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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

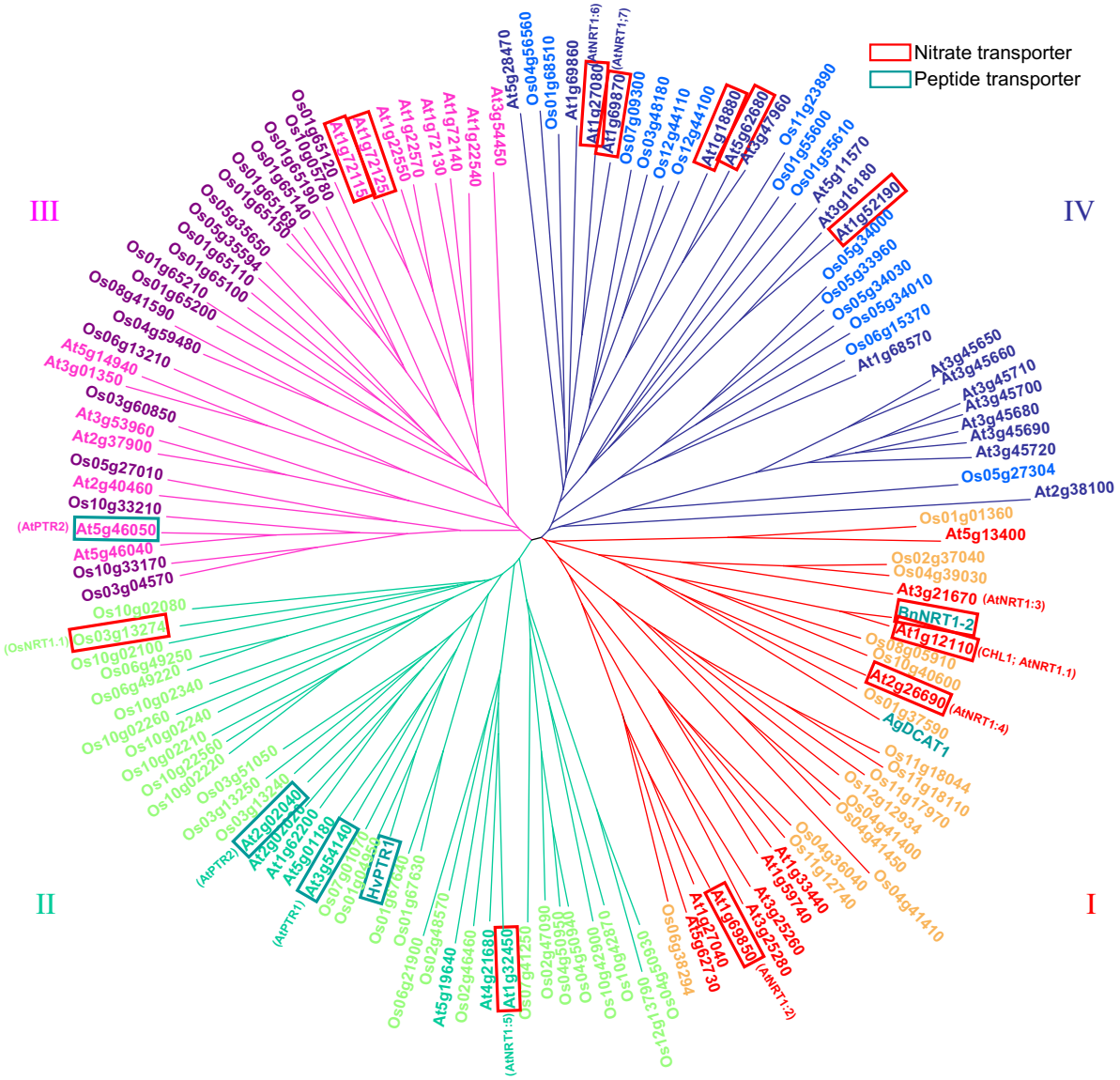

Fig. 1. Phylogenetic tree of the Arabidopsis and rice NRT1(PTR) family. Multiple sequence alignments of 53 Arabidopsis NRT1(PTR) transporters, 80 rice NRT1(PTR) transporters, and BnNRT1-2, AgDCAT1, and HvPTR1 were performed using the BLOSUM protein weight matrix and the phylogenetic tree was constructed using the neighbor-joining method of the ClustalX program [87]. The tree was displayed and manipulated using the MEGA3 program [88].

transport activity and only AtNRT1.1 (CHL1) showed dual-affinity nitrate transport activity. However, the sequence RXXT[101] was found in 36 of 53 Arabidopsis NRT1(PTR) transporters, including some of those shown to be pure low-affinity nitrate transporters, indicating that an additional sequence is required for the dual-affinity switch. BnNRT1-2 from *Brassica napus* and Os08g05910 and Os10g40600 from rice show a higher degree of sequence similarity to AtNRT1.1 (CHL1) than any of the Arabidopsis NRT1s, suggesting that they are orthologs of AtNRT1.1 (CHL1), and it will be interesting to determine whether these three transporters also function as dual-affinity nitrate transporters.

Ironically, high-affinity nitrate uptake was found to be normal in the first studies on the *chl1* mutant [23], and, at, that time, at which no gene involved in nutrient uptake had been identified, this different behavior of high- and low-affinity nitrate uptake in the *chl1* mutant was one of the strongest pieces of evidence supporting the hypothesis that the high- and low-affinity nutrient uptake systems in higher plants were genetically distinct. Many more channels and transporters have now been identified and found to be responsible only for high-affinity uptake or only for low-affinity uptake, demonstrating that the "genetically distinct model" is correct, and, in fact, AtNRT1.1 (CHL1) proved to be an exception to the rule.

Why was high-affinity nitrate uptake of *chl1* mutants sometimes found to be normal and sometime abnormal? This could be due to there being multiple genes involved in nitrate uptake. For example, in Arabidopsis, AtNRT1.1 (CHL1), AtNRT2.1, and AtNRT2.2 are known to be involved in high-affinity nitrate uptake [1,24,26–28], and AtNRT1.1 (CHL1) and AtNRT1.2 are known to be involved in low-affinity nitrate

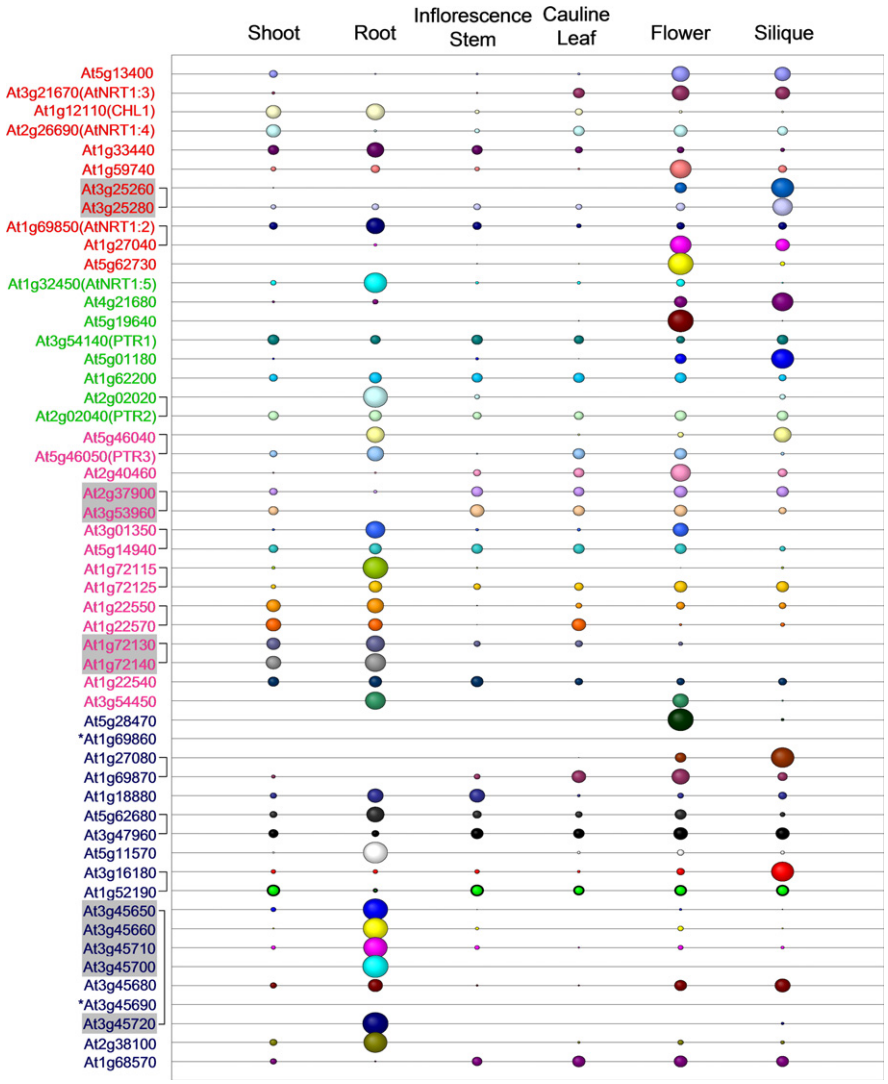

Fig. 2. Tissue-specific expression pattern of Arabidopsis *AtNRT1*(*PTR*) genes. Various tissues were collected for RT-PCR analyses of 53 *AtNRT1*(*PTR*) genes. Shoot and root tissues were collected from 14-day-old Arabidopsis grown hydroponically on nylon meshes in magenta box (Sigma) and the inflorescence stem, cauline leaf, flower, and silique were collected from 4-week-old pot-grown Arabidopsis. Images of RT-PCR analyses of AtNRT1 genes were quantified using a Luminescent Image Analyzer LAS1000plus (Fujifilm, Tokyo, Japan) and the software program, Image Gauge Ver. 4.0 (Fujifilm). The expression of the AtNRT1 genes was normalized to that of UBQ10. The sum of the expression of all tissues for each gene was taken as 100% and the expression in a given tissue expressed as a percentage of this (shown by the area of the circle). Each gene is represented by a distinct color. Genes for which no expression was detected in the RT-PCR analyses are indicated by an asterisk. Genes sharing the highest similarity and closely linked or located on duplicated blocks of the genome are indicated by a right-bracket (]); pairs or groups of genes with similar expression patterns are indicated by a light grey background.

uptake [12,15] (Fig. 3). The transcription levels of *AtNRT1.1* (*CHL1*) and *AtNRT2.1* have been shown to be differentially regulated by N-starvation [29,30], nitrite [31], and NR deficiency [29,30]. The determination of the relative contribution of AtNRT1.1 (CHL1), AtNRT2.1, and AtNRT2.2 to high-affinity nitrate uptake was made more complicated by the facts that phosphorylation of AtNRT1.1 (CHL1), which controls the switch between the high-affinity and low-affinity modes of action, is regulated by different concentrations of nitrate [25] and that gene compensation has been documented between *CHL1* and *AtNRT2.1* [32] and between *AtNRT2.1* and *AtNRT2.2* [28]. Thus, the contribution of AtNRT1.1 (CHL1), AtNRT2.1, and AtNRT2.2 to high-affinity nitrate uptake varies from one condition to the other, and the high-affinity nitrate uptake defect of the *chl1* mutant is only detected under conditions in which the contribution of AtNRT1.1 (CHL1) is dominant over that of AtNRT2.1 and AtNRT2.2. Indeed, the age of the plant (the plants used for different uptake studies ranged from 5-day-old to 6-week-old) [1,16,24,32], the N-status of the plant [15,16], and the uptake medium (with or without ammonium) [32,33] can all cause differences in uptake behavior of the *chl1* mutant. For example, two studies showing a high-affinity nitrate uptake defect of the *chl1* mutant used 5- to 12-day-old plants, an age when the high-affinity nitrate uptake of the *chl1* mutant is only 10–30% of the wild type level [1,24]. In contrast, the study which showed increased or normal high-affinity nitrate uptake activity in the *chl1* mutant used 6-week-old plants [32,33]. These

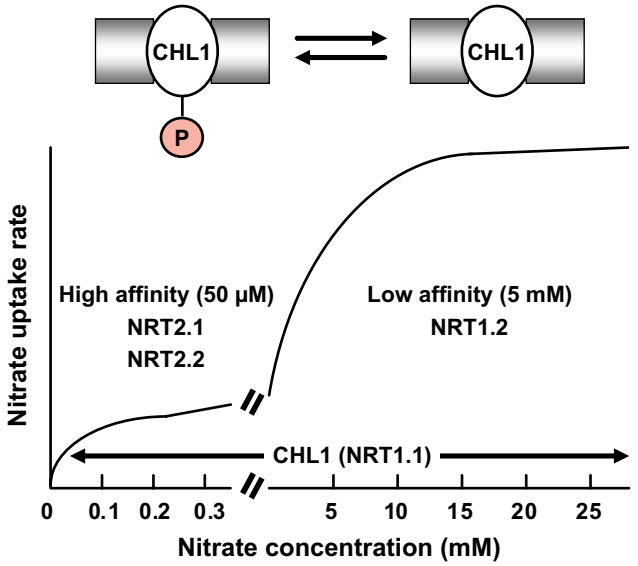

Fig. 3. Nitrate uptake in Arabidopsis. CHL1 (AtNRT1.1) is a dual-affinity nitrate transporter involved in both high- and low-affinity nitrate uptake. The mode of action of CHL1 is switched by phosphorylation and dephosphorylation. AtNRT2.1 and AtNRT2.2 are high-affinity nitrate transporters involved mainly in iHATS. AtNRT1.2 is a low-affinity nitrate transporter involved in cLATS.

results are consistent with the fact that *AtNRT1.1* (*CHL1*) is more highly expressed in the younger part of the root than the older part [15,34,35], while the converse is the case for *AtNRT2.1* [36,37]. In fact, no *AtNRT2.1* transcripts can be detected in 2- to 5-day-old plants [38].

In addition to nitrate uptake, AtNRT1.1 (CHL1) is also involved in nascent organ development [34], light-induced stomatal opening [39], repression of *AtNRT2.1* by high nitrate [32,33], relief of seed dormancy by nitrate [40], and stimulation of lateral root proliferation by high nitrate [35]. Some of these studies suggested that AtNRT1.1 (CHL1) may function as a nitrate sensor [32,33,35]. In yeast, several unique members of transporter families (Ssy1p for amino acids, Mep2p for ammonium, and Snf3p and Rgt2p for glucose) do function as extracellular nutrient sensors [41]. With the exception of Mep2p, these transporter-like sensors in yeast do not transport their respective nutrients. Since transport activity will alter cytosolic nutrient concentrations, it is, in fact, difficult to prove or disprove if a functional transporter also acts as a nutrient sensor.

2.2.2. AtNRT1.2 and cLATS. The basal level of nitrate uptake activity seen in nitrogen-starved plants or ammonium-grown plants is due to the "constitutive" components of nitrate uptake and the increase of several folds in activity after exposure to nitrate is due to the "inducible" components. There are four components of nitrate uptake, the constitutive high-affinity system (cHATS), the inducible high-affinity system (iHATS), the constitutive low-affinity system (cLATS), and the inducible low-affinity system (iLATS). In Arabidopsis, AtNRT1.2 is responsible for cLATS, as shown by the constitutive expression of *AtNRT1.2* and the defect in nitrate-induced membrane depolarization seen in the *atnrt1.2* antisense mutant grown in ammonium [12]. Although both AtNRT1.1 and AtNRT1.2 are involved in low-affinity nitrate uptake, these two transporters differ in three aspects: (1) expression of *AtNRT1.1* is induced by nitrate [3], while *AtNRT1.2* is constitutively expressed [12]; (2) AtNRT1.1 is a dual-affinity nitrate transporter, while AtNRT1.2 is a pure low-affinity nitrate transporter [1]; and (3) *AtNRT1.1* (*CHL1*) is expressed in the epidermis in the root tip [15], but in the cortex and external half of the endodermis in other regions, whereas *AtNRT1.2* mRNA is found only in the epidermis [12]. The physiological impact of the difference in cell type-specific expression remains to be analyzed.

Similar to *AtNRT1.2*, *OsNRT1* is a constitutive gene encoding a pure low-affinity nitrate transporter and is only expressed in the root epidermis [13]. However, phylogenetic analysis indicated that AtNRT1.2 and OsNRT1 belong to different groups of the NRT1(PTR) family (Fig. 1). If OsNRT1 is also responsible for cLATS, this then raises the question why OsNRT1, and AtNRT1.2 are orthologs, but belong to different groups of the NRT1(PTR) family.

2.2.3. AtNRT1.4 and petiole nitrate storage. After being taken up into the root cells, nitrate has to cross several cell membranes to be distributed in different cellular compartments and different tissues. Compared to nitrate uptake, less is known about how nitrate is transported to different cellular compartments and tissues. AtCLCa, a member of the chloride channel family (reviewed separately in this issue), functions as a nitrate/proton exchanger responsible for nitrate accumulation in vacuoles [42]. In addition, several *NRT1* genes in Arabidopsis are involved in nitrate distribution in different cellular compartments and tissues (Tsay, unpublished data). The low-affinity nitrate transporter gene, *AtNRT1.4*, is only expressed in the leaf petiole [11]. In the wild type, the petiole nitrate content is high, but NR activity is low, indicating that the petiole is a nitrate storage site. In the *atnrt1.4* mutant, the nitrate content of the petiole is reduced to half that of the wild type level, but that in the leaf lamina is slightly increased [11]. These studies on AtNRT1.4 show that the petiole has a unique function in nitrate homeostasis regulation. This could explain why some farmers use the petiole nitrate content to monitor the N-demand of crops.

2.3. Dipeptide transporters in the NRT1(PTR) family

In bacteria, yeasts, and animals, the ability to transport peptides, which plays a crucial role in nutrition in terms of carbon and nitrogen sources, is well established. However, in plants, the role of small peptides (2–6 amino acids) and their transporters is less defined. To date, three protein families have been identified as transporting small peptides in higher plants, the ABC-type transporters, the di/tripeptide transporters (PTR family), and the OPT family. Peptide transporters within the ABC superfamily have been reviewed by Stacey et al. [43]. In this review, we focus on recent data for the PTR and OPT families (Table 1). In addition, we will discuss some new insights into diverse possible roles of *Arabidopsis* PTRs in plant development, stress responses, and heavy-metal detoxification.

2.3.1. The PTRs in Arabidopsis. The first plant peptide transporter, AtPTR2 (At2g02040, formerly AtNRT1), was isolated by complementation of a yeast histidine transport-deficient mutant with an *Arabidopsis* cDNA library. However, no uptake of radiolabeled histidine could be measured in *S. cerevisiae* expressing AtPTR2 [7]. Later, *AtPTR2* was transformed into a yeast peptide transport-deficient mutant in which AtPTR2 displayed high-affinity, low-selectivity transport activity for di- and tripeptides [8]. AtPTR2 (formerly AtPTR2-B)

Table 1
Properties of Arabidopsis dipeptide and oligopeptide transporters

	AGI	Expression		Localization	Substrate specificity		Phenotypes
		mRNA[a]	Promoter-GUS		In yeast[b]	In oocytes	
AtPTRs							
AtPTR2	At2g02040	**Root**, **3d-germ**. **seed**, **silique;** other tissues	–		Di- and tripeptides*; phaseolotoxin	Di- and tripeptides	?[d]
AtPTR1	At3g54140	Weak expression in all tissues	***Vascular tissue*** throughout the plant	Plasma membrane	Dipeptides; phaseolotoxin	Di- and tripeptides; 4-APAA[c]	No unusual phenotype
AtPTR3	At5g46050	–	Cotyledons, leaves		Di- and tripeptides	–	Reduced defense against pathogens
AtOPTs							
AtOPT1	At5g55930	**Flower**; leaf, stem	***Vascular tissues***; pollen and pollen tubes		KLLLG	–	
AtOPT2	At1g09930	Equal in all tissues	–		–	–	
AtOPT3	At4g16370	**Flower**, **leaf**, **root**; stem	***Vascular tissues***; pollen and embryo		Cu^{2+}; Mn^{2+}; Fe^{2+}	–	Embryo arrested at the preglobular stage
AtOPT4	At5g64410	Equal in all tissues	***Vascular tissues***; embryonic cotyledons		KLLG; KLGL*; KLLLG	GGFL; GGFM; KLGL	
AtOPT5	At4g26590	**Flower**	–		KLLLG	–	
AtOPT6	At4g27730	**Flower**; root	***Vascular tissues***; ovules, embryo, stamen filaments and lateral root initiation		KLLLG; GSH*; GSSG	–	
AtOPT7	At4g10770	**Flower**; root	***Vascular tissues***; embryonic cotyledons		KLLLG	–	
AtOPT8	At5g53520	–	Pollen, early stages of embryogenesis		–	–	
AtOPT9	At5g53510	–	–	–	–		

[a]Words in bold indicate a stronger expression.
[b]Yeast growth complementation assays reveal possible substrates for the indicated AtOPT. Substrates further confirmed by uptake experiments are marked with an asterisk. Metal transport by AtOPT3 is nicotianamine-independent.
[c]4-APAA: Aminophenylacetic acid.
[d]Phenotypes observed in antisense mutants of AtPTR2 are delayed flowering and arrested seed development, however, the T-DNA insertion lines show no unusual phenotypes (personal communication).

was also cloned by Song et al. by functional complementation of a yeast peptide transport mutant with an *Arabidopsis* cDNA library [20]. Again, it was demonstrated that, when expressed in yeast [20] or Xenopus oocytes [14], it could mediate the uptake of various di- and tripeptides, but showed no His or nitrate uptake activity. AtPTR2 is expressed in most plant tissues, with high levels in green silique, root, and young seedlings [7,20]. In situ hybridization indicated that *AtPTR2* is expressed in the embryo at the heart stage of development [8]. It is noteworthy that the role of AtPTR2 in planta is still an open question, because the late flowering and seed abortion phenotype observed in antisense *AtPTR2* plants is not seen in the T-DNA-inserted *atptr2* mutant (G. Stacey, personal communication), indicating that the phenotype was caused by cross-silencing an unknown member(s) of the NRT1(PTR) family.

Functional analysis of AtPTR2 and fungus fPTR2 (formerly AtPTR2-A, isolated by complementing a yeast mutant with an Arabidopsis cDNA library, but later found to be a gene from a fungal contaminant [5,6]) in Xenopus oocytes under voltage clamp conditions revealed that both transport a broad spectrum of dipeptides, with K_ms ranging from 30 μM to 3 mM [14]. Similar to rabbit PepT1, AtPTR2 and fPTR2 prefer dipeptides; the tripeptide and amino acid transport activities being ~60% and 10%, respectively, of the dipeptide activity [14]. The low level of amino acid transport activity may explain why AtPTR2 was originally isolated by complementing a histidine transport-deficient mutant, but no histidine transport activity was observed. The substrate preferences of AtPTR2 and fPTR2 are quite similar. In addition, kinetic analysis suggests that both AtPTR2 and fPTR2 operate by a random binding, simultaneous transport mechanism [14].

Subsequently, AtPTR1 (At3g54140), which mediates the uptake of di- and tripeptides, was also identified by heterologous complementation of a yeast peptide transport-deficient mutant and found to recognize not only a wide spectrum of naturally occurring di- and tripeptides, but also the modified tripeptide, phaseolotoxin, and substrates lacking peptide bonds [21]. Transient expression analysis of a GFP fusion indicated that AtPTR1 is a plasma membrane protein and GUS staining analysis revealed strong expression of *AtPTR1* in vascular tissue throughout the plant, indicating a role in long-distance peptide transport [21].

AtPTR3 (At5g46050), a mechanical wounding-induced gene, was identified by screening mutant lines transformed with T-DNA containing a promoter trap vector carrying a GUS reporter [44]. Further study showed that *AtPTR3* expression is induced by salicylic acid (SA), and that wound-induced expression of *AtPTR3* was abolished in the SA signaling mutants, *NahG* and *npr1*. *AtPTR3* is able to complement the growth defect of the yeast dipeptide uptake mutant, *ptr2*, using dipeptides as the amino acid source [22]. One of the T-DNA inserted mutants, *atptr3-1*, showed increased susceptibility to the pathogen, *Erwinia carotovara* subsp. *carotovora*, but the phenotype was not so obvious in another mutant, *atptr3-2*, with a different ecotype background. The converse was true for another pathogen, *Pseudomonas syringae* with increased

susceptibility found in *atptr3-2* and no phenotype in *atptr3-1* [22]. Whether dipeptides are the primary substrate of AtPTR3, the role of AtPTR3 in pathogen defense, and whether these account for the ecotype-specific and pathogen-specific phenotypes remain to be determined.

2.3.2. The PTRs in barley. In the past few years, using degenerate primers designed to conserved regions of peptide transporters, homologous genes encoding peptide transporters have been identified in barley [18], *Vicia faba* [45], and the carnivorous plant, *Nepenthes*, [46]. The barley scutellar peptide transporter, HvPTR1, is the best characterized plant PTR, as peptide transport in germinating barley grain has been extensively studied using biochemical approaches [18,47,48]. Unlike *AtPTRs*, which are expressed in almost all tissues, expression of *HvPTR1* is highly tissue- and developmental stage-specific, with transcripts being detected in scutellar epithelial cells during germination [18]. All the evidence indicates that HvPTR1, localized in the plasma membrane of scutellar epithelial cells, is responsible for remobilizing small peptides, produced by the hydrolysis of storage protein in the endosperm, to the growing seedling [18,49]. In response to increased levels of amino acids (present at the later stage of germination), the dipeptide transport activity of the scutella is reduced and HvPTR1 protein is regulated at the post-translational level by phosphorylation [50]. This could be an important regulatory mechanism for controlling the amount of organic nitrogen transported from the endosperm to the embryo during seed germination.

2.4. Other substrates and potential substrates of the NRT1(PTR) transporters

In addition to nitrate and dipeptides, histidine and malate have been shown to be transported by some NRT1(PTR) transporters. RnPHT1, expressed in rat brain, exhibits high-affinity dipeptide and high-affinity histidine transport activity (K_m^{His} is about 20 μM), but no transport activity can be detected for other amino acids [51]. BnNRT1-2 from Brassica transports nitrate and histidine with similar K_ms (both in the mM range), but different pH dependencies [17]. AtPTR1 and AtPTR2 are high-affinity dipeptide transporters with low-affinity, low-capacity histidine transport activity [14,21]. RnPHT1 therefore transports dipeptide and histidine with equal efficiency, but AtPTR1 and AtPTR2 transport dipeptide much more efficiently than histidine. On the other hand, AgDCAT1, a member of the NRT1(PTR) family expressed in the actinorhizal nodules of alder, has been shown to be a dicarboxylate transporter, with a K_m of 70 μM for malate [19]. Located at the symbiotic interface, it may be responsible for providing the intracellular bacteria with dicarboxylates as carbon sources. It will be interesting to determine whether any of the Arabidopsis NRT1(PTR)s can transport malate.

What could be other potential substrates for NRT1(PTR) transporters accounting for such a large family in higher plants? IAA-amino acid conjugates, γ-glutamylcysteine, and glutathione, with similar structures to di- and tripeptides, are important molecules for plant development, nutrition, stress adaptation, and heavy metal detoxification. Using a reverse genetic approach, it was found that γ-glutamylcysteine and glutathione can be transported by one of the AtNRT1(PTR) transporters and that the T-DNA-inserted mutant was cadmium-sensitive (Tsai and Tsay, unpublished data).

3. NRT2 family

The *NRT2* family of high affinity nitrate transporters was first discovered in the chlorate-resistant mutant, *crnA*, now renamed *NRTA*, of *Aspergillus nidulans*: the nitrate uptake defect of this mutant is seen in the conidiospore and young mycelia stages, but not in older mycelia [52,53]. Subsequent searches led to the identification of an equivalent gene family in *Chlamydomonas* [54], marine cyanobacterium [55], and a variety of plants, including barley [56], tobacco [57], soybean [58], and *Arabidopsis* [30,38].

3.1. A two-component high-affinity nitrate uptake system

NRT2 protein contains 12 TM domains. In the fungus, *Aspergillus nidulans*, NRT2 protein is functional on its own. *A. nidulans* NRT2 cDNA expressed in Xenopus oocytes exhibits nitrate, nitrite, and chloride (nitrite analogue) uptake activity [59]. The nitrate-induced inward currents are pH-dependent, consistent with a proton-coupled mechanism. Mutagenesis analysis indicated that two conserved arginine residues (R87 and R459) in TM domains 2 and 8 are required for substrate binding, and intragenic suppression analysis revealed a functional interplay between R87 in TM 2 and N459 in TM 11 [60]. In contrast, in *Chlamydomonas* and higher plants, NRT2 protein alone does not show any nitrate transport activity and an additional component, NAR2, a protein with a single TM domain, is required. The involvement of NAR2 in high-affinity nitrate uptake was first identified genetically in *Chlamydomonas reinhardtii*, in which *NAR2* is next to *NRT2.1* in the nitrate-related gene cluster [54,61]. Xenopus oocytes co-injected with CrNAR2 and CrNRT2.1 show pH-dependent, nitrate-elicited currents, while oocytes injected with either one alone do not [62]. A direct interaction between NRT2 and NAR2 was further confirmed using the yeast split-ubiquitin system [63]. The interaction between NAR2 and NRT2 is very specific. For example, in barley, there are three *NAR2* genes, only one of which, *HvNAR2.3*, can form a functional unit with HvNRT2.1 [64].

3.2. Genes involved in high affinity nitrate uptake (HATS)

According to the physiological analyses, there are two high-affinity nitrate uptake systems, inducible HATS (iHATS) and constitutive HATS (cHATS). The V_{max} of iHATS is several folds higher than that of cHATS.

In Arabidopsis, there are seven *NRT2* genes. *AtNRT2.1* and *AtNRT2.2* are next to each other on the chromosome, and both are involved in high affinity nitrate uptake [26–28]. In the *nrt2.1* and *nrt2.2* mutants, iHATS is reduced by 50–72% and 19%, respectively, indicating that AtNRT2.1 plays a more dominant role and AtNRT2.2 a minor role in iHATS [28]. However, when *AtNRT2.1* is mutated, *AtNRT2.2* mRNA levels are increased three-fold to compensate the functional loss of AtNRT2.1 [28]. In Arabidopsis, there are two *NAR2* genes, *NAR2.1* (*AtNRT3.1*, At5g50200) and *NAR2.2* (*AtNRT3.2*, At4g24720). AtNAR2.1 is known to participate in high-affinity nitrate uptake [63,65]. In the *nar2.1* null mutant, cHATS is reduced by up to 89%, while iHATS is reduced by up to 96% [65]. It is noteworthy that, in a *nrt2.1 nrt2.2* double knock out mutant, cHATS was only reduced by 30–35% [28]. The severe defect of cHATS in the *nar2.1* mutant [65], but not the *nrt2.1 nrt2.2* double mutant [28], suggested that another

NRT2 gene(s) was probably responsible for cHATS. A mutant defective in cHATS, *chl8*, was isolated by chlorate selection at low concentration [66], but the gene mutated in *chl8* has not been identified. It will be interesting to determine whether one of the *NRT2* genes, particularly *NRT2.6* or *NRT2.7*, both of which are constitutively expressed in root [67,68], is responsible for the *chl8* phenotype.

3.3. Regulation of AtNRT2.1

AtNRT2.1 is involved in iHATS. The expression of *AtNRT2.1* matches the iHATS response pattern, increasing rapidly upon first provision of NO_3^- to nitrate-starved roots and decreasing when the NO_3^- supply is maintained [38]. NR mutants and block of NR activity by tungstate were used to determine whether nitrate itself or a reduced nitrogen metabolite was responsible for the feedback inhibition [29,30]. In NR mutants in which high levels of NO_3^- accumulate and low levels of reduced nitrogen metabolites are synthesized, *NRT2.1* transcript levels are increased, suggesting that NO_3^- is responsible for induction of *AtNRT2.1* expression and that its downstream metabolites are responsible for repression. Further studies using the glutamate synthase inhibitor, AZA, or exposure to NH_4^+ or various amino acids suggested that glutamine plays an important role in the downregulation of *NRT2.1* [36,38]. Similar results were obtained in expression analyses of *NpNRT2.1* in tobacco and *HvNRT2* in barley [57,69,70].

Although the increased accumulation of *NRT2.1* transcripts in NR mutants suggested that nitrate itself was not responsible for the feedback repression of *NRT2.1* [29,30,69,70], two recent studies indicated that, in the presence of ammonium, the expression of *AtNRT2.1* is repressed by a high concentration of nitrate [32,33]. More interestingly, the dual affinity nitrate transporter, AtNRT1.1 (CHL1), is required for this high-nitrate repression, as *AtNRT2.1* expression is de-repressed in the *chl1* mutant [32].

3.4. Role of AtNRT2.1 in root architecture

As with CHL1 (AtNRT1.1), some studies on root development suggested that AtNRT2.1 may act as a nitrate sensor or signal transducer. In the wild type, lateral root initiation is repressed by a high sucrose/nitrate ratio and this repression is overcome in the *lin1* mutant, which shows increased lateral root initiation under high sucrose/low nitrate conditions and carries a mutation in the *AtNRT2.1* gene [71]. De-repression of lateral root initiation in the *lin1* mutant is seen even in nitrate-free medium, showing that the phenotype of *lin1* is nitrate-independent and suggesting that AtNRT2.1 may act as a nitrate sensor or signal transducer in regulating root plasticity [71]. However, an independent study showed an opposite phenotype of the *atnrt2.1* mutant, with reduced lateral root initiation [37]. Since the growth conditions and medium composition were different in these two studies, no conclusive statement can be made about the roles of AtNRT2.1 in nitrate sensing and root development.

4. OPT family

In addition to PTRs, which transport di/tripeptides, a distinct transport system that can transport tetra- and pentapeptides (oligopeptide transporters, OPTs) was first discovered in *Candida albicans* [72]. Later, OPT orthologs were identified in higher plants (*Arabidopsis OPT1-OPT9*, rice *GT1*, and *Brassica juncea GT1*) by sequence similarity searches using the complete sequence of the *C. albicans OPT1* gene [73], or by RT-PCR, using primers corresponding to the conserved regions [74,75]. None of the OPT members show any significant sequence similarity to known PTRs.

OPTs and YS (yellow strip) are two different groups of a large transporter family [76,77]. ZmYS1 was shown to be a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metal complexes [78]. Since peptides, phytosiderophores, and nicotianamine are all amino acid derivatives, this could explain why these two types of transporter belong to the same family. It is noteworthy that members of this family (OPT/YS family) are found in fungi, bacteria, plants, and archaea, but not in animals [77]. The involvement of YS transporters in iron and metal transport is discussed in another review in this issue. In this review, we will focus on the OPTs.

4.1. OPTs in Arabidopsis

In Arabidopsis, there are nine *OPT* genes [73] (Table 1). *AtOPT* promoter-*GUS* fusion analyses revealed that the majority of *AtOPTs* are preferentially expressed in vascular tissues, suggesting a role in the long-distant transport of their respective substrates, and some *AtOPTs* show tissue-specific expression patterns, particularly during flower and seed development, suggesting distinct roles for specific OPTs in *Arabidopsis* [79]. Expression of AtOPT1, 4, 5, 6, and 7, but not AtOPT2 and 3, in a Leu auxotrophic yeast strain permitted prototrophic growth on the pentapeptide KLLLG, indicating that these five AtOPTs function as pentapeptide transporters [73]. The tetrapeptide and pentapeptide transport activity of AtOPT4 was confirmed by electrophysiological analysis of *AtOPT4*-injected Xenopus oocytes [80].

4.2. Role of Arabidopsis OPT3 in embryo development and metal transport

A T-DNA insertion mutation in one of the AtOPT homologs, *AtOPT3*, resulted in arrested embryo development at the early stage of embryogenesis [81]. Since so many nitrogen transporters [amino acid transporters, OPTs, and NRT1(PTRs)] are expressed in developing embryos, it is unlikely that the embryo lethality of the *opt3* mutant can be explained by a defect of nitrogen mobilization. Histochemical analysis of GUS activity showed that *AtOPT3* is highly expressed in pollen, developing embryos, and the vascular tissues of mature plants [81]. More interestingly, *AtOPT3* expression is highly upregulated by iron limitation, but not Zn or Cu deficiency [79,82]. AtOPT3 expression can rescue the growth defect of yeast mutants deficient in Cu, Fe, or Mn uptake, but growth is not affected by adding nicotianamine to the medium, suggesting that AtOPT3 can facilitate metal acquisition in yeast, but unlike ZmYS1, this is not mediated by transporting nicotianamine-chelated metal complexes [82]. The metal complexes transported by AtOPT3 must be determined to understand its role in embryo development and iron deficiency. This involvement of AtOPT3 in metal transport reduces the functional gap between OPTs and YS transporters.

4.3. Glutathione transport activity of OPTs

The yeast OPT transporter, ScOPT1 (HGT1), was found to be able to transport glutathione, oxidized glutathione, and

glutathione conjugates [80,83]. Similar to the behavior of OsGT1 and BjGT1 (OPTs from rice and *B. juncea*, respectively) [74,75], expression of AtOPT6, but not AtOPT7, was able to restore the growth defect of the yeast glutathione transport-deficient mutant, *hgt1*, using GSH or GSSG as the sole sulfur source [84]. Similar to OsGT1, AtOPT6 expressed in the *hgt1* mutant performed [^{3}H]GSH uptake, with two K_m values of 400 μM and 5 mM. The affinity and transport rate of AtOPT6 for GSH measured in yeast are much lower (6–8 times lower) than those of ScOPT1 (HGT1, K_m^{GSH} is 54 μM) [83,84]. Moreover, the [^{3}H]GST uptake activity of ScOPT1 (HGT1) can be inhibited by GST or GSSG with equal efficiency [83], whereas that of AtOPT6 is inhibited more efficiently by GSSG than by GSH itself [84], Thus, it is possible that GSSH, rather than GSH, is the primary substrate of AtOPT6.

4.4. Phytochelatin transport activity of OPTs

In addition to GSH, GSSG, and oligopeptides, ScOPT1 expressed in Xenopus oocyte also transports the phytochelatin PC_2, displaying the highest affinity for PC_2 [80]. PCs, formed from GSH, are involved in heavy metal detoxification. Recent studies involving complementing PC-deficient mutants with the PC synthase gene under the control of tissue-specific promoters have shown that PCs can be transported from the root to the shoot and from the shoot to the root [85,86]. In addition, grafting experiments also showed shoot to root transport of PCs [86]. The majority of AtOPTs are preferentially expressed in vascular tissue [79], making them perfect candidate genes for the long-distance transport of PCs. It will be interesting to determine whether any of the Arabidopsis OPTs, particularly AtOPT3, can transport PCs.

5. Concluding remarks

Physiological studies have shown that there are four nitrate uptake systems, iHATS, cHATS, iLATS, and cLATS, and molecular genetic studies have shown that four nitrate transporter genes, *AtNRT1.1* (*CHL1*), *AtNRT1.2* (*NTL1*), *AtNRT2.1*, and *AtNRT2.2*, are involved in nitrate uptake (Fig. 3). However, there is no simple one-to-one relationship between the genes and their corresponding uptake systems. Multiple genes are involved in each uptake system, and, sometimes, a single gene is involved in multiple uptake systems. For example, *CHL1*, *AtNRT2.1*, and *AtNRT2.2* are nitrate-inducible genes and have been shown to be involved in iHATS, but their basal levels of expression contribute to part of the cHATS [1,28]. The relative contributions of *CHL1*, *AtNRT2.1*, and *AtNRT2.2* to HATS depend on the age of the plant and the nitrogen composition of the growth medium and uptake medium. Gene compensation between *CHL1* and *ATNRT2.1* [32] and between *AtNRT2.1* and *AtNRT2.2* [28] make it more complicated to evaluate the relative contribution of each transporter. Further studies on the regulatory network controlling these genes and gene products at the transcriptional and post-transcriptional levels will help us to understand the beneficial effects of this redundancy.

With respect to substrate specificity, the members of the NRT1(PTR) family fall into two distinct subtypes, namely nitrate transporters and peptide transporters. To date, no nitrate transporters have been found to have peptide transport activity and no peptide transporters have been found to transport nitrate. This raises the questions whether none of the nitrate transporters in this family transport peptides and whether the feature responsible for substrate specificity can be used to predict the substrate specificity of new members of this family. This puzzle will be solved by identifying the structure determinants for the substrate specificity of NRT1(PTR) transporters.

Other than the nutritional role of dipeptide transporters in germinating seeds, less is known about the in planta functions of the PTR and OPT peptide transporters. T-DNA-tagged mutants of AtPTR1 and AtPTR2 show no difference in overall growth behavior compared to the wild type, but this is probably due to the functional redundancy of PTRs, and multiple-knockout mutants might be required for further investigation. It has been suggested that peptide-type hormones could be the substrates of PTRs or OPTs, but all peptide-type hormones so far identified are too large to be transported by either PTRs or OPTs. The identification of the substrates of PTRs and OPTs and the correlation of their transport activity with the mutant phenotype are key challenges in this field.

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References

[1] Liu, K.-H., Huang, C.-Y. and Tsay, Y.-F. (1999) CHL1 is a dual-affinity nitrate transporter of Arabidopsis involved in multiple phases of nitrate uptake. Plant Cell 11, 865–874.
[2] Doddema, H., Hofstra, J.J. and Feenstra, W.J. (1978) Uptake of nitrate by mutants of *Arabidopsis thaliana*, disturbed in uptake or reduction of nitrate I. Effect of nitrogen source during growth on uptake of nitrate and chlorate. Physiol. Plant 43, 343–350.
[3] Tsay, Y.-F., Schroeder, J.I., Feldmann, K.A. and Crawford, N.M. (1993) The herbicide sensitivity gene CHL1 of Arabidopsis encodes a nitrate-inducible nitrate transporter. Cell 72, 705–713.
[4] Fei, Y.-J. et al. (1994) Expression cloning of a mammalian proton-coupled oligopeptide transporter. Nature 368, 563–566.
[5] Steiner, H.-Y., Song, W., Zhang, L., Naider, F., Becker, J.M. and Stacey, G. (1994) An Arabidopsis peptide transporter is a member of a new class of membrane transport proteins. Plant Cell 6, 1289–1299.
[6] Steiner, H.-Y., Song, W., Zhang, L., Naider, F., Becker, J.M. and Stacey, G. (2000) Corrections. Plant Cell 12, 2295.
[7] Frommer, W.B., Hummel, S. and Rentsch, D. (1994) Cloning of an Arabidopsis histidine transporting protein related to nitrate and peptide transporters. FEBS Lett. 347, 185–189.
[8] Rentsch, D., Laloi, M., Rouhara, I., Schmelzer, E., Delrot, S. and Frommer, W.B. (1995) NTR1 encodes a high affinity oligopeptide transporter in Arabidopsis. FEBS Lett. 370, 264–268.
[9] Perry, J.R., Basral, M.A., Steiner, H.-Y., Naider, F. and Becker, J.M. (1994) Isolation and characterization of a *Saccharomyces cerevisiae* peptide transport gene. Mol. Cell. Biol. 14, 104–115.
[10] Hagting, A., Kunji, E.R.S., Leenhouts, K., Poolman, B. and Konings, W.N. (1994) The di- and tripeptide transport protein of Lactococcus lactis. J. Biol. Chem. 269, 11391–11399.
[11] Chiu, C.-C., Lin, C.-S., Hsia, A.-P., Su, R.-C., Lin, H.-L. and Tsay, Y.-F. (2004) Mutation of a nitrate transporter, AtNRT1:4, results in a reduced petiole nitrate content and altered leaf development. Plant Cell Physiol. 45, 1139–1148.
[12] Huang, N.-C., Liu, K.-H., Lo, H.-J. and Tsay, Y.-F. (1999) Cloning and functional characterization of an Arabidopsis nitrate

transporter gene that encodes a constitutive component of low-affinity uptake. Plant Cell 11, 1381–1392.
[13] Lin, C.-M., Koh, S., Stacey, G., Yu, S.-M., Lin, T.-Y. and Tsay, Y.-F. (2000) Cloning and functional characterization of a constitutively expressed nitrate transporter gene OsNRT1 from rice. Plant Physiol. 122, 379–388.
[14] Chiang, C.-S., Stacey, G. and Tsay, Y.-F. (2004) Mechanisms and functional properties of two peptide transporters, AtPTR2 and fPTR2. J. Biol. Chem. 279, 30150–30157.
[15] Huang, N.-C., Chiang, C.-S., Crawford, N.M. and Tsay, Y.-F. (1996) CHL1 encodes a component of the low-affinity nitrate uptake system in Arabidopsis and shows cell type-specific expression in roots. Plant Cell 8, 2183–2191.
[16] Touraine, B. and Glass, A.D.M. (1997) NO_3^- and ClO_3^- fluxes in the chl1-5 mutant of *Arabidopsis thaliana*. Plant Physiol. 114, 137–144.
[17] Zhou, J.-J., Theodoulou, F.L., Muldin, I., Ingemarsson, B. and Miller, A.J. (1998) Cloning and functional characterization of a *Brassica napus* transporter that is able to transport nitrate and histidine. J. Biol. Chem. 273, 12017–12023.
[18] West, C.E., Waterworth, W.M., Stephens, S.M., Smith, C.P. and Bray, C.M. (1998) Cloning and functional characterization of a peptide transporter expressed in the scutellum of barley grain during the early stages of germination. Plant J. 15, 221–229.
[19] Jeong, J. et al. (2004) A nodule-specific dicarboxylate transporter from alder is a member of the peptide transporter family. Plant Physiol. 134, 969–978.
[20] Song, W., Steiner, H.-Y., Zhang, L., Naider, F.G.S. and Becker, J.M. (1996) Cloning of a second Arabidopsis peptide transport gene. Plant Physiol. 110, 171–178.
[21] Dietrich, D., Hammes, U., Thor, K., Suter-Grotemeyer, M., Fluckiger, R., Slusarenko, A.J., Ward, J.M. and Rentsch, D. (2004) AtPTR1, a plasma membrane peptide transporter expressed during seed germination and in vascular tissue of Arabidopsis. Plant J. 40, 488–499.
[22] Karim, S., Holmstrom, K.O., Mandal, A., Dahl, P., Hohmann, S., Brader, G., Palva, E.T. and Pirhonen, M. (2007) AtPTR3, a wound-induced peptide transporter needed for defence against virulent bacterial pathogens in Arabidopsis. Planta 225, 1431–1445.
[23] Doddema, H. and Telkamp, G.P. (1979) Uptake of nitrate by mutants of *Arabidopsis thaliana*, disturbed in uptake or reduction of nitrate. Physiol. Plant 45, 332–338.
[24] Wang, R., Liu, D. and Crawford, N.M. (1998) The Arabidopsis CHL1 protein plays a major role in high-affinity nitrate uptake. Proc. Natl. Acad. Sci. USA 95, 15134–15139.
[25] Liu, K.-H. and Tsay, Y.-F. (2003) Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation. EMBO J. 22, 1005–1013.
[26] Cerezo, M., Tillard, P., Filleur, S., Munos, S., Daniel-Vedele, F. and Gojon, A. (2001) Major alterations of the regulation of root NO_3^- uptake are associated with the mutation of Nrt2.1 and Nrt2.2 genes in Arabidopsis. Plant Physiol. 127, 262–271.
[27] Filleur, S., Dorbe, M.F., Cerezo, M., Orsel, M., Granier, F., Gojon, A. and Daniel-Vedele, F. (2001) An Arabidopsis T-DNA mutant affected in Nrt2 genes is impaired in nitrate uptake. FEBS Lett. 489, 220–224.
[28] Li, W., Wang, Y., Okamoto, M., Crawford, N.M., Siddiqi, M.Y. and Glass, A.D. (2007) Dissection of the AtNRT2.1:AtNRT2.2 inducible high-affinity nitrate transporter gene cluster. Plant Physiol. 143, 425–433.
[29] Lejay, L.P.T., Lepetit, M., Olive, F., Filleur, S., Daniel-Vedele, F. and Gojon, A. (1999) Molecular and functional regulation of two NO_3^- uptake systems by N- and C-status of Arabidopsis plants. Plant J. 18, 509–519.
[30] Filleur, S. and Daniel-Vedele, F. (1999) Expression analysis of a high-affinity nitrate transporter isolated from *Arabidopsis thaliana* by differential display. Planta 207, 461–469.
[31] Loque, D., Tillard, P., Gojon, A. and Lepetit, M. (2003) Gene expression of the NO_3^- transporter NRT1.1 and the nitrate reductase NIA1 is repressed in Arabidopsis roots by NO_2^-, the product of NO_3^- reduction. Plant Physiol. 132, 958–967.
[32] Munos, S., Cazettes, C., Fizames, C., Gaymard, F., Tillard, P., Lepetit, M., Lejay, L. and Gojon, A. (2004) Transcript profiling in the chl1-5 mutant of Arabidopsis reveals a role of the nitrate transporter NRT1.1 in the regulation of another nitrate transporter, NRT2.1. Plant Cell 16, 2433–2447.
[33] Krouk, G., Tillard, P. and Gojon, A. (2006) Regulation of the high-affinity NO_3^- uptake system by NRT1.1-mediated NO_3^- demand signaling in Arabidopsis. Plant Physiol. 142, 1075–1086.
[34] Guo, F.Q., Wang, R., Chen, M. and Crawford, N.M. (2001) The Arabidopsis dual-affinity nitrate transporter gene AtNRT1.1 (CHL1) is activated and functions in nascent organ development during vegetative and reproductive growth. Plant Cell 13, 1761–1777.
[35] Remans, T. et al. (2006) The Arabidopsis NRT1.1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. Proc. Natl. Acad. Sci. USA A103, 19206–19211.
[36] Nazoa, P. et al. (2003) Regulation of the nitrate transporter gene AtNRT2.1 in *Arabidopsis thaliana*: responses to nitrate, amino acids and developmental stage. Plant Mol. Biol. 52, 689–703.
[37] Remans, T., Nacry, P., Pervent, M., Girin, T., Tillard, P., Lepetit, M. and Gojon, A. (2006) A central role for the nitrate transporter NRT2.1 in the integrated morphological and physiological responses of the root system to nitrogen limitation in Arabidopsis. Plant Physiol. 140, 909–921.
[38] Zhuo, D., Okamoto, M., Vidmar, J.J. and Glass, A.D. (1999) Regulation of a putative high-affinity nitrate transporter (Nrt2;1At) in roots of *Arabidopsis thaliana*. Plant J. 17, 563–568.
[39] Guo, F.Q., Young, J. and Crawford, N.M. (2003) The nitrate transporter AtNRT1.1 (CHL1) functions in stomatal opening and contributes to drought susceptibility in Arabidopsis. Plant Cell 15, 107–117.
[40] Alboresi, A., Gestin, C., Leydecker, M.T., Bedu, M., Meyer, C. and Truong, H.N. (2005) Nitrate, a signal relieving seed dormancy in Arabidopsis. Plant, Cell Environ. 28, 500–512.
[41] Forsberg, H. and Ljungdahl, P.O. (2001) Sensors of extracellular nutrients in *Saccharomyces cerevisiae*. Curr. Genet. 40, 91–109.
[42] De Angeli, A., Monachello, D., Ephritikhine, G., Frachisse, J.M., Thomine, S., Gambale, F. and Barbier-Brygoo, H. (2006) The nitrate/proton antiporter AtCLCa mediates nitrate accumulation in plant vacuoles. Nature 442, 939–942.
[43] Stacey, G., Koh, S., Granger, C. and Becker, J.M. (2002) Peptide transport in plants. Trends Plant Sci. 7, 257–263.
[44] Karim, S., Lundh, D., Holmstrom, K.O., Mandal, A. and Pirhonen, M. (2005) Structural and functional characterization of AtPTR3, a stress-induced peptide transporter of Arabidopsis. J. Mol. Model 11, 226–236.
[45] Miranda, M., Borisjuk, L., Tewes, A., Dietrich, D., Rentsch, D., Weber, H. and Wobus, U. (2003) Peptide and amino acid transporters are differentially regulated during seed development and germination in Faba bean. Plant Physiol. 132, 1950–1960.
[46] Schulze, W., Frommer, W.B. and Ward, J.M. (1999) Transporters for ammonium, amino acids and peptides are expressed in pitchers of the carnivorous plant Nepenthes. Plant J. 17, 637–646.
[47] Hardy, D.J. and Payne, J.W. (1991) Analysis of the peptide carrier in the scutellum of barley embryos by photoaffinity labelling. Planta 186, 44–51.
[48] Higgins, C.F. and Payne, J.W. (1981) The peptide pools of germinating barley grains: relation to hydrolysis and transport of storage proteins. Plant Physiol. 67, 785–792.
[49] Waterworth, W.M., West, C.E. and Bray, C.M. (2000) The barley scutellar peptide transporter: biochemical characterization and localization to the plasma membrane. J. Exp. Bot. 51, 1201–1209.
[50] Waterworth, W.M., Ashley, M.K., West, C.E., Sunderland, P.A. and Bray, C.M. (2005) A role for phosphorylation in the regulation of the barley scutellar peptide transporter HvPTR1 by amino acids. J. Exp. Bot. 56, 1545–1552.
[51] Yamashita, T., Shimada, S., Guo, W., Sato, K., Kohmura, E., Hayakawa, T., Takagi, T. and Tohyama, M. (1997) Cloning and functional expression of a brain peptide/histidine transporter. J. Biol. Chem. 272, 10205–10211.
[52] Brownlee, A.G. and Arst Jr., H.N. (1983) Nitrate uptake in *Aspergillus nidulans* and involvement of the third gene of the nitrate assimilation gene cluster. J. Bacteriol. 155, 1138–1146.
[53] Unkles, S.E., Hawker, K.L., Grieve, C., Campbell, E.I., Montague, P. and Kinghorn, J.R. (1991) crnA encodes a nitrate transporter in *Aspergillus nidulans*. Proc. Natl. Acad. Sci. USA 88, 204–208.

[54] Quesada, A., Galvan, A. and Fernandez, E. (1994) Identification of nitrate transporter genes in *Chlamydomonas reinhardtii*. Plant J. 5, 407–419.
[55] Sakamoto, T., Inoue-Sakamoto, K. and Bryant, D.A. (1999) A novel nitrate/nitrite permease in the marine *Cyanobacterium synechococcus* sp. strain PCC 7002. J. Bacteriol. 181, 7363–7372.
[56] Trueman, L.J., Richardson, A. and Forde, B.G. (1996) Molecular cloning of higher plant homologues of the high-affinity nitrate transporters of *Chlamydomonas reinhardtii* and *Aspergillus nidulans*. Gene 175, 223–231.
[57] Quesada, A., Krapp, A., Trueman, L.J., Daniel-Vedele, F., Fernandez, E., Forde, B.G. and Caboche, M. (1997) PCR-identification of a *Nicotiana plumbaginifolia* cDNA homologous to the high-affinity nitrate transporters of the crnA family. Plant Mol. Biol. 34, 265–274.
[58] Amarasinghe, B.H., de Bruxelles, G.L., Braddon, M., Onyeocha, I., Forde, B.G. and Udvardi, M.K. (1998) Regulation of GmNRT2 expression and nitrate transport activity in roots of soybean (Glycine max). Planta 206, 44–52.
[59] Zhou, J.J., Trueman, L.J., Boorer, K.J., Theodoulou, F.L., Forde, B.G. and Miller, A.J. (2000) A high affinity fungal nitrate carrier with two transport mechanisms. J. Biol. Chem. 275, 39894–39899.
[60] Unkles, S.E., Rouch, D.A., Wang, Y., Siddiqi, M.Y., Glass, A.D. and Kinghorn, J.R. (2004) Two perfectly conserved arginine residues are required for substrate binding in a high-affinity nitrate transporter. Proc. Natl. Acad. Sci. USA 101, 17549–17554.
[61] Galvan, A., Quesada, A. and Fernandez, E. (1996) Nitrate and nitrate are transported by different specific transport systems and by a bispecific transporter in *Chlamydomonas reinhardtii*. J. Biol. Chem. 271, 2088–2092.
[62] Zhou, J.J., Fernandez, E., Galvan, A. and Miller, A.J. (2000) A high affinity nitrate transport system from Chlamydomonas requires two gene products. FEBS Lett. 466, 225–227.
[63] Orsel, M., Chopin, F., Leleu, O., Smith, S.J., Krapp, A., Daniel-Vedele, F. and Miller, A.J. (2006) Characterization of a two-component high-affinity nitrate uptake system in Arabidopsis. Physiology and protein–protein interaction. Plant Physiol. 142, 1304–1317.
[64] Tong, Y., Zhou, J.J., Li, Z. and Miller, A.J. (2005) A two-component high-affinity nitrate uptake system in barley. Plant J. 41, 442–450.
[65] Okamoto, M., Kumar, A., Li, W., Wang, Y., Siddiqi, M.Y., Crawford, N.M. and Glass, A.D. (2006) High-affinity nitrate transport in roots of Arabidopsis depends on expression of the NAR2-like gene AtNRT3.1. Plant Physiol. 140, 1036–1046.
[66] Wang, R. and Crawford, N.M. (1996) Genetic identification of a gene involved in constitutive, high-affinity nitrate transport in higher plants. Proc. Natl. Acad. Sci. USA 93, 9297–9301.
[67] Okamoto, M., Vidmar, J.J. and Glass, A.D. (2003) Regulation of NRT1 and NRT2 gene families of *Arabidopsis thaliana*: responses to nitrate provision. Plant Cell Physiol. 44, 304–317.
[68] Orsel, M., Krapp, A. and Daniel-Vedele, F. (2002) Analysis of the NRT2 nitrate transporter family in Arabidopsis. Structure and gene expression. Plant Physiol. 129, 886–896.
[69] Krapp, A., Fraisier, V., Scheible, W., Quesada, A., Gojon, A., Stitt, M., Caboche, M. and Daniel-Vedele, F. (1998) Expression studies of Nrt2:1Np, a putative high-affinity nitrate transporter: evidence for its role in nitrate uptake. Plant J. 14, 723–731.
[70] Vidmar, J.J., Zhuo, D., Siddiqi, M.Y., Schjoerring, J.K., Touraine, B. and Glass, A.D. (2000) Regulation of high-affinity nitrate transporter genes and high-affinity nitrate influx by nitrogen pools in roots of barley. Plant Physiol. 123, 307–318.
[71] Little, D.Y., Rao, H., Oliva, S., Daniel-Vedele, F., Krapp, A. and Malamy, J.E. (2005) The putative high-affinity nitrate transporter NRT2.1 represses lateral root initiation in response to nutritional cues. Proc. Natl. Acad. Sci. USA 102, 13693–13698.
[72] Lubkowitz, M.A., Hauser, L., Breslav, M., Naider, F. and Becker, J.M. (1997) An oligopeptide transport gene from *Candida albicans*. Microbiology 143 (Pt 2), 387–396.
[73] Koh, S., Wiles, A.M., Sharp, J.S., Naider, F.R., Becker, J.M. and Stacey, G. (2002) An oligopeptide transporter gene family in Arabidopsis. Plant Physiol. 128, 21–29.
[74] Bogs, J., Bourbouloux, A., Cagnac, O., Wachter, A., Rausch, T. and Delrot, S. (2003) Functional characterization and expression analysis of a glutathione transporter, BjGT1, from *Brassica juncea*: evidence for regulation by heavy metal exposure. Plant, Cell Environ. 26, 1703.
[75] Zhang, M.Y., Bourbouloux, A., Cagnac, O., Srikanth, C.V., Rentsch, D., Bachhawat, A.K. and Delrot, S. (2004) A novel family of transporters mediating the transport of glutathione derivatives in plants. Plant Physiol. 134, 482–491.
[76] Curie, C., Panaviene, Z., Loulergue, C., Dellaporta, S.L., Briat, J.F. and Walker, E.L. (2001) Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. Nature 409, 346–349.
[77] Yen, M.R., Tseng, Y.H. and Saier Jr., M.H. (2001) Maize Yellow Stripe1, an iron-phytosiderophore uptake transporter, is a member of the oligopeptide transporter (OPT) family. Microbiology 147, 2881–2883.
[78] Schaaf, G., Ludewig, U., Erenoglu, B.E., Mori, S., Kitahara, T. and von Wiren, N. (2004) ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. J. Biol. Chem. 279, 9091–9096.
[79] Stacey, M.G., Osawa, H., Patel, A., Gassmann, W. and Stacey, G. (2006) Expression analyses of Arabidopsis oligopeptide transporters during seed germination, vegetative growth and reproduction. Planta 223, 291–305.
[80] Osawa, H., Stacey, G. and Gassmann, W. (2006) ScOPT1 and AtOPT4 function as proton-coupled oligopeptide transporters with broad but distinct substrate specificities. Biochem. J. 393, 267–275.
[81] Stacey, M.G., Koh, S., Becker, J. and Stacey, G. (2002) AtOPT3, a member of the oligopeptide transporter family, is essential for embryo development in Arabidopsis. Plant Cell 14, 2799–2811.
[82] Wintz, H., Fox, T., Wu, Y.Y., Feng, V., Chen, W., Chang, H.S., Zhu, T. and Vulpe, C. (2003) Expression profiles of *Arabidopsis thaliana* in mineral deficiencies reveal novel transporters involved in metal homeostasis. J. Biol. Chem. 278, 47644–47653.
[83] Bourbouloux, A., Shahi, P., Chakladar, A., Delrot, S. and Bachhawat, A.K. (2000) Hgt1p, a high affinity glutathione transporter from the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. 275, 13259–13265.
[84] Cagnac, O., Bourbouloux, A., Chakrabarty, D., Zhang, M.Y. and Delrot, S. (2004) AtOPT6 transports glutathione derivatives and is induced by primisulfuron. Plant Physiol. 135, 1378–1387.
[85] Chen, A., Komives, E.A. and Schroeder, J.I. (2006) An improved grafting technique for mature Arabidopsis plants demonstrates long-distance shoot-to-root transport of phytochelatins in Arabidopsis. Plant Physiol. 141, 108–120.
[86] Gong, J.M., Lee, D.A. and Schroeder, J.I. (2003) Long-distance root-to-shoot transport of phytochelatins and cadmium in Arabidopsis. Proc. Natl. Acad. Sci. USA 100, 10118–10123.
[87] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.
[88] Kumar, S., Tamura, K. and Nei, M. (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 5, 150–163.