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Minireview

Nitrate transporters and peptide transporters

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Abstract In higher plants, two types of nitrate transporters, NRT1 and NRT2, have been identified. In Arabidopsis, there are 53 *NRT1* genes and 7 *NRT2* genes. NRT2 are high-affinity nitrate transporters, while most members of the NRT1 family are low-affinity nitrate transporters. The exception is CHL1 (AtNRT1.1), which is a dual-affinity nitrate transporter, its mode of action being switched by phosphorylation and dephosphorylation of threonine 101. Two of the *NRT1* genes, *CHL1* and *AtNRT1.2*, and two of the *NRT2* genes, *AtNRT2.1* and *AtNRT2.2*, are known to be involved in nitrate uptake. In addition, *AtNRT1.4* is required for petiole nitrate storage. On the other hand, some members of the NRT1 family are dipeptide transporters, called PTRs, which transport a broad spectrum of di/tripeptides. In barley, HvPTR1, expressed in the plasma membrane of scutellar epithelial cells, is involved in mobilizing peptides, produced by hydrolysis of endosperm storage protein, to the developing embryo. In higher plants, there is another family of peptide transporters, called oligopeptide transporters (OPTs), which transport tetra/pentapeptides. In addition, some OPTs transport GSH, GSSH, GSH conjugates, phytochelatins, and metals.

Keywords: Nitrate transporter; Peptide transporter; NRT1; NRT2; PTR; OPT

1. Introduction

In higher plants, there are two types of nitrate transporters, known as NRT1s and NRT2s, and two types of small peptide transporters, known as PTRs (peptide transporters) and OPTs (oligopeptide transporters). NRT2s are high-affinity nitrate transporters, while most NRT1s are low-affinity nitrate transporters, with the exception of CHL1 (AtNRT1.1), which is a dual-affinity nitrate transporter [1]. PTRs are di/tripeptide transporters, while OPTs are tetra/pentapeptide transporters. Two plus two normally equals four; however, in this case, two plus two equals three, as NRT1s and PTRs belong to the same family, known as NRT1(PTR). In this review, we will discuss these three transporter families. No sequence homology is found between the NRT1(PTR) family and either the NRT2 family or the OPT family. Most of the in planta functions of the NRT1(PTR), NRT2, and OPT transporters have been identified in Arabidopsis, in which there are 7 *NRT2* genes, 53 *NRT1*(*PTR*) genes, and 9 *OPT* genes.

2. NRT1(PTR) family

The first *NRT1*(*PTR*) gene isolated was *CHL1* (*AtNRT1.1*). CHL1 stands for CHLorate resistant mutant 1. Chlorate, a nitrate analog, can be taken up by plants using nitrate uptake systems and converted by nitrate reductase (NR) into chlorite, which is toxic for plants. Mutants defective in nitrate uptake or NR activity are resistant to chlorate treatment. The low-affinity nitrate uptake mutant, *chl1*, was isolated in 1978 [2] and the *CHL1* (*AtNRT1.1*) gene was isolated using a T-DNA-tagged mutant in 1993 [3]. At that time, CHL1 was a novel protein showing no sequence similarity with any protein in the database. Using the Xenopus oocyte expression system, it was shown to be a proton-coupled nitrate transporter [3].

In 1994, five di/tripeptide transporter genes were identified independently in the rabbit (PepT1) [4], a fungus (fPTR2) [5,6], Arabidopsis (AtNTR1, renamed as AtPTR2) [7,8], yeast (PTR2) [9] and a bacterium (DtpT) [10] by functional cloning based on peptide transport activity when expressed in Xenopus oocytes (PepT1), complementation of a yeast mutant (fPTR2, AtPTR2 and yeast PTR2), or complementation of an *Escherichia coli* mutant (DtpT). These peptide transporters were found to share sequence similarity with the nitrate transporter CHL1, and, together, they form a new transporter family, called NRT1 (PTR).

All the evidence indicates that nitrate transporters cannot transport peptide [11–13], while peptide transporters cannot transport nitrate [14], i.e. peptide transporters and nitrate transporters are functionally distinct. Nitrate and peptides are very different in structure. The question why peptides and nitrate share the same family of transporter has puzzled workers in the field ever since the identification of NRT1(PTR) family. This puzzle should be solved in the future by structure determination of the nitrate transporters and peptide transporters in this family by mutagenesis or crystal structure studies. The common feature of peptides and nitrate is that both are nitrogen sources: nitrate is the primary nitrogen source in higher plants, while di/tripeptides are the nitrogen sources in animals. CHL1 (AtNRT1.1) is involved in taking nitrate from the soil [15,16], and PepT1, expressed in the intestine, is involved in absorption of the di/tripeptide products of protein digestion [4]. Most secondary transporters in animals are sodium-coupled, but PepT1, like NRT1, is a proton-coupled transporter. Since all the NRT1(PTR) transporters identified

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in organisms other than higher plants are di/tripeptide transporters, it is more likely that nitrate transport activity evolved from an ancient peptide transporter.

2.1. NRT1(PTR) family in Arabidopsis and rice

Another remarkable feature of the NRT1(PTR) family is the number of *NRT1*(*PTR*) genes in higher plants. In contrast to the low number in other organism (six in humans, four in *C. elegans*, three in Drosophila, and one in yeast), Arabidopsis has 53 *NRT1*(*PTR*) genes and rice 80, suggesting that this family plays some unique function in higher plants. We can ask whether transport of nitrate and/or peptide is sufficient to account for the large numbers of *NRT1*(*PTR*) genes in higher plants or whether there are any unidentified substrates or functions for this family.

All of the NRT1(PTR) transporters in higher plants contain 12 putative transmembrane (TM) spanning regions, with a large hydrophilic loop between TM domains 6 and 7. At1g72120 was originally predicted to encode a protein with 24 TM domains, but Northern blot analysis and RT-PCR using various primers indicate that At1g72120 should be split into two genes, At1g72115 and At1g72125, each encoding a protein with the typical 12 TMs (Wang, Yang, and Tsay, unpublished data). The position of the long hydrophilic loop between TMs 6 and 7 is unique to higher plant NRT1(PTR) and rat PHTs [14]. In most animal NRT1(PTR) transporters, the long loop is located between TMs 9 and 10, while, in fungi, it is between TMs 7 and 8. However, the function of the long hydrophilic loop in the NRT1(PTR) transporters has not been elucidated.

Phylogenetic analysis of NRT1(PTR) transporters in Arabidopsis and rice, together with BnNRT1-2 from Brassica [17], HvPTR1 from barley [18], and AgDCAT1 from alder [19] shows that they can be classified into four subgroups I, II, III and IV (labeled, respectively in red, green, pink, and blue in Figs. 1 and 2). Four clusters in the phylogenetic tree (Os11g18044–Os04g41410, Os04g50930–Os07g41250, Os10g02220–Os10g02080, and Os04g59480–Os01g65120) are rice specific and two clusters (At1g72115–At1g22540 and At3g45650–At3g45720) are Arabidopsis specific indicating that the genes in these clusters evolved by duplication after speciation events. Indeed, genes in the Arabidopsis-specific clusters are either closely linked or located in the duplicated blocks of the genome.

RT-PCR analysis using gene-specific primer shows that 51 of the 53 Arabidopsis *NRT1*(*PTR*) genes are expressed, and that only two (At1g69860 and At3g45690), for which no transcript could be detected in the tissues tested, might be pseudogenes (Fig. 2). Seven *NRT1*(*PTR*) genes are tandemly clustered on chromosome 3 (At3g45650–At3g45720), and five of these are root-specific, indicative of functional redundancy. In addition, there are 12 pairs of genes, marked with brackets in Fig. 2, which (1) share the highest degree of sequence similarity with each other, and (2) are either closely linked or located on duplicated blocks of the genome. When the tissue-specific expression patterns are compared between the genes in each pair, identical patterns are seen with only three pairs (marked with a gray background in Fig. 2). Thus, most of the 53 genes, even those sharing a high degree of sequence similarity, exhibit different tissue expression patterns and may play unique functions in Arabidopsis.

So far, using Xenopus oocyte system for functional studies, 13 plant *NRT1*(*PTR*) genes (*AtNRT1.1* [At1g12110] [3], *BnNRT1-2* [17], *AtNRT1.2* [At1g69850, *NTL1*] [12], and *AtNRT1.4* [At2g26690, *NTL3*] [11] in group I, *OsNRT1.1* [13] and At1g32450 [*AtNRT1.5*, *NTL2*] in group II, At1g72115 and At1g72125 in group III, and At1g27080 [*AtNRT1.6*, *NTL9*], At1g69870 [*AtNRT1.7*, *NTL4*], At1g18880, At5g62680 and At1g52190 [*NTL8*] in group IV [our unpublished data]) have been proven to encode nitrate transporters (Fig. 1). Nitrate transporters are found in all four groups. On the other hand, using yeast and/or Xenopus oocytes for functional studies, three of the plant *NRT1*(*PTR*) genes (*AtPTR2* [8,14,20], *HvPTR1* [18], and *AtPTR1* [21]) were found to encode peptide transporters. All three belong to a cluster in group II (Fig. 1). In addition, *AtPTR3* (At5g46050) in group III has been shown to be able to complement a yeast dipeptide uptake mutant [22], but its dipeptide transport activity has not been directly tested in either yeast or oocytes. In summary, nitrate transporters are found in all four groups, while dipeptide transporters mainly belong to group II, with one member AtPTR3 in group III.

2.2. Nitrate transporters in the NRT1(PTR) family

2.2.1. CHL1 (AtNRT1.1).

CHL1 (*AtNRT1.1*) was not only the first *NRT1*(*PTR*) gene to be identified, but is also the most extensively studied. The nitrate concentration in the soil can vary by four orders of magnitude from the μM to mM range. To counteract this fluctuation, plants have evolved two nitrate uptake systems, one high-affinity, with a K_m in the μM range, and one low-affinity, with a K_m in the mM range (Fig. 3). When the *chl1* mutant was first isolated, nitrate uptake studies showed that it was defective in low-affinity nitrate uptake, but had normal high-affinity nitrate uptake activity [23]. In addition, based on the currents elicited by different concentrations of nitrate, the K_m, calculated in *CHL1*-injected oocytes, was about 5 mM, in the low-affinity range [15]. On the basis of these two pieces of evidence, the low- and high-affinity nitrate uptake systems were for a long time thought to be genetically distinct, and CHL1 was thought to be a low-affinity nitrate transporter. However, two later independent studies showed that high-affinity nitrate uptake was also defective in the *chl1* mutant [1,24]. In addition, Xenopus oocytes expressing AtNRT1.1 (CHL1) were found to exhibit two phases of nitrate uptake, with a K_m of about 50 μM for the high-affinity phase and a K_m of about 4 mM for the low-affinity phase, indicating that CHL1 is a dual-affinity nitrate transporter [1].

The mode of action of AtNRT1.1 (CHL1) is switched by phosphorylation and dephosphorylation of threonine 101 (Fig. 3). Xenopus oocytes expressing the T101A mutant, which cannot be phosphorylated, exhibit only low-affinity nitrate uptake activity; while oocytes expressing the T101D mutant, which mimics the phosphorylated form, exhibit only high-affinity nitrate uptake activity [25]. This indicates that phosphorylated AtNRT1.1 (CHL1) functions as a high-affinity nitrate transporter, and dephosphorylated CHL1 functions as a low-affinity transporter. The phosphorylation levels of AtNRT1.1 (CHL1) are regulated in response to the changes of the external nitrate concentrations [25].

Other Arabidopsis NRT1s have been tested for high-affinity nitrate transport activity ([1,11,13] and our unpublished data). Of the 12 tested, eleven showed pure low-affinity nitrate