Cloning and Functional Characterization of a \textit{Brassica napus}
Transporter That Is Able to Transport Nitrate and Histidine*

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A full-length cDNA for a membrane transporter was
isolated from \textit{Brassica napus} by its sequence homology
to a previously cloned \textit{Arabidopsis} low affinity nitrate
transporter. The cDNA encodes a predicted protein of
589 amino acid residues with 12 putative transmembrane
domains. The transporter belongs to a multigene
family with members that have been identified in bacte-
ria, fungi, plants, and animals and that are able to
transport a range of different nitrogen-containing sub-
strates, including amino acids, peptides, and nitrate. To
identify the substrates of this plant gene, we have ex-
pressed the protein in \textit{Xenopus} oocytes. The properties
of the transporter are consistent with a proton cotrans-
port mechanism for nitrate, and the voltage dependence
of the \(K_m\) for nitrate was determined. The \(K_m\) for nitrate
was shown to increase from 4 to 14 mM as the mem-
brane voltage became more negative from \(-40\) to \(-180\) mV.
Oocytes expressing the gene could accumulate internal
nitrate to concentrations higher than those measured in
water-injected controls. A range of different substrate
molecules for the transporter was tested, but of these,
histidine gave the largest currents, although the affinity
was in the millimolar range. The pH dependence of the
activity of the transporter was different for the sub-
strates, with histidine transport favored at alkaline and
nitrate at acid external pH. Kinetic analysis of the me-
chanism of histidine transport suggests a cotransport of
protons and the neutral form of the amino acid, with the
\(K_m\) for histidine decreasing at more negative mem-
brane voltages. This gene is the first member of this family
of transporters for which the transport of two very differ-
ent types of substrate, nitrate and histidine, has been
demonstrated.

A family of mammalian peptide transporters (1, 2) has been
identified, and sequence comparisons have shown that it
includes plant peptide transporters (3–5). The mammalian pep-
tide transporters have been shown to transport a broad range
of substrates, including di- and tripeptides (2) and free amino
acids (6). The family members are characterized by all having
a consensus motif, and they have been named the proton-de-
dependent glioglycptide transporter (POT) family (7), or as most
members are peptide transporters, they have also been called
the PTR family (8). However, the family also includes plant
members that have been identified as nitrate transporters (9, 10).
In this paper, we show that another member of this family,
isolated from the plant \textit{Brassica napus}, can transport both the
amino acids and nitrate when expressed in \textit{Xenopus} oocytes.

In soil, the nitrate concentrations can vary from \(>1\) \(\mu\)M to
\(>10\) mM depending on factors such as rainfall and fertili-
sing supply (11). Soil also contains other forms of nitrogen, includ-
ing ammonium and amino acids, and these may also be nitro-
gen sources available to plants (e.g. Ref. 12). Nitrate uptake by
plants has been shown to have biphasic kinetics, with different
affinities for external nitrate; one uptake system has \(K_m\) values
for nitrate in the micromolar range, and the other in the mil-
limolar range (13). Furthermore, these transporters show differ-
fing patterns of induction by nitrate, with two different high
affinity systems, only one of which is nitrate-inducible; the
other is constitutively expressed (14). Examples of each type of
gene have been cloned: a low affinity transport system (9) and
a high affinity transport system (15).

After isolating a transporter gene, the best way to charac-
terize the electrophysiological properties of the protein is to
heterologously express the transporter in \textit{Xenopus} oocytes. The
advantage of this approach is that the activity of the trans-
porter in the oocyte plasma membrane can then be assayed by
measuring the nitrate-elicted current. In addition, in these
experiments, the membrane voltage, a parameter that is usu-
ally variable in most uptake experiments, can be controlled.
The control of membrane voltage is achieved by using the
two-electrode voltage-clamp technique on an oocyte that is
expressing the transporter. A low affinity nitrate transporter
from \textit{Arabidopsis} has been cloned, and its \(K_m\) for nitrate
was determined when expressed in \textit{Xenopus} oocytes. The \(K_m\)
for nitrate of the AtNRT1 (formerly called \textit{CHL}) gene expressed
in oocytes was measured as 8.5 mM at \(-60\) mV, but the voltage
dependence of this property was not determined (16). This
characterization was performed using oocytes in a mannitol-
based external solution, in place of the more usual NaCl-based
oocyte saline. This solution was chosen because AtNRT1 may
also be able to transport chloride (17), and a nitrate-elicted
current may be hidden in the background chloride current that
would be present in a more typical saline. However, we have
used a typical saline to demonstrate nitrate transporter activ-
ity in oocytes injected with \textit{BnNRT1:2} (alternative name
\textit{BnNRT1B}) cRNA. Furthermore, we employed the activity of
endogenous anion channels to demonstrate that oocytes ex-
pressing this related transporter from \textit{Brassica napus}
Nitrate and Histidine Transporter

(BnNRT1;2) can accumulate nitrate, but not chloride. These results show that this low affinity nitrate transporter is able to transport several different nitrogen-containing molecules; however, the transport requires high external concentration of the substrate, suggesting that if present in root cells, it is most likely to function in uptake of nitrate from the soil when it is available at high concentrations. However, the production of mRNA for the transporter is induced in roots by treatment with only low external concentrations of nitrate.

EXPERIMENTAL PROCEDURES

Plant Material—Oilseed rape seedlings (Brassica napus L., cv. Kentan nova) were grown hydroponically for 1 week and supplied with a nitrogen-free but otherwise complete nutrient solution at pH 6.5 (18). The plants were maintained in a growth chamber under a photon flux density of 200 μmol m⁻² s⁻¹ (400–700 nm) with a diurnal light cycle of 16 h of light followed by 8 h of darkness. The temperature and humidity were kept at 25 °C and 80%, respectively.

Isolation of RNA and Synthesis and Cloning of cDNA—Total RNA from roots supplied with 25 mM KNO₃ for 3 h was phenol/chloroform-extracted and purified according to Oehlen et al. (15) and subsequently used for preparing poly(A)⁺ RNA with oligo(dT)₃⁴-Dynabeads (Dynal, Oslo, Norway). The mRNA was converted to cDNA using a TimeSaver cDNA synthesis kit (Pharmacia Biotech, Uppsala, Sweden), ligated to dephosphorylated Agt10 arms, and in vitro packaged (Stratagene). Approximately 2.0 × 10⁶ plaques from the unamplified agt10 cDNA library were selected at medium hybridization stringency. The [³²P]cIdCTP-labeled probe was prepared from CHL1 cDNA (kindly provided by Dr. N. Crawford) using a DECAprime DNA labeling kit (Ambion Inc.). Positive clones of interest were selected into pBluescript II KS⁺ (Stratagene), amplified in XL1-Blue, and sequenced using the Sequenase II kit (Amersham International, Buckinghamshire, United Kingdom) and synthetic oligonucleotide primers.

Ribonuclease Protection Assay— cRNA Preparation—A full-length cDNA for the BnNRT1;2 transporter in the Xenopus oocytes, the cDNA insert was excised from pBluescript, blunted, and inserted into the BgII site of the dephosphorylated Xenopus expression vector pSP64T. This vector provides 5'- and 3'-flanking sequences from the Xenopus β-globin gene to any cDNA that provides its own initiation codon (19, 20).

RIBONUCLEASE PROTECTION ASSAY (RPA)—The expression of BnNRT1;2 mRNA in roots was deduced by a ribonuclease protection assay (Hyb-Speed™ RPA, Ambion Inc.). A probe for the 3'-untranslated region was used to minimize cross-hybridization to related transcripts since cDNA cloning and Southern analyses indicated that there are several homologous genes in the rape genome. The RPA analysis was performed according to the manufacturer's recommendations using 25 μg of root total RNA and 3 × 10⁵ cpm of the gel-purified riboprobe. All samples were normalized to 50 μg of RNA with yeast total RNA. The 388-base pair RNA probe was transcribed and labeled with [³²P]UTP to high specific activity (MAXIscript, Ambion Inc.) after digestion of the plasmid with BpaAI. Digestion of the probe target mRNA was performed with RNase T only, as the 3'-untranslated region is rather AU-rich. RNA was extracted as described above, before as well as 1 and 6 h after the addition of 100 μM KNO₃.

cRNA Preparation—A full-length cDNA for the B. napus gene BnNRT1;2 was constructed in a Xenopus expression vector (pSP64T). The construct was linearized by digestion with BamHI, and cDNA was transcribed and capped using an SP6 mRNA mMachine™ in vitro transcription kit (Ambion Inc.) according to the manufacturer's instructions.

Oocyte Preparation and Injection—Oocytes were removed and treated as described previously (21). Stage V or VI oocytes (22) were chosen for injection with 50 nl of BnNRT1;2 cRNA (1 μg µl⁻¹) or 50 nl of diethyl pyrocarbonate-treated water and were assayed for transporter activity 4–5 days after injection. For the measurement of internal nitrate concentrations, we decided to use a more usual frog saline containing 116 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 5 mM HEPES (pH 7.2). Other details of the electrophysiology methods have been described previously (21).

RESULTS

Isolation of Clones and Sequence Analysis—The screening of the agt10 library demonstrated that cDNA homologous to the AtNRT1 cDNA (CHL1) was rather abundant; ~10 positive plaques of 100,000 were obtained. Sequence analysis revealed that one of the cDNA clones (BnNRT1;2) (26) practically represented a full-length clone, 2.0 kilobase pairs in length. It contains an open reading frame corresponding to a 65-kDa protein consisting of 589 amino acids. By sequence comparison, BnNRT1;2 was shown to be highly homologous to AtNRT1 both at the nucleotide level (85%) and at the level of deduced amino acid sequence. The protein corresponding to the 2.0-kilobase pair clone contains 12 hydrophobic amino acid segments. These putative membrane-spanning regions are separated into two groups, six in each, with a long putative cytoplasmic loop in the middle containing charged amino acids. The proposed topology of the protein is almost identical to the one

1 The abbreviations used are: ribonuclease protection assay; MES, 2-(N-morpholino)ethanesulfonic acid.
predicted for AtNRT1 (CHL1) (see Ref. 9), and it retains the consensus motif (FYXXINXGSX) described for the protein family (7, 8). A comparison of the amino acid identity between BnNRT1;2 and some other members of the family is shown in Table I. The comparison shows the most similarity to the Arabidopsis (9) and tomato (10) homologues, which are 97 and 82% homologous to BnNRT1;2, suggesting that both of these are nitrate transporters. The identity comparison with other members of the family suggests that they are more distantly related, and they have all been shown to be peptide transporters (Table I).

Root mRNA Expression—Significant hybridization of the 3′-untranslated region of the BnNRT1;2 clone to total RNA from roots was only obtained if the roots had been pretreated with nitrate, as deduced from RPA analysis (Fig. 1). This result clearly demonstrates that the gene coding for this specific protein is highly nitrate-regulated. The same pattern, as seen here at 100 μM nitrate, can also be seen after the addition of 10 μM nitrate (data not shown), indicating that the sensitivity of the system for nitrate perception is very high.

Steady-state Nitrate-dependent Currents in Xenopus Oocytes Injected with BnNRT1;2 cRNA—The steady-state currents of the transporter activity were measured as a function of membrane voltage and nitrate concentration in oocytes that had been previously injected with BnNRT1;2 cRNA. No nitrate-elicited currents for water-injected oocytes could be measured (data not shown). Fig. 2A shows the I-V difference curves obtained from a cRNA-injected oocyte on treatment with sodium nitrate ranging from 1 to 20 mM at pH 7.5. The currents varied between oocytes and were typically in the range 200–300 nA (see Table II). The measured accumulation of nitrate large enough to demonstrate that ion equilibrium potential (−18 mV), assuming an internal concentration of 62 mM (27). This result suggests that the internal chloride concentrations are not significantly different between cRNA- and water-injected oocytes. However, when external chloride was replaced by a similar concentration of nitrate, the reversal potentials were significantly different between BnNRT1;2 and water-injected oocytes: −18.8 mV for the former and −50.5 mV for the later (Table II). Using the Nernst equation, the estimated internal nitrate concentrations from the reversal potentials are 17 mM in water-injected oocytes and 57 mM nitrate in BnNRT1;2-injected oocytes (Table II). The oocytes were voltage-clamped at −50 mV when exposed to 120 mM nitrate before the tail current measurements. At this membrane potential, the oocytes could passively accumulate 17 mM nitrate, which is much lower than that estimated from the reversal potential in BnNRT1;2-injected oocytes. However, the resting potentials of oocytes prior to voltage clamping are also shown in Table II; from these, the equilibrium concentrations could also be calculated, and these are shown Table II. The measurements of internal nitrate were confirmed using nitrate-selective microelectrodes (Table II). The results in Table II show that cRNA-injected oocytes accumulate more nitrate than similar water-injected oocytes. However, in no case were the accumulations of nitrate large enough to demonstrate that active transport of nitrate is occurring (i.e., the measured accumulation was significantly larger than that predicted from the Nernst equation or equilibrium potential).

Other Substrates for the Transporter—Although the gene was isolated by its sequence homology to a low affinity nitrate transporter, it belongs to a family of transporters that includes peptide and amino acid proton cotransporters, we tested other substrates in oocytes that had been injected with BnNRT1;2 cRNA. These experiments showed that the oocytes showed larger currents when supplied with histidine than when treated with nitrate. However, several different substrates were tested, including other basic amino acids and anions. The results of these currents relative to the histidine currents for a range of different amino acids are shown in Table III. Only the basic amino acids lysine and arginine appeared to be transported; the other amino acids tested did not elicit significant currents in oocytes injected with BnNRT1;2 cRNA when compared with water-injected controls (Table III). Micromolar concentrations of ammonium did not elicit currents, and concentrations above 0.5 mM gave large currents in both cRNA- and water-injected oocytes. Also, D-histidine, free imidazole, and

| Name          | Identity in the amino acid sequence to BnNRT1;2 | Substrate and source reference |
|---------------|-----------------------------------------------|--------------------------------|
| AtNRT1 (CHL1) | 96.9                                          | Nitrate transport (9)          |
| LeNRT         | 82.4                                          | Nitrate transport (10)         |
| AtPTR2B       | 65.9                                          | Peptide transport (3, 26)      |
| HPEPT1        | 56.0                                          | Peptide transport (2)          |
| PepT1         | 51.7                                          | Peptide/histidine transport (6) |
| PHT1          | 51.2                                          | Peptide transport (1)          |
| AtPTR2A       | 50.9                                          | Peptide transport (4)          |

FIG. 1. Northern blot analysis showing root-specific expression and nitrate induction of BnNRT1;2. Shown are the results from RPA analysis of total RNA extracted from B. napus roots before (0 h) as well as 1 and 6 h after the addition of 100 μM KNO3. The left part of the autoradiogram shows the probe yeast RNA control, undigested and after digestion. All digestions were carried out with RNase T, and the same amount of probe was added to each lane.
the dipeptide histidine-leucine failed to elicit significant current in cRNA-injected oocytes (data not shown). Other anions tested included nitrite, cyanate, and chlorate, all of which elicited small negative currents, which were not significantly different from those obtained in control water-injected oocytes.

Fig. 4 shows the result of experiments in which cRNA-injected oocytes were incubated in radiolabeled L-histidine at pH 5.5; these oocytes accumulated significantly more histidine. Fig. 4B shows the I-V difference curve for the same cRNA-injected oocytes treated with L-histidine, nitrate, and L-histidine plus nitrate at pH 5.5. The I-V curve shows that 10 mM L-histidine elicited a larger current than 10 mM nitrate, but when nitrate and L-histidine were applied at the same concentration in the same solution, the current obtained was the same size as that obtained with histidine alone.

**pH Dependence of Nitrate- and Histidine-elicited Currents**—Fig. 5 shows nitrate-elicited currents at two different pH values in a BnNRT1;2-injected oocyte. Nitrate applied externally at 10 mM elicited larger currents (negative currents, cation-inward) at more acid pH (pH 5.0) than at pH 7.2 (Fig. 5A). In contrast, L-histidine transport had a very different pH optimum compared with nitrate; the largest currents were obtained at more alkaline pH (Fig. 5B). Similar treatments elicited no more than 1-nA currents in water-injected oocytes.

**Kinetics of Histidine Transport**—The concentration dependence of the histidine-elicited currents at pH 5.5 could be fitted to a Michaelis-Menten function at membrane voltages more negative than −100 mV. However, in these oocytes, the expression of BnNRT1;2 was lower than that in previous experiments; for example, the $i_{\text{max}}$ value for nitrate-elicited currents at pH 5.5 was only 10–20 nA (data not shown). The fitted lines and values at −100, −120, −160, and −180 mV are shown in Fig. 6A. The voltage dependence of the kinetic parameters obtained from the fitted data is shown in Fig. 6B. Both $K_m$ and
Calculated internal \( [\text{NO}_3^-] \) at pH 6 mean and measured mean compared to theoretical nernstian concentrations for water- and BnNRT1;2-injected oocytes.

Reversal potentials were determined from the tail currents (24) measured in either 120 mM sodium chloride or nitrate in the external solution, which also contained 2 mM KCl, 2 mM CaCl\(_2\), 1 mM MgCl\(_2\), and 5 mM MES (pH 5.5). The electrical potential values are shown as the difference relationships for the same oocyte that had been previously injected with cRNA for BnNRT1;2; however, we will begin by discussing whether there are other interpretations of these observations.

One explanation for the apparent transport of two such different substrates is that the expression of BnNRT1;2 stimulates the expression or activity of an endogenous transporter present in the oocyte plasma membrane. There are already a few examples of this phenomenon; the injection of cRNA for minK (28) and IsK (29) has been shown to activate endogenous oocyte channels. However, if the two different substrates were each transported by different membrane proteins, then the currents obtained when both substrates are supplied simultaneously should be additive, and this was not the case (see Fig. 4B). Furthermore, water-injected control oocytes did not show any significant histidine- or nitrate-elicited currents and so do not provide any evidence for either endogenous transport system in oocytes. Another possibility is that the positively charged form of histidine can donate or substitute for protons in the cotransport mechanism. This would seem unlikely because histidine transport can occur without any nitrate in the external solution, but uncoupled proton transport has been described for a \( \text{H}^+ / \text{sucrose cotransporter} \) (30).

The gene BnNRT1;2 belongs to the emerging PTR family of transporters, which have been shown to transport various substrates ranging from peptides and amino acids to nitrate (8). Although, mammalian and plant members of the family have been shown to transport peptides and histidine (3, 6), this is first example that has been shown to transport both nitrate and amino acids.

The gene BnNRT1;2 codes for a transporter that, when expressed in oocytes, can transport both nitrate and amino acids.

### Table II

| Parameter | Water-injected oocyte | BnNRT1;2 RNA-injected oocyte |
|----------|-----------------------|-----------------------------|
| \( E_0 \) (mV) | \(-20.3 \pm 0.9 \) (4) | \(-15.7 \pm 2.1 \) (6) |
| \( E \) (mV) | \(-50.5 \pm 6.4 \) (4) | \(-18.8 \pm 4.2 \) (6) |
| Measured mean resting potential (mV) | \(-42.2 \pm 5.4 \) (4) | \(-31.9 \pm 5.6 \) (6) |
| Calculated internal \( [\text{NO}_3^-] \) (mM) from \( E \) | 16.7 (13, 22) | 57 (49, 65) |
| Theoretical nernstian internal \( [\text{NO}_3^-] \) (mM) | 23 (19, 29) | 34.5 (28, 43) |
| Measured internal \( [\text{NO}_3^-] \) (mM) | 8 (3, 15) (4) | 41 (29, 55) (4) |

### Table III

| Substrate | Histidine current at \(-160 \) mV % |
|-----------|----------------------------------|
| Arginine  | 50 |
| Lysine    | 47 |
| aa mixture 1\(^a\) | 14 |
| aa mixture 2\(^b\) | 15 |

\(^a\) aa mixture 1 contained alanine, leucine, glycine, glutamine, and threonine.  
\(^b\) aa mixture 2 contained asparagine, proline, valine, aspartic acid, isoleucine, and tyrosine.

**Fig. 4. Histidine and nitrate uptake by oocytes.** A, radiolabeled histidine uptake by BnNRT1;2 cRNA- and water-injected oocytes in saline at pH\(_5\) 5.5. B, \( I-V \) difference curve showing histidine-elicited current compared with nitrate-elicited current from the same oocyte. The \( I-V \) difference relationships for the same oocyte that had been previously injected with cRNA for BnNRT1;2 were determined at pH\(_5\) 5.5 with 10 mM nitrate (\( \bullet \)), 10 mM histidine (\( \bigcirc \)), or 10 mM histidine + 10 mM nitrate (\( \square \)).
and amino acids. Furthermore, the I-V difference curves show that both types of substrate generate inward cation currents, which would be consistent with the idea that transport is proton-coupled, as is found for the members of this family that have been characterized. Comparative analysis of the amino acid sequence has indicated that the family is distinct from other families of secondary transporters and can be divided into two subfamilies (8). The genes \(BnNRT1;2\), \(AtNRT1\), and \(AtPTR2-B\) are all classified in one subfamily, suggesting that the nitrate transport activity we have observed may also be found for \(AtPTR2-B\) (3). However, nitrate did not compete with radiolabeled dileucine uptake in yeast cells expressing \(AtPTR2-B\) (5). The results shown in Fig. 4 suggest that supplying both substrates together may not necessarily decrease the uptake of one of them because the histidine-elicited current was not altered by the addition of nitrate to the bathing solution.

A membrane protein that is able to transport such very different types of substrate is unusual in biology. These substrates have very different sizes, and so presumably they have two different binding sites on the protein. As each substrate-elicited current was not additive when supplied together, some models for the transport can be discounted. For example, there cannot be two different forms of the protein, each transporting the different substrates, because this would also result in additive currents when both substrates were supplied. However, both substrates were required in millimolar concentrations, and other plant transporters have been identified that have higher affinities for both substrates (e.g. Refs. 15 and 31). The in vivo biphasic uptake observed for nitrate (13) and the multiphasic amino acid uptake kinetics (32) are explained by the activity of transporters with differing substrate affinities. The related plant peptide transporter \(AtPTR2-B\) also required millimolar concentrations of histidine to complement the yeast amino acid uptake mutant (33), but it is not known whether the amino acid transport activity is important in vivo (3).

The rat peptide/histidine transporter was shown to have a \(K_m\) for histidine of 17 \(\mu\)M (6), but in contrast, much higher (millimolar) concentrations of histidine were required for the currents obtained in this work and for yeast complementation

![Graph A: pH dependence of nitrate- and histidine-elicited currents in BnNRT1;2 cRNA- and water-injected oocytes.](image)

**Fig. 5.** pH dependence of nitrate- and histidine-elicited currents in \(BnNRT1;2\) cRNA- and water-injected oocytes. A, the I-V difference relationships were determined at pH 7.2 (●) and pH 6.0 (▲) when the oocyte was exposed to 10 mM nitrate after previously allowing a 5-min adjustment period to each different proton concentration. The I-V difference relationship of a water-injected oocyte is also shown (●). B, I-V difference relationships of histidine-elicited currents in \(BnNRT1;2\) cRNA-injected oocytes at pH 5.5 (●) and pH 8.5 (▲). The I-V difference relationship of a water-injected oocyte is also shown (●).

**Fig. 6.** Kinetics of histidine-elicited currents at pH 8.5 in \(BnNRT1;2\) cRNA-injected oocytes. A, the I-V difference relationships were determined for oocytes treated with differing concentrations of histidine. The voltage-response curves were obtained by plotting histidine-elicited currents against external histidine concentrations, and the data were fitted to the Michaelis-Menten equation (see “Experimental Procedures”) at four different membrane voltages (−100, −120, −160, and −180 mV). B, voltage dependence of the \(K_m\) (●) and \(i_{\text{max}}\) (▲) for histidine. These parameters were determined from the fitted curves like those shown in A.
Nitrate and Histidine Transporter

by AtPTR2-B (33). Furthermore, the uptake of radiolabeled histidine was greatest at pH 5.5 (6), which again contrasts with the pH optimum for the activity of BnNRT1;2 (Fig. 5B). At pH 5.5, the 1+ form of histidine is the chief ionic species present in the solution, although a smaller amount of the zwitterion will also be present (0.28 times less). The other ionic forms of histidine will be far less abundant, and in a millimolar solution of histidine, only micromolar concentrations of the 2+ form and even less of the 1− form will be present. More alkaline pH will increase the amounts of the zwitterion and the 1− form as the histidine-elicted currents increase, so it seems likely that one or both of these species are transported, but it is difficult to identify which ionic species of histidine is transported. The alkaline pH optimum does not suggest a histidine channel mechanism for uptake because the positively charged forms are less abundant. However, at these pH values, there will also be a smaller proton gradient to drive the cotransport, but the uptake can be driven by a more negative membrane potential. This idea is supported by the actual shape of the I-V relationship shown in Fig. 5E: at the more alkaline pH, the slope of the line increased, indicating that the histidine current has become more voltage-dependent. Another unrelated plant amino acid transporter (AAP5) has been shown to transport the neutral form of histidine in cotransport with protons (34). Therefore, it is possible that BnNRT1;2 also transports histidine as the zwitterion in cotransport with protons, and the kinetic analysis in Fig. 6 supports this hypothesis. The transport of the neutral form of histidine could explain the requirement for relatively high concentrations of histidine at pH 5.5. It is unclear whether the low affinity amino acid transport activity of BnNRT1;2 is a major function of the nitrate transporter in planta.

For more information on the function of BnNRT1;2 in vivo, it will be important to identify in which root cell type this transporter is expressed because the external concentrations of substrates will determine its role in the plant. Genes homologous to BnNRT1;2 are expressed in the root hair cells of tomato, sugarcane, and the roots of Arabidopsis (33). However, BnNRT1;2 is expressed in the root and so could be involved in supplying the growing roots with histidine or the uptake of nitrate. The diverse pH optima for each of the substrates may indicate distinct roles in different parts of the plant according to the external pH, perhaps as a nitrate transporter at the soil interface or a histidine transporter into developing cells of the root tip. There may be other substrates for the transporter, such as particular peptides, that have yet to be determined. The low affinity nitrate transporter is induced by very low external concentrations of nitrate outside the roots, so the production of low affinity nitrate transporters does not require the presence of high external concentrations of nitrate. This result may indicate that the induction of nitrate transport in plants is dependent on the presence of nitrate in the environment and not the actual concentration of available nitrate.

The transport of two such different substrates by a single protein suggests that there may be other carriers with this type of multipurpose function. The design of the oocyte experiments is limited by the substrates that are offered by the experimenter, and usually the choice of these depends on the assignment of a gene to a family and therefore a particular function, and appropriate substrates are applied to assay activity. Perhaps some already characterized peptide and amino acid transporters of this family have other anion substrates yet to be identified. The affinity of the transporter may be very important in defining the in vivo function; for example millimolar concentrations of some substrates, particularly peptides, may not exist in vivo. Finally, the naming of transporter genes according to their substrate becomes very difficult, and the most likely in vivo substrate could depend on where the gene is expressed.

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