A Novel H⁺-coupled Oligopeptide Transporter (OPT3) from Caenorhabditis elegans with a Predominant Function as a H⁺ Channel and an Exclusive Expression in Neurons*

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We have cloned and functionally characterized a novel, neuron-specific, H⁺-coupled oligopeptide transporter (OPT3) from Caenorhabditis elegans that functions predominantly as a H⁺ channel. The opt3 gene is ~4.4 kilobases long and consists of 13 exons. The cDNA codes for a protein of 701 amino acids with 11 putative transmembrane domains. When expressed in mammalian cells and in Xenopus laevis oocytes, OPT3 cDNA induces H⁺-coupled transport of the dipeptide glycyl-sarcosine. Electrophysiological studies of the transport function of OPT3 in Xenopus oocytes show that this transporter, although capable of mediating H⁺-coupled peptide transport, functions predominantly as a H⁺ channel. The H⁺ channel activity of OPT3 is ~3-4-fold greater than the H⁺/peptide cotransport activity as determined by measurements of H⁺ gradient-induced inward currents in the absence and presence of the dipeptide using the two-microelectrode voltage clamp technique. A downhill influx of H⁺ was accompanied by a large intracellular acidification as evidenced from the changes in intracellular pH using an ion-selective microelectrode. The H⁺ channel activity exhibits a $K_{0.5}$ of 1.0 $\mu$M at a membrane potential of ~50 mV. At the level of primary structure, OPT3 has moderate homology with OPT1 and OPT2, two other H⁺-coupled oligopeptide transporters previously cloned from C. elegans. Expression studies using the opt3::gfp fusion constructs in transgenic C. elegans demonstrate that opt3 gene is exclusively expressed in neurons. OPT3 may play an important physiological role as a pH balancer in the maintenance of H⁺ homeostasis in C. elegans.

Peptide transport across the plasma membrane as one of many transmembrane activities is mediated by specific integral membrane proteins (carriers) and has been demonstrated to be a widely distributed phenomenon throughout nature in both prokaryotes and eukaryotes, such as bacteria, yeast, plants, and animals (1–3). Active transport of organic solutes in biological systems is primarily linked to a transmembrane ion gradient. The electrochemical proton gradient in microbes is the primary driving force for the transport of amino acids, sugars, and small peptides. In contrast, in animal kingdoms the predominant coupling ion for the active transport of the organic solutes seems to be Na⁺ rather than H⁺. The electrochemical Na⁺ gradient provides the driving force for active transport of most nutrients. However, in the peptide transport process it appears that this ancient driving mechanism has not been shifted during the evolution (2). It is this transmembrane H⁺ gradient that energizes the active transfer of oligopeptides. In the past several years, two different peptide transporters actively translocating di- and tripeptides, namely PEPT1 and PEPT2, have been cloned and characterized from the mammalian intestine and kidney, respectively (4–11). The major difference between these two transporters, in terms of transport function, is in the affinity toward their common substrates, the former being a low affinity type and the latter a high affinity type.

Previously, two oligopeptide transporters (OPT) from Caenorhabditis elegans, namely OPT1 and OPT2, homologous to the mammalian counterparts, PEPT1 and PEPT2, respectively, were identified, isolated, and characterized in our lab (12). Subsequently, a database search revealed that there might be another OPT isoform encoded by the gene F56P4.5 on chromosome I in C. elegans. Here we report the cloning and functional characterization of this OPT isoform designated OPT3. OPT3 is capable of mediating the H⁺-coupled transport of peptides when expressed heterologously in mammalian cells and Xenopus laevis oocytes. Interestingly, this isoform also functions as a H⁺ channel, and the H⁺ gradient-dependent H⁺ channel activity is much more predominant than the H⁺-coupled peptide transport activity. OPT3 is expressed exclusively in neurons. These studies suggest that OPT3 might play an important role in H⁺ homeostasis in C. elegans in addition to its likely role in the clearance of peptides arising from neuropeptide metabolism. In mammals, PEPT2 is expressed in the brain, and a similar role for this transporter in the clearance of peptide products arising from the hydrolysis of neuropeptides within the brain has been postulated (13–16).

EXPERIMENTAL PROCEDURES

Nematode Culture and Poly(A)⁺ mRNA Isolation—A wild type nematode strain, C. elegans N2 (Bristol) was obtained from the Caenorhab-
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ditis Genetics Center (St. Paul, MN). Nematode culture was carried out using a standard procedure with a large scale liquid cultivation protocol (17). To remove the main contaminants, the nematodes were cleaned by a sedimentation method through 15% (w/v) Ficoll 400 in 0.1 M NaCl. The pellet was used for total RNA preparation. Total RNA was isolated from transformed TR7 reagent from Life Technologies, Inc. Poly(A)+ mRNA was purified by a double affinity chromatography using oligo(dT)-cellulose (12).

**Reverse Transcriptase-Polymerase Chain Reaction**—A pair of the PCR primers specific for the putative *C. elegans* opt3 gene was designed: 5′-CCG GTA TTT TTT ATG C-3′ (forward primer) and 5′-CCG ACG TTT TTT ATG C-3′ (reverse primer). 1.5 μg RNA (~0.5 μg) isolated from mixed stage C. elegans worms was taken as template to perform RT-PCR using an RT-PCR kit from Perkin-Elmer (Norwalk, CT). A single DNA fragment was obtained by RT-PCR with an estimated size of ~0.7 kb as predicted by the distance between these two primers. Following PCR, the amplified DNA fragment was gel purified and cloned into a pGEM T Easy-vector (Promega Madison, WI), and its identity was established by sequencing. The 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 Life Technologies, Inc. was used to establish the cDNA library using the poly(A)+ RNA from *C. elegans*. The transformation of the ligated cDNA into E. coli was performed by electroporation (4, 20, 21). Intracellular ion activity was measured as the difference between the ion-selective electrode (pH) and a KCl voltage electrode (4, 20, 21). Intracellular pH measurement—Ion-selective microelectrodes were used to monitor intracellular pH ([H+]i) of oocytes as described previously (4, 20, 21). Intracellular ion activity was measured as the difference between the ion-selective electrode (pH) and a KCl voltage electrode impaled into the oocyte, and membrane potential (Vm) was measured with the two-microelectrode voltage clamp method.

**In Vitro Transcription, Oocyte Expression, and Electrophysiological Studies**—OPT3 was expressed heterologously in *X. laevis* oocytes by microinjection of OPT3 cDNA. The procedures for in vitro transcription, oocyte isolation, and microinjection have been described previously (4, 13, 19). The function of OPT3 in oocytes was monitored by the uptake of the radiolabeled dipeptide [14C]Gly-Sar and uptake of cationic peptides of the putative promoter of the opt3 gene (GenBankTM accession number AF142441). The partial PCR-cloned DNA fragment was used as a hybridization probe to screen a *C. elegans* (mixed stages) cDNA library to obtain the full-length cDNA clone. The OPT3 cDNA sequence is approximately ~2.5 kilobase pairs long with a poly(A) tail. The 5′-untranslated and the 3′-untranslated regions are 66 and 316 base pairs long, respectively. The polyadenylation signal sequence AATAAA is located at 21 nucleotides upstream from the poly(A) tail in the cloned cDNA. The cDNA sequence of *C. elegans* OPT3 has been deposited in GenBankTM with accession number AF142441. The OPT3 transporter protein deduced from the cDNA sequence contains 701 amino acid residues (Fig. 1A) and has a molecular mass of ~78.0 kDa, with an isoelectric point 7.45. According to the Kyte-Doolittle hydrophat plot, this protein possesses multiple transmembrane domains (transmembrane domain 11, with a window size of 21 amino acids). The large extracellular loop is predicted to be located between transmembrane domains 8 and 9.

The opt3 gene from *C. elegans* is localized on chromosome I and contains, at least, ~4.4-kb nucleotides. The distance between the opt3 gene and the upstream gene, T27A3.1, is estimated to be ~7.0 kb long, where the potential regulatory region of the opt3 gene is located (*C. elegans* data base, ACeDB, version 4.3, data version WS2.4–17). The presence of 13 exons in the opt3 gene is deduced by a comparison between the sequences of the cloned cDNA and the opt3 gene (GenBankTM deposit F65645). The exon-intron organization of the gene is shown in Fig. 1B. There is no identifiable transsplicing leader present in the OPT3 cDNA. This indicates that the mRNA is directly transcribed from the opt3 gene, similar to the first two oligopeptide transporters (OPT1 and OPT2) previously reported (12).

**Proton-coupled Peptide Transport by OPT3**—OPT3, when heterologously expressed in Xenopus oocytes or in mammalian cells, demonstrated transport activity toward peptide sub-
A novel peptide transporter from *C. elegans* was identified and characterized. The transporter, named OPT3, was found to be expressed in *C. elegans* HRPE cells and was shown to be a functional peptide transporter. OPT3 expression in mammalian cells (HeLa and HRPE cells) was monitored by the uptake of [14C]Gly-Sar, a model substrate. The transport activity of OPT3 was found to be pH-dependent, with a significant increase in activity when the pH was decreased from 8.0 to 5.5. The transport activity was also dependent on the concentration of the peptide substrate. The transporter was found to be functional in oocytes, where it was shown to increase the uptake of [14C]Gly-Sar.

The kinetic characteristics of the transport activity were also studied. The uptake of [14C]Gly-Sar was found to be saturable with respect to the peptide concentration at a pH of 7.5. The transport activity was also found to be dependent on the concentration of H⁺ in the perfusion buffer.

The functional expression of OPT3 in mammalian cells was also studied. The transport activity of OPT3 was found to be pH-dependent and concentration-dependent. The magnitude of the transport activity was found to increase as the pH was decreased and as the peptide concentration was increased.

The transporter was also found to be functional in oocytes, where it was shown to increase the uptake of [14C]Gly-Sar. The transport activity was also found to be pH-dependent, with a significant increase in activity when the pH was decreased from 8.0 to 5.5. The transport activity was also found to be dependent on the concentration of the peptide substrate. The transporter was found to be functional in oocytes, where it was shown to increase the uptake of [14C]Gly-Sar.

The functional expression of OPT3 cDNA was also performed in a mammalian expression system. Transient expression of OPT3 in both HeLa cells and HRPE cells was monitored by the transport of [14C]Gly-Sar. When studied in the pH range of 5.5 to 8.0, Gly-Sar influx in control cells transfected with vector alone was negligible, indicating that neither HeLa cells nor HRPE cells themselves have significant levels of peptide transport activity. In contrast, Gly-Sar influx in OPT3 cDNA-transfected cells was much higher than in control cells. Furthermore, the cDNA-dependent influx showed a marked dependence on pH. The influx increased several-fold when the pH was changed from 8.0 to 5.5. The pH dependence was seen in HeLa cells as well as HRPE cells. These data show clearly that OPT3 is a H⁺-coupled peptide transporter driven by a transmembrane H⁺ gradient.

Proton- and Peptide-induced Currents in OPT3-expressing Oocytes—The H⁺-coupled Gly-Sar transport *via* OPT3 was then studied using electrophysiological approaches in *Xenopus* oocytes expressing this transporter heterologously. The transporter-associated current measurements were made using the two-microelectrode voltage clamp protocol. These studies have revealed an interesting phenomenon associated with OPT3. In the absence of the peptide substrate, changing the pH of the perfusion buffer from 7.5 to 5.0 induced large inward currents in OPT3-expressing oocytes (Fig. 3). The magnitude of this pH-dependent inward current varied between 350–1,000 nA in different oocytes. This characteristic is unique to OPT3. When the pH of the perfusion buffer was changed from 7.5 to 5.0, the magnitude of inward currents seen in oocytes expressing *C. elegans* OPT1 or OPT2 was typically 15–55 nA (data not shown). The same is true in water-injected oocytes (the last current curve on the right in Fig. 3) or in oocytes expressing mammalian PEPT1 or PEPT2. Addition of Gly-Sar to the perfusion buffer at pH 5.0 caused a further increase in the magnitude of the inward current in OPT3-expressing cells. But the peptide-induced current was only about 20–25% of the H⁺-induced current. These studies also showed that the H⁺-induced current as well as the peptide-induced current was dependent on the concentration of H⁺ in the perfusion buffer. The magnitude of these currents decreased as the H⁺ concentration decreased in the buffer, as evidenced from the changes in currents associated with the changes in pH from 5.0 to 6.5.

The H⁺-induced currents and the peptide-induced currents of OPT3 were further analyzed in terms of their dependence on membrane potential. Steady-state currents in OPT3-expressing oocytes measured at three different conditions are shown in Fig. 4, namely pH 7.5 (A), pH 5.0 (B), and pH 5.0 plus 2 mM Gly-Sar (C). These steady-state currents, plotted as a function of membrane potential, are shown in Fig. 4D. These values were used to analyze the current-membrane potential relationship independently for the H⁺-induced current (Fig. 4E) and peptide-induced current (Fig. 4F). The magnitude of both currents increased markedly with the hyperpolarization of membrane potential. The H⁺-induced current was about 300 nA at a membrane potential of −50 mV, and this value increased to about 1,000 nA when the membrane potential was hyperpolarized to −150 mV. The corresponding values for peptide-induced current were 110 and 940 nA. At lower membrane potential, the H⁺-induced current was more predominant than the peptide-induced current. The difference between these two components appeared to become smaller when the membrane potential was hyperpolarized.

**Saturation Kinetics of H⁺-induced Currents**—We analyzed the kinetic characteristics of OPT3-mediated H⁺-dependent currents (Fig. 5). H⁺-induced currents were measured at increasing concentrations of H⁺ in the perfusion buffer and at different testing membrane potentials. All these measurements were made in the absence of peptide substrates. Fig. 5A describes the current-membrane potential relationships at different H⁺ concentrations in the range of 30 nM to 10 μM. Fig. 5B shows that the H⁺-induced current is saturable with respect to H⁺ concentration in this range. The relationship between H⁺ concentration and the magnitude of the current induced was hyperbolic at all testing membrane potentials. Fig. 5 (C and D) describes the influence of membrane potential on the kinetic parameters of the H⁺-induced current. The *K₅₀* value for H⁺ (i.e. H⁺ concentration at half-maximal current) increased as the membrane potential was depolarized. At a membrane potential of −50 mV, the *K₅₀* for H⁺ was 1.0 ± 0.2 μM. Hyperpolarization of the membrane potential decreased the *K₅₀* value for H⁺ to a significant extent. The *K₅₀*

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**Figure 1**

Amino acid sequence of OPT3. Transmembrane domains determined by hydrophobicity plot are boxed. Stop codon is indicated by filled boxes and numbered; introns are indicated by solid lines. The untranslated exons and the introns in the figure are drawn to the exact scale.

**Table 1**

| Exon | Structure | Function |
|------|-----------|----------|
| 1    | Exon 1    | H⁺ influx |
| 2    | Exon 2    | H⁺ influx |
| 3    | Exon 3    | H⁺ influx |
| 4    | Exon 4    | H⁺ influx |
| 5    | Exon 5    | H⁺ influx |
| 6    | Exon 6    | H⁺ influx |

**Figure 3**

Current-voltage relationships in OPT3-expressing oocytes. The current was measured at different membrane potentials. A: pH 7.5, B: pH 5.0, C: pH 5.0 plus 2 mM Gly-Sar. The corresponding values for peptide-induced current are shown in Fig. 4E. The magnitude of both currents increased markedly with the hyperpolarization of membrane potential.
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value for H\(^+\) was 0.3 ± 0.1 μM at a membrane potential of −150 mV.

Saturation Kinetics of Peptide-induced Currents—The kinetics of peptide-induced currents was analyzed for two peptide substrates, Gly-Sar and carnosine. The peptide-induced currents were also saturable with respect to peptide concentration. The relationship was hyperbolic for Gly-Sar as well as carnosine (data not shown). The kinetic parameters \(I_{\text{max}}\) and \(K_{0.5}\) were calculated, and the influence of membrane potential on these parameters is described in Fig. 6. The \(I_{\text{max}}\) increased in magnitude as the membrane potential was hyperpolarized (Fig. 6A). The \(K_{0.5}\) value for Gly-Sar decreased significantly when the membrane potential was hyperpolarized. The \(K_{0.5}\) for Gly-Sar was 0.74 ± 0.11 mM at −50 mV, and this value decreased to 0.36 ± 0.06 mM at −150 mV (Fig. 6B). The influence of membrane potential on \(K_{0.5}\) for carnosine was similar except that when the membrane potential was depolarized beyond −30 mV, the \(K_{0.5}\) for carnosine decreased. Under similar conditions, the \(K_{0.5}\) for Gly-Sar increased.

**OPT3-associated Intracellular acidification**—OPT3-mediated H\(^+\)-influx was demonstrated by an intracellular pH (pH\(_i\)) measurement protocol using a pH-sensitive microelectrode impaled into oocytes to continuously record pH changes, along with another microelectrode to monitor variations of the membrane potential (V\(_m\)) simultaneously. The results obtained with an OPT3-expressing oocyte are described in Fig. 7A. A switch of pH of the perfusion buffer from 7.5 to 5.5 without any dipeptide substrate caused a marked decrease in pH\(_i\), from pH\(_i\) 7.35 to ~7.15) accompanied by a marked depolarization of the membrane (from ~58 mV to +2 mV). The addition of a dipeptide substrate (Gly-Leu) to the perfusion buffer did not alter the intracellular acidification and caused a slight additional depolarization of the membrane. Removal of the dipeptide substrate from the perfusion buffer reversed the membrane depolarization. A switch of pH of the perfusion buffer from 5.5 to 7.5 resulted in a significant recovery of intracellular pH and a complete reversal of the membrane potential to the original value (~58 mV). For comparison, a similar experiment was carried out with an OPT2-expressing oocyte (Fig. 7B). A switch of pH of the perfusion buffer from 7.5 to 5.5 without Gly-Leu caused an intracellular acidification and a depolarization. The addition of Gly-Leu to the perfusion buffer resulted in marked intracellular acidification, i.e., decreased pH\(_i\), accompanied by a significant change in membrane potential (from ~5 mV to +55 mV). Removal of the dipeptide substrate stopped the intracellular acidification and reversed the depolarization markedly. A switch of pH of the perfusion buffer from 5.5 to 7.5 returned the membrane potential to the original value. However, there was only a partial recovery of pH\(_i\).

Three significant differences can be noted between the oocytes expressing OPT3 and OPT2. First, a switch of pH\(_i\) from 7.5 to 5.5 alone caused a rapid and marked acidification in the OPT3-expressing oocyte (0.25 pH units in <5 min) but a slower and smaller acidification in OPT2-expressing oocyte (0.1 pH units in 10 min). These data show that OPT3 exhibits a much higher level of peptide-uncoupled H\(^+\) conductance than OPT2. Second, the addition of a dipeptide substrate did not alter the H\(^+\) conductance pathway of OPT3, i.e., no further pH\(_i\) decrease. In contrast, the dipeptide substrate caused intracellular acidification in the OPT2-expressing oocytes, showing that OPT2 mediates a high level of peptide/H\(^+\) co-transport. H\(^+\) influx via this co-transport pathway was much higher than the H\(^+\) influx via H\(^+\) conductance pathway in the OPT2-expressing oocyte. Third, the recovery of pH\(_i\) when pH\(_o\) was changed from 5.5 to 7.5 was markedly different between the oocytes expressing OPT3 and OPT2. In the OPT3-expressing oocyte, the recovery was marked, indicating that OPT3-mediated H\(^+\) efflux was facilitated by the outwardly directed electrochemical H\(^+\) gradient. This pronounced recovery of pH\(_i\) was not seen in the OPT2-expressing oocyte. This suggests that OPT2-mediated H\(^+\) efflux is not as sensitive to a favorable electrochemical H\(^+\) gradient as OPT3-mediated H\(^+\) efflux.
Tissue-specific Expression Pattern of opt3 Gene—The tissue distribution pattern of the expression of the opt3 gene was studied by analyzing the expression of a GFP reporter construct with the expression of the reporter gene under the control of the opt3 gene promoter. As evidenced in Fig. 8, GFP was expressed exclusively in the interneuron AVA that is involved in the backward locomotion in C. elegans. Fig. 8A shows the location of the AVA interneuron with respect to that of amphid neurons. The AVA interneuron was identified by green fluorescence because of GFP expression, and the amphid neurons were identified by staining with Dil. The relative location of the GFP-positive neuron with that of the surrounding amphid neurons (ASK, ADL, ASI, ASH, and ASJ) establishes the identity of the GFP-expressing neuron as AVA. A fluorescence micros-
copy image with a standard fluorescein isothiocyanate filter is displayed in Fig. 8B, showing AVAR and AVAL neuron cell bodies (arrows) and the processes (arrowheads). The GFP expression in these neurons was detected as early as L1 larva stage and persisted into the adult stage.

DISCUSSION

In this paper we report the identification, structural and functional characterization, and tissue-specific distribution of a new member in the OPT family, namely OPT3. The opt3 gene was identified through a GenBank™ data base search, and the cDNA was isolated from a mixed stage C. elegans cDNA library. We were able to functionally express its transport activity using two different in vitro heterologous systems: X. laevis oocyte expression system and mammalian cell expression system. Sequence analysis indicates that OPT3 is a new member in the OPT family. OPT3 contains two signature sequence motifs specific for the OPT family, which might be related to the essential properties of the oligopeptide transporters (1, 26). Signature I begins from residue 71 in the OPT3 transporter protein with a consensus sequence written as (G/A)(G/A/S) (L/I/V/M/F/Y/W/A)(L/I/V/M)(G/A/S)DX(L/I/V/M/F) GSIMAD+VFGX3VIX3SXLX3G, and signature II from residue 154 with a consensus (F/Y/T)X2(L/M/F/Y) (F/Y/V)(L/I/V/M/F/Y/ W/A)X(I/V/G)N(L/I/V)(F)X2FYFXINGGSL (26). A sequence comparison at amino acid level with the other two OPT members previously isolated from C. elegans (12) has revealed that the similarity and identity between OPT3 and OPT1 (CPTA) are 49 and 38%, respectively. The similarity and the identity between OPT3 and OPT2 (CPTB) are 48 and 39%, respectively.

OPT3 possesses a broad spectrum of substrate specificity toward a variety of small peptides regardless of their charge in a H⁺-dependent and Na⁺- and Cl⁻-independent manner (data not shown). The apparent affinity (K_{0.5}^p) of OPT3 for di- and tripeptide substrates is in the micromolar range, comparable with that of another high affinity peptide transporter, OPT1, previously isolated from C. elegans (12). As far as the peptide transport activity is concerned, OPT3 appears to function similar to the other members in the OPT family. However, OPT3 is unique among these family members in terms of the H⁺-induced currents associated with the transporter.

Generally, when water-injected oocytes are subjected to a pH switch from 7.5 to 5.0 in the perfusion buffer, there is an insignificant depolarization of the membrane potential. This depolarization usually leads to a −15–25-nA inward current.
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FIG. 6. Steady-state peptide-induced currents as a function of substrate concentration in an OPT3-expressing oocyte. The steady-state peptide-dependent currents were measured at different concentrations of Gly-Sar (GS) and carnosine (CAR) in the range of 0.05–10 mM and at different testing membrane potentials using a pulse protocol. The peptide-evoked currents were saturable with respect to peptide concentrations and the relationship followed Michaelis-Menten kinetics. The data were fitted to the Michaelis-Menten equation, and the kinetic parameters were calculated. A, the effects of the testing membrane potentials on the apparent affinity of OPT3 for peptide substrates (K_{app})

B, the effects of the testing membrane potentials on the apparent affinity of OPT3 for peptide substrates (K_{app}).

detected by the two-microelectrode voltage clamp technique. A similar situation occurs in oocytes heterologously expressing the mammalian peptide transporters PEPT1 and PEPT2 or the C. elegans peptide transporters OPT1 and OPT2. Thus, the H^{+}-induced currents are minimal for these peptide transporters in the absence of peptide substrates. In the case of OPT3, the H^{+}-induced currents in the absence of peptide substrates are significantly large, varying in the range of 250–1,000 nA in different oocytes. It is this feature that has led us to classify OPT3 as a unique and novel member in the OPT family. The peptide-evoked currents at pH 5.0 are only about 50–120 nA in different oocytes. On average, the magnitude of the peptide-induced current in SGLT1 has been established and the physiological functions related to the new H^{+} transporter in the nervous system remain to be investigated.

Ectopic GFP reporter gene studies in the transgenic C. elegans have demonstrated that the opt3 gene is expressed in the cell bodies and processes of interneuron AVA (R and L), which tentatively suggests that OPT3 is a neuron-specific peptide transporter. Laser ablation experiments have shown that killing the interneurons, AVB and PVC, which innervate VB/DB motor neurons leads to defective forward movements in adults, whereas ablating the interneurons, AVA and AVD, which innervate VA/DA neurons results in defective backward movements (32). These observations strongly support a model in which DB/VB motor neurons and their associated interneurons mediate forward movement and the DA/VA motor neurons and their associated interneurons mediate backward movement. The physiological functions related to the new H^{+}/peptide symporter OPT3 in the nervous system remain to be investigated.
Another high affinity peptide transporter, PEPT2, has been shown to be present in the central nervous system by Northern blot and in situ hybridization studies in mammals (9, 14, 15). The ectopic expression approach proves to be particularly useful for pinpointing the precise time and place of GFP expression because cell lineages can be simultaneously observed using differential interference contrast imaging. However, it has to be pointed out that reintroduced transgene might be expressed sometimes in a different pattern and at different levels depending on the constituent elements used to establish the recombinant DNA. Discrepancies in expression pattern have been observed qualitatively or quantitatively between transgene and endogenous counterpart (22). Therefore, transgene expression pattern cannot be used as an exclusive means to determine the physiological expression pattern of the endogenous opt genes. The GFP expression study remains to be confirmed by immunocytochemistry.

The physiological function of the peptide transporters in the nervous system has not been understood very well. In contrast to the intestinal and the renal peptide transporters, there is no evidence at present indicating that a peptide transport system in glias or astrocytes might be of nutritional importance. In the nervous system, peptides are the most abundant chemical messengers. Potentially, this transporter could act as a general scavenger system to remove biologically active dipeptides or tripeptides (e.g. Kyotorphin; Tyr-Arg). Most likely this transport system might be related to the catabolic pathway of neuropeptides. For example, after inactivation of peptidergic signal substances by the membrane-affiliated peptidases, the resultant peptide fragments could effectively be removed by the transporter to avoid further degradation of the peptide fragments and the formation of neuroactive substances, e.g. glutamate and glycine (14, 15, 33–37).

**Fig. 7.** Proton transport by OPT3 and OPT2. Simultaneous recordings of intracellular pH (pHᵢ) and membrane potential (Vᵢ) of oocytes expressing either OPT3 (A) or OPT2 (B) are shown. For both experiments, oocytes were superfused and equilibrated in a pH 7.5 uptake solution (see “Experimental Procedures”). After pHᵢ and Vᵢ stabilized, the bath solution was switched to the 5.5 uptake solution and later to 1 mM Gly-Leu at pHᵢ 5.5. Note that the rates of pHᵢ changes are directly comparable between A and B since the time scales of the two experiments are identical.

**Fig. 8.** GFP-expression pattern driven by the opt3 gene promoter in stably transformed transgenic C. elegans. A shows a double-exposed image of AVA cell body in green (fluorescein isothiocyanate filter) indicated by an arrow, superimposed with that of the surrounding amphid neurons (ASK, ADL, ASI, ASH, and ASJ) and their processes (arrowhead) stained with DiI (Texas Red filter) in the vicinity. The intestines are also shown because of auto-fluorescent granules. B shows both AVAR and AVAL neuron cell bodies (arrows) and the processes (arrowheads). The camera lucid drawing on the right depicts the positions of the neurons and processes for clarity.
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