The neurons are color-coded based on their synaptic outputs: the D-type DD and VD motor neurons innervate the body muscles; RME neurons innervate the head muscles; AVL and DVB innervate the enteric muscles; and RIS is an interneuron. Reproduced, with permission, from Ref. [4] © (1993) Macmillan Magazines Limited (http://www.nature.com/nature).

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Available online 28 May 2004

 0166-2236/$ - see front matter doi:10.1016/j.tins.2004.05.005

glutamate-gated Cl^- channels belong to a related subbranch of ligand-gated Cl^- channels [13] (B. Bamber, pers. commun.).

To determine the *in vivo* behavioral role of the GABA neurons, laser ablation of the single or multiple neurons was performed [4]. Three phenotypes were observed. Animals in which the DD or VD motor neurons were ablated exhibited abnormal locomotion. Laser ablation of the RME neurons resulted in animals that had foraging defects. AVL and DVB laser-ablated animals exhibited abnormal defecation behavior. Animals in which RIS had been ablated exhibited no visible phenotype. The genetic basis for foraging behavior is unexplored; however, the cellular and genetic bases for locomotion and defecation have been well-studied. Based on these studies, as will be described, it was concluded that GABA acts as both an inhibitory and excitatory neurotransmitter in *C. elegans*.

Inhibitory GABA function

The 19 ventral cord D-type neurons inhibit contraction of the ventral and dorsal body wall muscles during locomotion [4]. A bend in the body is made by contracting muscles on one side of the body while relaxing muscles via GABA innervation on the opposite side (Figure 2a). Movement is driven by propagating such a body bend as a sinusoidal body wave from one end of the animal to the other (Figure 2a). Because the VD and DD neurons are required for relaxation of body wall muscles, when these neurons are killed, the operated worm has only excitatory input into its muscles. When such an animal is touched on the nose it 'shrinks' – that is, it pulls in its head and its body shortens owing to hypercontraction of the body wall muscles on both sides of the body (Figure 2b). Therefore, GABA released from the ventral cord D-type neurons is required to relax muscles to reset posture when changing directions.

Excitatory GABA function

The GABA motor neurons AVL and DVB stimulate the enteric muscles during defecation (Figure 2b) [4]. Every 50 s, a worm undergoes a stereotypic pattern of muscle contractions, which ultimately leads to the expulsion of intestinal contents [14] (Figure 3a). The first muscle contraction, the posterior body contraction (pBoc), occurs in the tail and pushes the food in the intestine towards the anterior of the worm. The second contraction, the anterior body contraction (aBoc), occurs in the head and compacts the food at the posterior of the worm. The final contraction, the enteric muscle contraction (Emc), opens the anus so that the intestinal contents can be expelled. AVL and DVB are required in the last step to contract the enteric

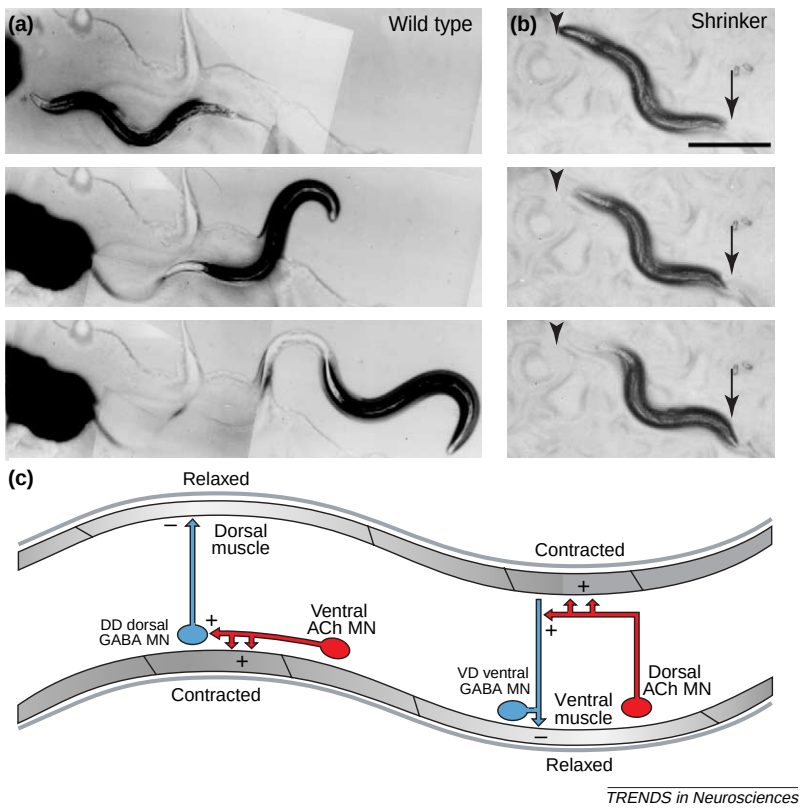

Figure 2. The D-type motor neurons are inhibitory. **(a)** When a wild type worm is tapped on the nose it moves backwards by propagating a sinusoidal wave from the tail to the head. **(b)** When a mutant defective for GABA transmission is tapped on the nose, it contracts muscles on both sides of its body causing the animal to shrink (arrows point to the tail of the worm; arrowheads indicate where the head was before the nose was tapped). Scale bar in (b), 0.5 mm (a,b). Figure courtesy of J. White. **(c)** Motor neuron (MN) connectivity. Motor neuron cell bodies are in the ventral cord. The DD and VD GABA neurons (blue) synapse to the dorsal and ventral body muscles, respectively. Cholinergic motor neurons (red) send inputs to the ventral and dorsal body muscles, as well as to the GABA motor neurons. Release of ACh leads to contraction of the body wall muscle on one side and stimulates GABA release onto muscles on the opposite side. This stimulation and contralateral inhibition causes the body to bend and leads to coordinated locomotion. Panels (a,c) reproduced, with permission, from Ref. [4] © (1993) Macmillan Magazines Limited (http://www.nature.com/nature).

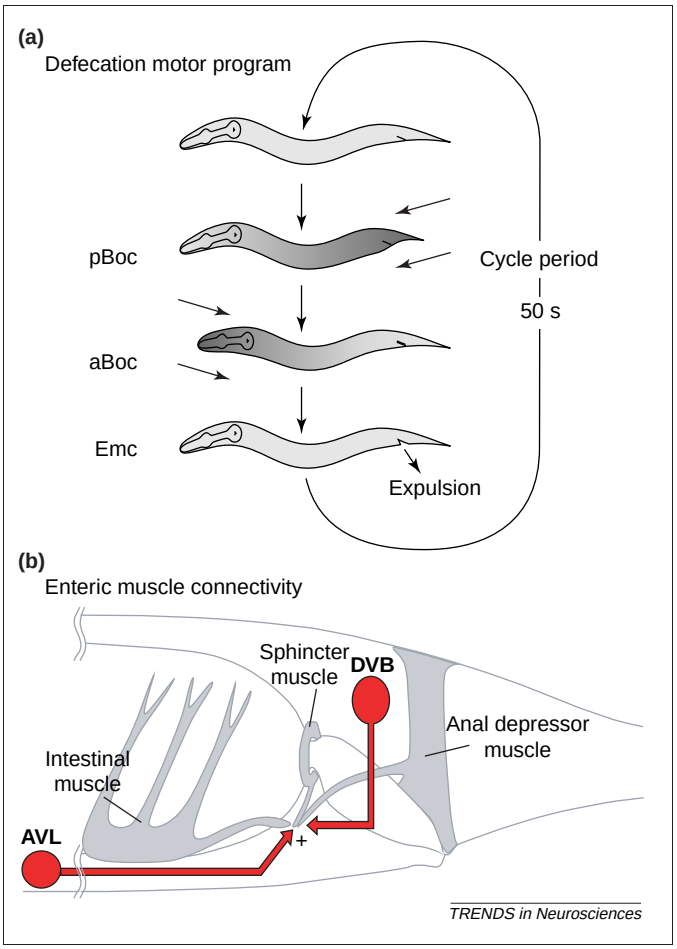

Figure 3. AVL and DVB motor neurons are excitatory. **(a)** Schematic diagram of the defecation motor program [14]. Every 50 s, an animal initiates a defecation cycle, which consists of the three stereotyped, independent muscle contractions. The cycle initiates with the posterior body contraction (pBoc), which pushes the food in the intestine towards the anterior of the worm. The second contraction, the anterior body contraction (aBoc), occurs in the head and compacts the food at the posterior of the worm. The final contraction, the enteric muscle contraction (Emc), opens the anus so that the intestinal contents can be expelled. **(b)** Schematic of the enteric muscles. The enteric muscles consist of four cells: the anal depressor, the sphincter, and two bilaterally symmetric intestinal cells. These cells are connected by gap junctions, and each cell sends a muscle arm to the pre-anal ganglion, where DVB makes synaptic contact. AVL, which is located anteriorly in the ventral head ganglion, sends a process along the ventral nerve cord abutting DVB and the enteric muscles. Release of GABA from AVL and DVB causes the anal depressor and intestinal muscles to contract.

muscles [4] (Figure 3b). When these neurons are killed using a laser, the worm fails to contract its enteric muscles and becomes bloated with food. Therefore, GABA released from AVL and DVB is required to excite muscle contraction.

Genes required for GABA function

To identify genes required for GABA function, mutants were identified that resembled worms in which the GABA neurons had been killed [5,14]. A total of six genes were identified in which mutations caused all or a subset of the behavioral defects (Table 1). Three mutants, *unc-25*, *unc-46* and *unc-47*, both shrink and lack enteric muscle contractions, suggesting the mutated genes encode proteins that are required for both the inhibitory and the excitatory GABA functions. Two mutants, *unc-30* and *unc-49*, exhibit the 'shrinker' phenotype, suggesting the proteins are required for only the inhibitory GABA function. One mutant, *exp-1*, is defective for enteric muscle contractions, suggesting the EXP-1 protein is required only for excitatory GABA function [14]. By analyzing the phenotypes of the mutants further, it was possible to predict which protein was likely to be defective before cloning the gene.

Presynaptic or postsynaptic?

To determine whether a mutant had a presynaptic or a postsynaptic defect, mutant animals were exposed to the GABA receptor agonist muscimol. If the postsynaptic GABA receptors are present, then muscimol will activate the GABA receptor constitutively. When wild-type animals are bathed in muscimol, contractions cease in the body muscles and the animal becomes flaccid [5]; by contrast, the enteric muscles undergo repetitive contractions (E. Jorgensen, unpublished). The mutants *unc-49* and *exp-1*were resistant to the effects of muscimol on the body and enteric muscles, respectively; therefore, these genes were predicted to encode postsynaptic GABA receptors [5] (E. Jorgensen, unpublished). Specifically, *unc-49* was predicted to encode an inhibitory GABA receptor because these mutants are defective only for locomotion, the inhibitory GABA function. By contrast, *exp-1* was predicted to encode an excitatory GABA receptor because mutants are defective only for enteric muscle contraction, the excitatory GABA function. The mutants that are sensitive to muscimol – *unc-25*, *unc-30*, *unc-46* and *unc-47* – were predicted to encode proteins required in the presynaptic neuron because the muscles in these mutants responded normally to the GABA receptor agonist.

GABA neurons synthesize GABA and then package the neurotransmitter into synaptic vesicles. An animal that is not capable of synthesizing or packaging GABA should be defective for both the inhibitory and the excitatory GABA functions. Three of the four 'presynaptic' mutants that gave normal muscimol responses – *unc-25*, *unc-46* and *unc-47* – fall into this class. To determine whether GABA was synthesized normally in these three mutants, animals were stained with anti-GABA antibodies. Two predictions can be made. First, if a mutant is defective for synthesis of GABA, then no GABA staining should be observed. Second, if a mutant is defective for packaging of GABA so that it cannot be released, then there will be an increase in GABA staining. As expected, one of the three mutants, *unc-25*, lacked GABA expression, suggesting that this gene encoded the biosynthetic enzyme glutamic acid decarboxylase (GAD). *unc-47* mutants showed increased levels of GABA, suggesting that this gene encoded the vesicular transporter VGAT. *unc-46* mutants had the same level of GABA expression as wild-type worms, making it difficult to assign a specific function for the affected protein.

The final mutant, *unc-30*, is defective for the inhibitory GABA function of locomotion but the animals are sensitive to muscimol, indicating that the defect is presynaptic. In addition, staining with anti-GABA antibodies indicated that *unc–30* mutants do not express GABA in the VD and DD neurons. Because these neurons were generated but do not synthesize the correct neurotransmitter, it appeared

Table 1. GABA mutant phenotypes[a,b]

Mutant	Inhibitory function	Excitatory function	Muscimol sensitive	GABA levels	Protein	Refs
unc-25	−	−	Yes	(−)	GAD	[4,5,15]
unc-30	−	+	Yes	VDs and DDs[c]: (−) RMEs, AVL, DVB, RIS: (+)	Transcription factor	[4,5,7]
unc-46	−	−	Yes	(+)	Unknown	[4,5]
unc-47	−	−	Yes	(++++)	VGAT	[4–6]
unc-49	−	+	No	(+)	Anionic GABA receptor	[4,5,16]
exp-1	+	−	No	(+)	Cationic GABA receptor	[9,14]

[a]Key to symbols: −, defective behavior; +, wild-type behavior; (+), wild-type GABA level; (−), low GABA level; (++++), high GABA level.
[b]Abbreviations: GAD, glutamic acid decarboxylase; VGAT, vesicular GABA transporter.
[c]DD and VD motor neurons innervate the dorsal and ventral body muscles, respectively; RME motor neurons innervate the head muscles; the AVL and DVB motor neurons innervate the enteric muscles; and RIS is an interneuron.

that the UNC-30 protein was required specifically for GABA cell identity in these neurons.

All but one of the six genes (the exception is *unc-46*) have been cloned and, remarkably, all five of the cloned genes encode proteins that were predicted from the phenotypic analysis (Table 1). Specifically, *unc-25* encodes GAD, *unc-47* encodes VGAT, *unc-30* encodes a homeodomain transcription factor that is required for *unc-25* and *unc-47* expression in the VD and DD neurons, *unc-49* encodes an inhibitory GABA-gated anion channel, and *exp-1* encodes an excitatory GABA-gated cation channel [6,7,9,15,16]. In the next section, each of these proteins will be discussed in more detail. But first, it is worth discussing the things that were not identified.

In the genetic screens, mutants were identified that caused a severe shrinking defect or a lack of enteric muscle contractions; mutations that did not result in these phenotypes would not have been recovered. Examination of the genome indicates that there are several genes involved in GABA function that were not identified in the screens. First, there are multiple GABA receptor subunits in the genome that were not mutated. Notably, there are α and β $GABA_A$ receptor subunits, another UNC-49-like receptor subunit, another EXP-1-related receptor subunit and a metabotropic GABA receptor in the genome (E. Jorgensen, unpublished). The GABA-mediated behaviors controlled by these three additional GABA receptors are unknown; however, one of these receptors might be responsible for mediating foraging behavior, which is defective in *unc-25*/GAD mutants but is not defective in either *unc-49* mutants or *exp-1* mutants. Targeted knockouts of these genes might reveal their functions. Second, the plasma membrane GABA transporter present in the genome was not identified in the genetic screens. This membrane transporter is required to remove secreted GABA from the synaptic cleft. Transport of GABA across the plasma membrane can be used to rescue *unc-25*/GAD mutants: simply bathing mutants in GABA can restore GABA to the AVL and DVB neurons and can rescue function from these neurons [5]. Nipecotic acid blocks the plasma membrane GABA transporter. This drug blocks transport of GABA into AVL and DVB and blocks rescue of the *unc-25* mutant. However, blocking GABA transport in the wild type with nipecotic acid does not result in a phenotype, suggesting that synthesis of GABA alone – in the absence of GABA recycling– provides sufficient neurotransmitter for enteric muscle contractions (E. Jorgensen, unpublished).

unc-25: is GABA required for development?

The *unc-25* gene was cloned and found to encode the biosynthetic enzyme for GABA, glutamic acid decarboxylase (GAD) [15] (Figure 4). Previously, *in vitro* data had indicated that GABA could be involved in neuronal development, specifically in cell proliferation, cell migration, neurite extension or synapse formation [17]. A test of this hypothesis is to determine whether defects in

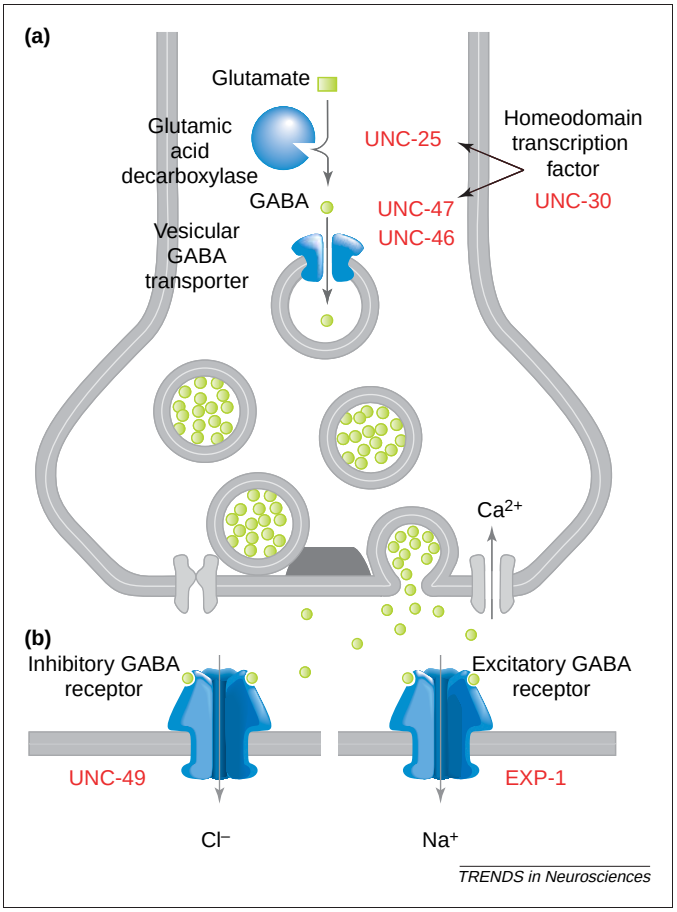

Figure 4. Proteins required for GABA function in *Caenorhabditis elegans*. (a) Proteins required for presynaptic GABA function. GABA is synthesized in the cytoplasm of the neuron from glutamate by glutamic acid decarboxylase (GAD), which is encoded by the *unc-25* gene. GABA is then transported into the synaptic vesicle by the vesicular GABA transporter (VGAT), which is encoded by the *unc-47* gene. UNC-46 is likely to have a modulatory role in vesicular GABA loading. The UNC-30 transcription factor is required for UNC-25 and UNC-47 expression in the GABA motor neurons. (b) Proteins required for postsynaptic GABA function. GABA release from the DD and VD motor neurons activates the inhibitory $GABA_A$-like UNC-49 receptor; the influx of Cl^- causes relaxation of body muscles. GABA release from the AVL and DVB motor neurons activates the novel excitatory EXP-1 GABA receptor; the influx of Na^+ causes contraction of the enteric muscles.

development are present in a mutant that eliminates GABA synthesis.

GABA is not required for axonal outgrowth or synaptic development in *C. elegans*. Specifically, GABA neuron axon morphology appeared normal in adult *unc-25* mutants [15]. In addition, multiple criteria showed that synapse formation was normal in the absence of GABA. At the light level, fluorescently tagged synaptic vesicles appeared to cluster normally in GABA neurons [15]. The postsynaptic inhibitory GABA receptor also appeared to have a normal distribution in *unc-25* mutants [18]. A reconstruction of the ventral nerve cord from serial electron micrographs demonstrated that the synaptic structure and connectivity were correct [15]. Definitive proof that these synapses are functional was provided by rescuing *unc-25* function as an adult: addition of exogenous GABA provided the AVL and DVB neurons with an external source of GABA, GABA was pumped into cells via the plasma membrane transporter, and normal behavior was restored [5]. These data demonstrate unequivocally that GABA release is not required for axon outgrowth, synapse formation or synapse maintenance in *C. elegans*.

The absence of a role for GABA in nervous system development has also been observed during fetal development in mice. Mice have two abundant isoforms of GAD, GAD67 (encoded by *Gad1*) and GAD65 (encoded by *Gad2*) and mice lacking either GAD isoform are viable at birth [19–22]. *Gad1 Gad2* double-knockout mice are also born alive, although they die soon after birth owing to a cleft palate, similar to the *Gad2* single-mutant mice [23]. There was only a trace level of GABA observed in these double mutants. The development of dendrites and axons in the cerebellum, neocortex and hippocampus of the GAD double-mutant mice appeared normal, although detailed analyses of post-fetal synaptic development were not possible in these mutants. Thus, in *C. elegans* and mice, GABA does not appear to be required for neurogenesis or axon outgrowth.

unc-47: what protein is the vesicular GABA transporter?

The vesicular GABA transporter was first identified by cloning the *unc-47* gene in *C. elegans* [6] (Figure 4). The development of a genetic strategy was a fortuitous advance for identification of this transporter because alternative approaches were unattractive. Expression cloning would have been unwieldy because UNC-47 is a rather ineffective transporter – the K_m for transport of GABA is ~5mM [6,24–26]. Moreover, molecular strategies based on the sequence of the vesicular ACh and vesicular monoamine transporters would have failed because VGAT is unrelated to these other transporters. By contrast, a genetic strategy does not rely on a robust biochemical assay, or on assumptions of homology, but instead relies only on the assumption that if you break a machine, it no longer functions. When *unc-47* was cloned it was found to encode a novel multipass transmembrane protein with weak sequence homology to plasma membrane amino acid transporters in plants. To prove that this membrane protein was VGAT, it was demonstrated that the gene was expressed in GABA neurons and that the protein was localized to synaptic vesicles. In addition, the rat homolog of UNC-47 was capable of transporting GABA into vesicular compartments of PC12 cells with the same kinetics of transport that was shown for synaptic vesicles purified from rat brain [6,27].

Searches for homologs among sequenced genomes of metazoans revealed dozens of proteins of unknown function related to VGAT. Over the past few years it has been shown that these proteins are required at plasma, lysosomal or vacuolar membranes for amino acid transport [28–33]. For example, some members of this family of proteins are thought to be important for the circuitous transport of released glutamate back into the neuron. In this model, released glutamate is taken up by astrocytes and converted to glutamine [34,35]. Glutamine is exported from the glia via the neutral amino acid transport system N. Then, the neutral amino acid transport system A transports glutamine into the neuron [28–30], where it is converted back to glutamate [36].

unc-46: is UNC-46 an auxiliary protein required for transport?

unc-46 mutants are defective for both the inhibitory and excitatory functions of GABA, which suggests that UNC-46 acts presynaptically [5]. Interestingly, overexpression of the UNC-47 GABA transporter in an *unc-46* mutant background partially rescues the defects in these animals (K. Schuske and E. Jorgensen, unpublished). Thus, UNC-46 could regulate the transport of GABA into vesicles (Figure 4). However, GABA levels are not as severely increased in *unc-46* mutants as in *unc-47* mutants (S. McIntire, pers. commun.), and the phenotype of *unc-46* mutants is weaker than *unc-47* mutants. Thus, it is likely that the UNC-46 protein has a modulatory role in GABA packaging. Molecular characterization of the gene should help to elucidate the role of the UNC-46 protein in GABA neuronal function.

unc-30: is the function of *unc-30* transcription factor conserved in vertebrates?

unc-30 mutants are defective for only VD and DD motor neuron function and, unlike *unc-49* mutants, the defect was shown to be presynaptic. First, the UNC-30 protein was required for GABA synthesis in the VD and DD neurons [5]. Second, it was required for proper outgrowth and synapse formation of these neurons (J. White, pers. commun.). The *unc-30* gene was cloned and found to encode a homeodomain transcription factor [7] (Figure 4). The UNC-30 protein was expressed in the VD and DD neurons as well as in six non-GABA neurons. Ubiquitous expression of the UNC-30 protein led to the ectopic synthesis of GABA in many non-GABA neurons as well as in some non-neuronal tissues. UNC-30 was shown to bind to promoter elements in both the *unc-25* (GAD-encoding) and *unc-47* (VGAT-encoding) genes, and these elements were necessary for DD and VD neuronal expression [37]. Therefore, UNC-30 is required for GABA neuronal specification in the D-type neurons.

Is this role of UNC-30 conserved in other organisms? UNC-30 was the founding member of a class of homeodomain-containing proteins that include Pitx1, Pitx2 and

Pitx3. The function of these transcription factors might have been conserved during evolution. First, Pitx2 can substitute for UNC-30 when expressed in *C. elegans* [8]. The behavioral and neuronal development defects in *unc-30* mutants are partially restored by expression of Pitx2 in these mutants. Moreover, activation of the *unc-25*/GAD promoter is observed in these transgenic animals. Second, regulatory domains in the human and mouse *Gad1* gene contain binding sequences for the UNC-30 class of homeodomain proteins and the mouse *Gad1* promoter is activated in neuroblastoma cells cotransfected with expression vectors containing *unc-30* or Pitx2 cDNAs [8]. However, recently published Pitx2 knockout mice were shown to have grossly normal GABA expression [38]. Therefore, if the function of Pitx2 for specifying GABA neuronal identity is conserved with UNC-30, then Pitx2 is likely to be acting redundantly with Pitx1 or Pitx3, or with another transcription factor.

How is UNC-30 acting to control axon outgrowth and synapse formation in DD and VD motor neurons? This is perhaps the most interesting role of *unc-30*, but to date none of the genes that act downstream of *unc-30* for axon outgrowth or synapse formation are known. However, it is known that the genes required for GABA neuronal function are not required for GABA neuronal development. Axon morphology of GABA neurons has been analyzed using transgenic constructs that express green fluorescent protein (GFP), and synaptic distribution in GABA neurons has been analyzed using constructs that express the synaptic vesicle protein synaptobrevin tagged with GFP [39]. Both *unc-25* and *unc-47* mutants have normal axon morphology and a normal distribution of synaptic vesicles [15,18]. Interestingly, it was recently shown that Pitx2 knockout mice have defects in axon migration in midbrain neurons, including GABA neurons, suggesting a possible conservation of this role for UNC-30 and Pitx2 [38]. Identification of targets of UNC-30 regulation will be of great interest because these genes are required specifically for GABA neuron outgrowth and synapse formation.

unc-49: is the *C. elegans* GABA receptor pharmacologically similar to the vertebrate $GABA_A$ receptor?

The *unc-49* gene encodes the GABA receptor that mediates body muscle inhibition during locomotion [16,40] (Figure 4). The *unc-49* locus encodes three distinct GABA receptor subunits by splicing a common N-terminal ligand-binding domain to one of three alternative C-terminal domains, producing the UNC-49A, UNC-49B and UNC-49C subunits [16]. This unusual gene structure is conserved in the distantly related nematode *Caenorhabditis briggsae*. The UNC-49A subunit is barely detectable *in vivo*, and does not heteromultimerize with UNC-49B or UNC-49C to form a functional receptor *in vitro*. By contrast, the UNC-49B and UNC-49C subunits are strongly expressed at neuromuscular junctions from the D-type GABA motor neurons [16,18]. When these two subunits are coexpressed in *Xenopus* oocytes they form a functional GABA-gated Cl^- channel, suggesting that the UNC-49B– UNC-49C heteromer is the native receptor *in vivo* [16]. Nevertheless, UNC-49B alone can form a homomeric receptor when expressed by itself in oocytes.

Analysis of the pharmacology of the UNC-49 receptor might reveal the structural basis for the action of allosteric regulators of $GABA_A$ receptors. The UNC-49B and UNC-49C subunits are 67% identical; nevertheless, these receptor subunits confer very different pharmacological properties [41]. Thus, the sequence differences in the UNC-49C subunit are in functionally important regions. For example, UNC-49B homomers are very sensitive to the open-channel blocker picrotoxin, whereas UNC-49B– UNC-49C heteromers are strongly resistant [41]. The differential picrotoxin sensitivity between the receptors can be accounted for by a single residue difference within the M2 domain, which has been previously demonstrated to confer picrotoxin sensitivity to vertebrate $GABA_A$ receptors [42,43]. Because $GABA_A$ receptors are pharmacological targets of anesthetics, anti-epileptics and anti-anxiety medications, further comparative analysis of the UNC-49 receptors might help elucidate agonists and antagonist action at $GABA_A$ receptors.

exp-1: how can GABA act as an excitatory neurotransmitter?

exp-1 mutants are unique among the GABA mutants because they exhibit defects only in excitatory GABA functions. Specifically, *exp-1* mutants lack enteric muscle contractions yet move normally, and thus resemble animals in which just the AVL and DVB GABA motor neurons have been killed [9,14]. The *exp-1* gene encodes a novel GABA-gated cation channel [9] (Figure 4) that is localized to the enteric muscles, adjacent to the synaptic inputs of AVL and DVB. How can GABA cause muscle contraction, when it is normally an inhibitory neurotransmitter? Electrophysiological characterization of the EXP-1 GABA receptor demonstrated that the channel is selective for cations [9], in contrast to all the other GABA-gated ion channels previously characterized, which are anion selective [44]. EXP-1 resembles conventional GABA receptors; however, the pore-forming domain, which determines ion selectivity, is divergent. How did a GABA receptor evolve cation selectivity? *In vitro* mutagenesis experiments of ligand-gated ion channels have demonstrated that three (or fewer) residues are crucial in determining ion selectivity. Specifically, Galzi and colleagues first demonstrated that changes at three residues in the α7 nicotinic ACh receptor pore-forming domain were sufficient to convert the normally cation-selective channel to an anion-selective channel [45]. The reciprocal changes were demonstrated to convert the α1 glycine receptor from anion-selective to cation-selective [46,47]. These crucial residues that are sufficient to convert the α1 glycine receptor ion selectivity are observed in the pore-forming region of EXP-1 [9], thus demonstrating that evolution is a passable molecular biologist – albeit a very slow one.

Concluding remarks

Over the past ten years, genetic screens in the nematode *C. elegans* have identified six genes required for GABA function. Although one of these genes encodes an unusual GABA-gated cation channel that does not appear to be in

vertebrates, the components involved in cell identity, biosynthesis and packaging are conserved in the vertebrate nervous system. Notably, these studies identified the genes encoding VGAT and the UNC-30 homeodomain transcription factor; genome comparisons identified the vertebrate orthologs of these genes.

Nematodes and vertebrates diverged >800 million years ago. Nevertheless, the proteins governing the mechanisms of exocytosis and neurotransmitter function are conserved in the nematode and vertebrate nervous systems. This conservation indicates that the components of the nervous system were not gradually perfected as more complex organisms arose. Rather, an elaborate nervous system evolved using the full complement of neurotransmitters, receptors and cell differentiation factors. This simple organism, perhaps a worm, then gave rise to more complex organisms, whose nervous systems differ little from those of worms except in size.

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