Amino Acids (2006) 30: 113–120
DOI 10.1007/s00726-005-0248-z

Amino Acids
Printed in Austria

Expression and transcriptional regulation of amino acid transporters in plants

Review Article

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Received June 19, 2005
Accepted July 18, 2005
Published online March 10, 2006;

Summary. Recent studies have shown that there are more than 50 amino acid transporter genes in the *Arabidopsis* genome. This abundance of amino acid transporters implies that they play a multitude of fundamental roles in plant growth and development. Current research on the expression and regulation (i.e., tissue-specific expression and regulation of expression in response to nutrient and environmental changes) of these genes has provided useful information about the functional significance of plant amino acid transport systems.

Keywords: Amino acid – Transporter – Gene expression – *Arabidopsis thaliana*

Introduction

Amino acids are the currency of nitrogen exchange between source to sink tissues in plants (Bush, 1999). Amino acid transporters have been reported to play a variety of essential roles in growth and development. They function in long-distance transport, acquisition by import-dependent cells, and intracellular partitioning between different compartments in the cell. The biochemical properties of plant amino acid transporters were first described using purified plasma membrane vesicles (Bush, 1993, for review), and the first cDNAs encoding plant amino acid transporters were cloned using functional complementation of yeast transport mutants (Frommer et al., 1993; Hsu et al., 1993). More recently, two superfamilies of amino acid transporters have been identified; the amino acid, polyamine and choline transporters superfamily (APC) and the amino acid transporter family (ATF) (Fischer et al., 1998; Ortiz-Lopez et al., 2000; Su et al., 2004).

There are 14 APC transporters in the *Arabidopsis* genome. Among these transporters, nine members of the cationic amino acid transporters (AtCAT1-9) are well described in *Arabidopsis* (Frommer et al., 1995; Su et al., 2004). AtCATs contain 14 putative transmembrane (TM) domains and they are high-affinity basic amino acid transporters. The other 5 members in APC superfamily, including a putative gamma-amino butyric (GABA) transporter, have 12 putative TM domains.

ATF amino acid transporters were first described in plants. In *Arabidopsis*, this superfamily (approximately 46 members) contains at least five subclasses of transporter gene families including: the amino acid permeases (AtAAPs) (Frommer et al., 1993; Hsu et al., 1993), the lysine/histidine transporters (AtLHTs) (Chen and Bush, 1997), the proline transporters (AtProTs) (Rentsch et al., 1996), the aromatic and neutral amino acid transporters (AtANTs) (Chen et al., 2001) and the putative auxin transporters (AtAUXs) (Bennett et al., 1996).

Given the large number of amino acid transporters in plants, it seems clear they are functionally differentiated based on substrate specificity, cell and tissue specific expression patterns, and developmental and environmental control of expression and activity. Because of this complexity, investigators are examining in detail the transport properties, expression patterns and regulation of many members of each transporter gene family. Results to date strongly support the notion that functional significance is defined not only by biochemical activity (i.e., amino acid transport) but also by expression pattern as controlled

by developmental programs, and by differential responses to environmental signals, such as alterations in nutrient availability. In this review, recent advances describing the expression and regulation of plant amino acid transporter genes are summarized and possible directions for future research are discussed.

Expression of amino acid transporters

Knockout and/or suppression mutants (i.e., T-DNA or transposon insertion, antisense, or RNAi transgenic plants) are commonly used to characterize the function of plant gene products. These approaches have not been very successful for plant amino acid transporters. For example, homozygous T-DNA insertion mutants of *AtAAP3* showed no difference from wild type under the growth conditions used (Okumoto et al., 2004). RNAi suppression of *AtAAP1* in *Arabidopsis* did not exhibit any significant changes in phenotype (Guo, 2004). It has been shown that a number of plant amino acid transporters exhibit some level of transport activity (often less than 10% of that for their primary substrate) for a broad spectrum of substrates. In this case, *AtAAP2*, *AtAAP5*, and *AtAAP6* may compensate for *AtAAP3* because they are co-expressed in roots and can transport similar amino acids as *AtAAP3*. *AtAAP5* has a similar expression pattern as *AtAAP1* (expressed in source leaf, flower, and fruit) and may function for *AtAAP1* in the null mutant. Thus, the absence of easily scored phenotypes in null mutants of amino acid transporters may be due to functional compensation by one or more other transporters. Alternatively, visible phenotypes may be missed or are only induced under certain growth conditions. Therefore, detailed analysis of gene expression and regulation is needed, along with biochemical descriptions, to fully understand the physiological contributions of individual transporters in plants.

Expression is generally characterized by RT-PCR, promoter-GUS constructions, and/or in situ RNA hybridization. For subcellular localization, immunolocalization and/or expression of a transporter::GFP fusion protein are used (Bush, 2004, for review of methods). The expression of plant amino acid transporters was previously summarized by Fischer et al. (1998) and Ortiz-Lopez et al. (2000). We summarize recent descriptions of plant amino acid transporter expression (mostly from *Arabidopsis*) in the following section.

It has been suggested that AtAAP1, AtAAP2, AtAAP4, and AtAAP5 might be involved in phloem loading of amino acids from source tissues since their expression was observed in source leaves (Hsu et al., 1993; Fischer et al., 1995; Hirner et al., 1998). These might also be involved in transporting amino acids into the developing embryo because their expression was also detected in stems and flowers (Ortiz-Lopez et al., 2000). Expression of both *AtAAP1* and *AtAAP2* have been detected in siliques. Transgenic plants expressing *AtAAP1* or *AtAAP2* promoter-GUS constructs showed that *AtAAP1* was only expressed in seeds and *AtAAP2* was specifically expressed in the vascular tissues of siliques (Hirner et al., 1998). Furthermore, *AtAAP2* expression was also observed in major veins of leaves and stems of mature plants, and in the peduncle. These results suggested that AtAAP2 might play important roles in long-distance transport of amino acids in *Arabidopsis*. AtAAP3, expressed specifically in Arabidopsis roots, may be involved in amino acid uptake from the phloem or in retrieving amino acids from the soil (Fischer et al., 1998). Recently, the expression pattern of *AtAAP3* was analyzed by Okumoto et al. (2004) using *AtAAP3* promoter-GUS transgenic plants. *AtAAP3* expression was observed for a short period in the connective tissue of the stamen before dehiscence, although it was mainly expressed in the root vascular tissue. AtAAP6 is a high-affinity transporter for most acidic and neutral amino acids (Fischer et al., 2002). Expression of *AtAAP6* was mainly detected in roots, sink leaves and cauline leaves (Fischer et al., 1995). Promoter-GUS analysis showed that *AtAAP6* is expressed in xylem parenchyma, suggesting that AtAAP6 might function in uptake of amino acids from the xylem because a high-affinity transporter may be required due to the low amino acid concentration in xylem sap (Okumoto et al., 2002). Histochemical analysis of *AtAAP8* promoter-GUS transgenic plants showed that *AtAAP8* is expressed in young siliques and developing seeds, suggesting a role in amino acid acquisition in developing seeds.

AtLTH1 expression was detected on the surface of roots in young seedlings and in pollen using whole mount, in situ hybridization, suggesting it might be involved in the uptake of amino acids from the soil and/or into sink tissues (Chen and Bush, 1997). AtLHT2 has been recently characterized as a high-affinity proline and aspartate transporter that plays an important role in partitioning of amino acids for microspore because it was specifically expressed in tapetum cells in *Arabidopsis* (Lee and Tegeder, 2004). Three proline transporter genes (*AtProT1*, *AtProT2*, and *AtProT3*) were found to be expressed ubiquitously in *Arabidopsis* and *AtProT2* expression was regulated by salt stress (Rentsch et al., 1996; Grallath et al., 2005). *AtProT1* was highly expressed in roots, stems and flowers. The expression of *AtProT1* in flowers was observed

mainly in the stalk phloem that enters the carpals (Rentsch et al., 1996). Transgenic plants containing promoter-GUS constructs revealed that *AtProT2* was expressed in the epidermis and cortex cells in roots, whereas expression of *AtProT3* was only detected in the above-ground organs, such as leaves, flowers, and siliques (Grallath et al., 2005). Only one aromatic and neutral amino acid transporter (AtANT1) has been characterized to date (Chen et al., 2001). *AtANT1* is expressed in all organs with highest abundance in flowers and cauline leaves.

In the APC superfamily, AtCAT1 was first characterized by Frommer et al. (1995). *AtCAT1* is expressed in leaves, flowers and developing siliques. Transgenic tobacco plants expressing an *AtCAT1* promoter-GUS construct showed that GUS expression was detected in several floral tissues (i.e., peduncle, sepal, corolla, pistil and ovaries). Promoter-GUS analysis revealed that the transcripts of *AtCAT1* were specifically localized in major veins of leaves and roots. These results suggested that AtCAT1 might play multiple roles in phloem physiology, from phloem loading to providing amino acids for developing embryos. Recently, expression of several other *AtCAT*s have been characterized (Su et al., 2004). Each member of this family has a specific tissue or organ distribution, although most of *AtCAT*s have a broad expression pattern in *Arabidopsis*. *AtCAT6* was preferably expressed in roots, and *AtCAT7* was expressed at a very low level under normal conditions. *AtCAT5* promoter-GSU transgenic plants revealed that *AtCAT5* was also expressed in seeds, suggesting AtCAT5 might function in seed germination or seedling development by loading of basic amino acids from seed stalks into seeds. A high level of *AtCAT8* expression was observed in rapidly dividing cells of both shoot and root apical meristems. Of those examined, most amino acid transporters have been localized in the plasma membrane. However, AtCAT2::GFP was localized at the vacuole, suggesting it is a vacuolar amino acid transporter in *Arabidopsis*.

The expression of the orthologous genes of amino acid transporters may not be similar because the pool of amino acids available for phloem transport is differentially regulated in different species (Delrot et al., 2001). Transcription levels of *RcAAP1* and *RcAAP2* were found to be highest in cotyledons and relatively low in roots (Bick et al., 1998). Very low expression of both genes was detected in hypocotyl, endosperm, sink and source leaves. In contrast, *RcAAP3* expression was observed in all plant tissues examined including sink and source tissues (Neelam et al., 1999). Both RcAAP1 and RcAAP3 share highest sequence similarity to AtAAP3, and RcAAP2 is most closely related to AtAAP6 in *Arabidopsis*. Although RcAAP1 may serve a similar function as AtAAP3 because of comparable expression patterns, RcAAP2 and RcAAP3 may function differently from their orthologs in *Arabidopsis*. Differential expression of orthologous transporters in different species may provide information for gene origination and evolution.

Proline transporters (LeProT1, LeProT2, and LeProT3) have been identified in tomato that are similar to those found in *Arabidopsis* (AtProT1 and AtProT2). LeProT1 transports proline and GABA with low affinity, and glycinebetaine with high affinity (Schwacke et al., 1999). LeProT1 might be involved in pollen nutrition because of its strong expression in mature and germinating pollen.

VfAAP1 expression was detected at a high level in cotyledons during early development (Miranda et al., 2001). Thus, it might play an important role in providing amino acids for storage protein biosynthesis. *VfAAP1* was also expressed in other sink tissues. *VfAAP3* was expressed in most tissues such as roots, stems, gynoecia, pods and seed coats at different developmental stages (Miranda et al., 2001).

Regulation of amino acid transporter gene expression by environmental signals

Amino acid transport has been shown to be highly regulated by environmental signals, such as light (Guo, 2004), osmotic changes (Rentsch et al., 1996; Girousse et al., 1996; Ueda et al., 2001; Popova et al., 2003), and pathogen attack (Carginale et al., 2004). Similarly, amino acid composition and biosynthesis are highly regulated by light (for review see Oliveira et al., 2001; Sitt et al., 2002). However, little is known about light regulation of plant amino acid transporters. Preliminary results showed that *AtAAP1* expression was induced within 6 hours when dark-adapted plants were exposed to light (Ortiz-Lopez et al., 2000) but it is not clear whether the induction was caused by a light signal or the photosynthesis-dependent increase in sugar levels. Recent experiments confirmed that light was (at least) part of the signals for induction of *AtAAP1* expression using DCMU (photosynthesis inhibitor) treatment (Guo, 2004). Moreover, sugars were also shown to induce expression, and regulation by light and sugar exhibited an additive effect.

Generally, plants synthesize and accumulate osmoprotectants such as proline, glycinebetaine and sugar polyols under stress conditions. For example, proline concentrations in the phloem sap of alfalfa increased up to 60 times under water stress conditions (Girousse et al., 1996).

Proline content in tomato flowers is much higher than in any other organs (Schwacke et al., 1999). Likewise, increased deposition of proline at the root apex in water stressed plants (Voetberg and Sharp, 1991) might due to phloem transport of proline (Girousse et al., 1996). Up-regulation of proline transporter gene expression in response to water and salt stresses has been reported in many plant species. For example, *AtProT2* in *Arabidopsis thaliana* (Rentsch et al., 1996), *HvProT* in barley (Ueda et al., 2001), and *McAAT1* in *Mesembryanthemum crystallinum* (Popova et al., 2003) were found to be strongly induced by water and salt stress. Transgenic *Arabidopsis* over-expressing *AhProT* (a proline transporter from *Atriplex hortensis* L.) had increased proline content in root tips and showed salt tolerance (Shen et al., 2002).

Expression of other amino acid transporters has also been reported to be regulated by osmotic stress. For example, *AtAAP4* and *AtAAP6* expression was down-regulated by water and salt stresses in *Arabidopsis* (Rentsch et al., 1996). Induction of proline transport and repression of other amino acid transport may be important for plants to overcome the stress conditions. In *M. crystallinum*, increased proline concentration was observed in both root and leaf tissues in response to high salinity (Popova et al., 2003). Expression of two amino acid transporters was regulated differentially under salt stress. In unstressed conditions, *McAAT1* (ProT subfamily) was expressed only in leaves but *McAAT2* (LHT subfamily) was specifically expressed in roots. When plants were salt stressed for 6 hours, *McAAT1* expression was induced in leaves whereas *McAAT2* transcript was down-regulated. This agreed with the previous finding that expression of *AtAAPs* was repressed by water and salt stresses (Rentsch et al., 1996). However, it is interesting that expression of *McAAT2* could be induced by osmotic stress in the epidermis of root tips and mature roots (Popova et al., 2003).

Regulation of amino acid transporter gene expression by nutrients

Regulation of transporter gene expression in response to variation in nutrient availability has been reported. *AtAAP1* expression was found to be up-regulated by either 30 mM sucrose or glucose (Guo, 2004). In plants, there are two major sugar signaling pathways: a specific pathway for sucrose (Chiou and Bush, 1998; Vaughn et al., 2002), and at least one glucose-specific hexokinase-mediated pathway (Jang et al., 1997; Moore et al., 2002). Induction of *AtAAP1* expression by glucose was blocked in HXK1-antisense plants but sucrose induction was unaffected. Those results showed that glucose induction of *AtAAP1* expression required *HXK1* expression, whereas sucrose induction is *HXK1* independent (Guo, 2004).

Nitrogen availability in the soil solution frequently limits plant growth and development. It has been known for a long time that nitrate deficiency in the soil induces increased nitrate transport activity (Coruzzi and Bush, 2001, for review of nutrient signaling) and, with the successful cloning of several nitrate transporters, enhanced transport activity has been shown be the result of increased transporter gene expression. Although regulation of nitrate reductase and transporter gene expression has been studied extensively in plants (Stitt, 1999; Wang et al., 2003), little is known about comparable regulation of amino acid transport expression. Expression of *AtAAP1* in nitrogen-starved plants

Table 1. Regulation of amino acid transporter gene expression in response to nitrate[1]

Gene	150 μM KNO_3 20 min		4 mM KNO_3 20 min		40 mM KNO_3 20 min		4 mM KNO_3 2 h		4 mM KNO_3 24 h	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
AtAAP1	2.2[2]	1.1	1.0	0.81	2.4	4.6	1.7	0.46	2.0	1.7
AtAAP4	0.76	0.70	0.4	0.56	0.95	1.49	0.46	0.36	1.1	0.64
AtLHT1	1.9	3.7	1.5	1.5	1.8	14.1	1.1	1.6	1.6	24.0
AtProT2	0.50	0.40	0.90	0.70	1.8	12.0	0.85	0.30	0.89	0.70
AtANT1	1.1	0.52	0.79	0.47	1.5	6.2	1.1	0.67	0.95	0.77
AtCAT1	0.45	0.92	0.85	1.1	1.87	0.81	0.34	0.81	1.56	1.6

[1] *Arabidopsis* (Columbia) plants were grown hydroponically in MS salt solution containing 2 mM NH_4NO_3, 2 mM KNO_3 and 0.5% of sucrose for 11 days and 4 h at 22°C (16 h/8 h, day/night). Plants were then subject to nitrogen starvation for 24 h under same growth condition by transferring them into nitrogen free MS medium. The nitrogen starved plants were treated with 150 μM, 4 mM, or 40 mM KNO_3. Control plants were treated with the same concentration of KCl. Total RNA was isolated separately from seedling roots and shoots

[2] The ratio of message abundance, determined with quantitative real-time PCR, in nitrate treated sample to its control sample

was found to be induced by nitrate within 30 minutes (Ortiz-Lopez et al., 2000). The induction of *AtAAP1* expression was not affected by tungstate (nitrate reductase inhibitor), suggesting that nitrate is the signaling molecule for regulation of *AtAAP1* expression (Guo, 2004). The regulation of *AtAAP1* expression in response to nitrate was also detected using microarray (Guo, 2004) and real-time PCR (Table 1).

Nitrate regulation of expression of six amino acid transporters – AtAAP1 (Frommer et al., 1993; Hsu et al., 1993), AtAAP4 (Fischer et al., 1995), AtANT1 (Chen et al., 2001), AtCAT1 (Frommer et al., 1995), AtLHT1 (Chen and Bush, 1997) and AtProT2 (Rentsch et al., 1996), representing five families of amino acid transporters, was analyzed using real-time PCR (Table 1). Only *AtAAP1* expression was found to be up-regulated by either 0.2 mM or 40 mM of nitrate in both seedling roots and shoots within 20 minutes. Expression of *AtANT1*, *AtLHT1* and *AtProT2* could only be induced by high concentration of nitrate (40 mM) in seedling shoots within 20 minutes. Interestingly, only *AtLHT1* expression was induced in seedling shoots when plants were treated with 4 mM nitrate for two hours (Table 1).

Ammonium and amino acid (i.e., Glu and Gln) induction of *AtAAP1* expression has been demonstrated in *Arabidopsis* (Guo, 2004). In contrast, *VfAAP1* expression was repressed in cotyledons cultured in the presence of glutamine and cysteine (Miranda et al., 2001). In microarray experiments, only *AtAAP2* was found to be induced by Glu (>2 fold) and only *AtAAP6* expression was observed to be significantly down-regulated by Gln (Guo, 2004). Generally, the effect of nitrate and ammonium on regulation of amino acid transporter expression in seedling shoots was much greater than that in seedling roots.

Promoter analysis

As described above, plant amino acid transporter genes exhibit complex patterns of expression that are differentially regulated in response to a variety of developmental and environmental signals. In spite of the growing body of evidence documenting this complexity, little is known about the regulatory elements in transporter gene promoters that are responsible for controlling expression. Sequence analysis of the *AtAAP1* promoter (Chang, 1998) revealed a GATA element for NIT-2 recognition (Fu and Marzluf, 1990) and an AT-rich element (Hwang et al., 1997). These two cis elements might be related to nitrogen regulation of *AtAAP1* expression (Guo, 2004). In the *AtCAT5* promoter, a TGACGT motif

Table 2. *Cis*-elements analysis of the 1 kb promoter regions of amino acid transporter genes in *Arabidopsis*

Gene	RY-element (Ref. 1)	CGTAC-motif (Ref. 2)	AuxRR (Ref. 3)	ABRE (Ref. 4)	DRE (Ref. 4)	A-Box (Ref. 5)	TATCCATC motif (Ref. 5)	TATC Box (Ref. 6)	GATA (Ref. 7)	As1 (Ref. 4)
AAP1	1	0	0	1	0	1	1	1	0	0
AAP2	0	0	0	1	0	3	1	1	2	0
AAP3	2	0	0	1	1	1	2	2	0	1
AAP4	1	0	0	2	0	3	0	1	1	0
AAP5	0	0	0	0	0	1	3	0	1	1
AAP6	0	1	1	0	0	0	1	1	4	1
AAP7	0	1	1	5	0	2	2	2	0	0
AAP8	0	0	0	3	0	0	2	2	3	0
ProT1	0	1	0	2	0	2	6	1	2	0
ProT2	0	2	0	1	1	1	2	1	3	1
LHT1	1	0	0	0	0	0	4	1	2	1
LHT2	1	3	0	0	1	2	3	1	2	1
ANT1	0	1	1	4	0	3	1	1	1	1
CAT1	3	2	0	1	0	0	2	1	3	0
CAT2	0	2	3	2	0	2	1	4	2	0
CAT3	0	0	1	0	0	2	1	1	0	0
CAT4	0	1	2	2	0	2	1	2	3	1
CAT5	1	2	0	1	1	1	0	0	2	0
CAT6	0	2	1	1	0	2	2	1	2	0
CAT7	0	1	3	6	1	1	2	0	0	0
CAT8	0	3	0	3	0	1	1	1	0	0
CAT9	0	0	0	0	0	2	1	2	1	0

Ref. 1: Prieto-Dapena et al., 1999; Ref. 2: Rouster et al., 1997; Ref. 3: Sakai et al., 1996; Ref. 4: Yamahuchi-Shinozaki and Shinozaki, 1994; Ref. 5: Huang et al., 1990; Ref. 6: Jacobsen et al., 1995; Ref. 7: Arguello-Astorga and Herrera-Estrella, 1995

was identified at −177 before start codon (Su et al., 2004). *AtCAT5* was expressed during early seedling development because TGACGT was reported to strongly drive α-amylase expression during seedling development (Yamauchi, 2001).

For a better understanding of the molecular determinants regulating plant amino acid gene expression, we analyzed 1 kb of the promoter sequence for 22 plant amino acid transporter genes (Table 2) using PlantCare (http://intra.psb.ugent.be:8080/PlantCARE/, Lescot et al., 2002). Seven genes (*AtAAP1*, *AtAAP3*, *AtAAP4*, *LHT1*, *LHT2*, *AtCAT1*, *AtCAT5*) have the putative seed-specific nitrogen-responsive GCN4-/RY-like element (Bäumlein et al., 1992). A GCN4/RY-like motif is a likely candidate for conferring nitrogen responsiveness for these genes. In plants, the GCN4 elements act as enhancers in several storage protein genes, and as silencers in amino acid biosynthetic and non-storage protein genes. The GCN4-like RY motifs play a key role in seed-specific gene regulation in coordination with other *cis*-acting elements (Bäumlein et al., 1992).

Induction of *ProTs* expression by osmotic stress has been observed in several plant species. We found a significant drought-responsive element (DRE) in *AtProT2* promoter (Table 2). C-repeat/DRE element, a target sequence for an AP2 domain containing transcription factor CBF1, is responsible for the induction of gene expression under salt stress and water deficit (Yamahuchi-Shinozaki and Shinozaki, 1994; Stockinger et al., 1998). The promoters of three other transporter genes (*AtAAP3*, *LHT2*, and *AtCAT5*) also contain a DRE motif. It would be interesting to test whether expression of these three genes is regulated by osmotic stress. An As1 motif, for root-specific expression, is present in the promoters of eight genes (*AtAAP3*, *AtAAP5*, *AtAAP6*, *AtProT2*, *AtLHT1*, *LHT2*, *AtANT1*, and *AtCAT5*) and, significantly, expression of all eight genes was observed in *Arabidopsis* roots (Fischer et al., 1998; Ortiz-Lopez et al., 2000; Su et al., 2004).

A number of amino acid transporter genes have putative plant hormone (auxin, abscisic acid, and jasmonate) responsive elements in their promoter regions (Table 2). Interestingly, except for *AtCAT5*, all promoters have a TATC-core element motif, and most of the promoters (16 out of 22) have both an A-box and a TATC-core element. The A-box and TATC-core elements are conserved *cis* elements found in the promoter regions of alpha-amylase genes (Huang et al., 1990). The TATCCA element is responsible for sugar repression and it is also important for the gibberellin induction (Jacobsen et al., 1995). Its antisense sequence GATA motif may be involved in nitrogen regulation of gene expression. These data show that plant amino acid transporter gene expression is regulated by a complex array of *cis* elements and transcription factors involving cross-talk between sugar, nitrogen and plant hormone signaling pathways.

Future perspective

Biochemical descriptions of plant amino acid transporters in purified membrane vesicles (see Bush, 1993 for review) and the successful cloning of the first transporters yielded a period of rapid growth in our understanding of these critical transporters (Frommer et al., 1993; Hsu et al., 1993). Multiple gene families have been identified and several transporters have been described in detail. Although we have a much clearer picture about the number and types of amino acid transporters found in plants, our understanding of the physiological function for the majority of these transporters is still very limited. The complexity of individual expression patterns and broad substrate specificity are two major challenges to providing functionally relevant descriptions of many amino acid transporters in the plants. As noted above, disruption of one amino acid transporter gene may not affect plant growth and development. Making double mutants or knocking out a group of the transporters (i.e., these transporters have similar expression patterns or similar biochemical function) using RNAi may overcome these difficulties. Over-expression or ectopic expression is another approach for pushing forward. For example, transgenic plants over-expressing *AtAAP1* were found to have severe phenotypes such as deformed leaf shape, small leaf size, short siliques and late flowering (Guo, 2004). These results suggest that AtAAP1 plays important roles that have yet to be fully appreciated. Moreover, over-expression of *AtAAP1* may cause imbalances of amino acid distribution that adversely affect plant growth and development.

Although there is a substantial amount we do not understand about plant amino acid transporters, there are many opportunities for applying what we have learned toward new approaches for improving crop productivity or nutritional value. For example, transgenic plants (*Vicia narbonensis* and pea) ectopically expressing *VfAAP1* in seeds were found to have increased storage proteins (Rolletscheck et al., 2005). Targeted enhancement of amino nitrogen content in nitrogen-poor seeds could have a significant impact on nutritional value.

References

Arguello-Astorga GR, Herrera-Estrella LR (1996) Ancestral multipartite units in light-responsive plant promoters have structural features correlating with specific phototransduction pathways. Plant Physiol 112: 1151–1655

Bäumlein H, Nagy I, Villarroel R, Inze D, Wobus U (1992) *cis*-Analysis of a seed protein gene promoter: the conservative RY repeat CATGCATG within the legumin box is essential for tissue-specific expression of a legumin gene. Plant J 2: 233–239

Bennett MJ, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schulz B, Feldmann KA (1996) *Arabidopsis* AUX1 gene: a permease-like regulator of root gravitropism. Science 273: 948–950

Brenner ICO, Chiu J, Hsieh MH, Kouranov A, Lam HM, Shin MJ, Coruzzi G (2001) Metabolite and light regulation of metabolism in plants: lessons from the study of a single biochemical pathway. Braz J Med Biol Res 34: 567–575

Bush DR (1993) Proton-coupled sugar and amino acid transporters in plants. Annu Rev Plant Physiol Plant Mol Biol 44: 513–542

Bush DR (1999) Amino acid transport. In: Singh BK (ed) Plant amino acids: biochemistry and biotechnology. Marcel Dekker, New York, pp 305–318

Bush DR (2004) Functional analysis of proton-coupled sucrose transport. In: Blatt MR (ed) Membrane transport in plants. Blackwell, Oxford, pp 135–147

Chang HC (1998) Topology and expression regulation of neutral amino acid transporter (NAT2). PhD dissertation, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Chang HC, Bush DR (1997) Topology of NAT2, a prototypical example of a new family of amino acid transporters. J Biol Chem 272: 30552–30557

Carginale V, Maria G, Capasso C, Ionata E, La Cara F, Pastore M, Bertaccini A, Capasso A (2004) Identification of genes expressed in response to phytoplasma infection in leaves of *Prunus armeniaca* by messenger RNA differential display. Gene 332: 29–34

Chen L, Bush DR (1997) LHT1, a lysine- and histidine-specific amino acid transporter in *Arabidopsis*. Plant Physiol 115: 1127–1134

Chen L, Ortiz-Lopez A, Jung A, Bush DR (2001) ANT1, an aromatic and neutral amino acid transporter in *Arabidopsis*. Plant Physiol 125: 1813–1820

Chiou TJ, Bush DR (1998) Sucrose is a signal molecule in assimilate partitioning. Proc Natl Acad Sci USA 95: 4784–4788

Coruzzi G, Bush DR (2001) Nitrogen and carbon nutrient and metabolite signaling in plants. Plant Physiol 125: 61–64

Delrot S, Rochat C, Tegader M, Frommer W (2001) Amino acid transport. In: Lea P, Morot-Gaudry JF (eds) Plant nitrogen. Springer, Berlin, pp 213–236

Dundar E (2003) Molecular and physiological analysis of amino acid transporters in *Arabidopsis thaliana*. PhD dissertation, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Fischer WN, Kwart M, Hummel S, Frommer WB (1995) Substrate specificity and expression profile of amino acid transporters (AAPs) in *Arabidopsis.* J Biol Chem 270: 16315–16320

Fischer WN, André B, Rentsch D, Krolkiewicz S, Tegeder M, Breitkreuz K, Frommer WB (1998) Amino acid transport in plants. Trends Plant Sci 3: 188–195

Fischer WN, Loo DD, Koch W, Ludewig U, Boorer KJ, Tegeder M, Rentsch D, Wright EM, Frommer WB (2002) Low and high affinity amino acid H^+-cotransporters for cellular import of neutral and charged amino acids. Plant J 29: 717–731

Frommer WB, Hummel S, Riesmeier JW (1993) Expression cloning in yeast of a cDNA encoding a broad specificity amino acid permease from *Arabidopsis thaliana*. Proc Natl Acad Sci USA 90: 5944–5948

Frommer WB, Hummel S, Unseld M, Ninnemann O (1995) Seed and vascular expression of a high-affinity transporter for cationic amino acids in *Arabidopsis*. Proc Natl Acad Sci USA 92: 12036–12040

Fu YH, Marzluf GA (1987) Molecular cloning and analysis of the regulation of nit-3, the structural gene for nitrate reductase in *Neurospora crassa*. Proc Natl Acad Sci USA 84: 8243–8247

Gazzarrini S, Lejay L, Gojon A, Ninnemann O, Frommer WB, von Wiren N (1999) Three functional transporters for constitutive, diurnally regulated, and starvation-induced uptake of ammonium into *Arabidopsis* roots. Plant Cell 11: 937–947

Girousse C, Bournoville R, Bonnemain JL (1996) Water deficit-induced changes in concentrations in praline and some other amino acids in the phloem sap of alfafa. Plant Physiol 111: 109–113

Grallath S, Weimar T, Meyer A, Gumy C, Suter-Grotemeyer M, Neuhaus JM, Rentsch D (2005) The AtProT family. Compatible solute transporters with similar substrate specificity but differential expression patterns. Plant Physiol 137: 117–126

Guo MG (2004) Molecular and genomic analysis of nitrogen regulation of amino acid permease I (AAP1) in *Arabidopsis*. PhD dissertation, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Hirner B, Fischer WN, Rentsch D, Kwart M, Frommer WB (1998) Developmental control of H^+/amino acid permease gene expression during seed development of *Arabidopsis*. Plant J 14: 535–544

Hsu LC, Chiou TJ, Chen L, Bush DR (1993) Cloning a plant amino acid transporter by functional complementation of a yeast amino acid transport mutant. Proc Natl Acad Sci USA 90: 7441–7445

Hwang JS, Yang J, Pittard AJ (1997) Critical base pairs and amino acid residues for protein-DNA interaction between the TyrR protein and TyrP operator of *Escherichia coli*. J Bacteriol 179: 1051–1058

Jacobsen JV, Gubler F, Chandler PM (1995) Gibberellin and abscisic acid in germinating cereals In: Davies PJ (ed) Plant hormones: physiology, biochemistry and molecular biology. Kluwer, Boston, pp 246–271

Jang JC, Leon P, Zhou L, Sheen J (1997) Hexokinase as a sugar sensor in higher plants. Plant Cell 9: 5–19

Koch W, Kwart M, Laubner M, Heineke D, Stransky H, Frommer WB, Tegeder M (2003) Reduced amino acid content in transgenic potato tubers due to antisense inhibition of the leaf H^+/amino acid symporter StAAP1. Plant J 33: 211–220

Lee YH, Tegeder M (2004) Selective expression of a novel high-affinity transport system for acidic and neutral amino acids in the tapetum cells of *Arabidopsis* flowers. Plant J 40: 60–74

Lescot M, Dehais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouze P, Rombauts S (2002) PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. Nucleic Acids Res 30: 325–327

Miranda M, Borisjuk L, Tewes A, Heim U, Sauer N, Wobus U, Weber H (2001) Amino acid permeases in developing seeds of *Vicia faba* L.: expression precedes storage protein synthesis and is regulated by amino acid supply. Plant J 28: 61–71

Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, Hwang I, Jones T, Sheen J (2003) Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science 300: 332–336

Ortiz-Lopez A, Chang HC, Bush DR (2000) Amino acid transporters in plants. Biochim Biophys Acta 1465: 275–280

Okumoto S, Koch W, Tegeder M, Fischer WN, Biehl A, Leister D, Stierhof YD, Frommer WB (2004) Root phloem-specific expression of the plasma membrane amino acid proton co-transporter AAP3. J Exp Bot 55: 2155–2168

Okumoto S, Schmidt R, Tegeder M, Fischer WN, Rentsch D, Frommer WB, Koch W (2002) High affinity amino acid transporters specifically expressed in xylem parenchyma and developing seeds of *Arabidopsis*. J Biol Chem 277: 45338–45346

Piechulla B, Merforth N, Rudolph B (1998) Identification of tomato Lhc promoter regions necessary for circadian expression. Plant Mol Biol 38: 655–662

Popova OV, Dietz KJ, Golldack D (2003) Salt-dependent expression of a nitrate transporter and two amino acid transporter genes in *Mesembryanthemum crystallinum*. Plant Mol Biol 52: 569–578

Prieto-Dapena P, Almoguera C, Rojas A, Jordano J (1999) Seed-specific expression patterns and regulation by ABI3 of an unusual late embryogenesis-abundant gene in sunflower. Plant Mol Biol 39: 615–625

Rentsch D, Hirner B, Schmelzer E, Frommer WB (1996) Salt stress-induced proline transporters and salt stress-repressed broad specificity amino acid permeases identified by suppression of a yeast amino acid permease-targeting mutant. Plant Cell 8: 1437–1446

Rolletschek H, Hosein F, Miranda M, Heim U, Gotz KP, Schlereth A, Borisjuk L, Saalbach I, Wobus U, Weber H (2005) Ectopic expression of an amino acid transporter (VfAAP1) in seeds of *Vicia narbonensis* and pea increases storage proteins. Plant Physiol 137: 1236–1249

Rouster J, Leah R, Mundy J, Cameron-Mills V (1997) Identification of a methyl jasmonate-responsive region in the promoter of a lipoxygenase 1 gene expressed in barley grain. Plant J 11: 513–523

Sakai T, Takahashi Y, Nagata T (1996) Analysis of the promoter of the auxin-inducible gene, parC, of tobacco. Plant Cell Physiol 37: 906–913

Schwacke R, Grallath S, Breitkreuz KE, Stransky E, Stransky H, Frommer WB, Rentsch D (1999) LeProT1, a transporter for proline, glycine betaine, and gamma-amino butyric acid in tomato pollen. Plant Cell 11: 377–391

Shen YG, Zhang WK, Yan DQ, Du BX, Zhang JS, Chen SY (2002) Overexpression of proline transporter gene from Halophyte confers tolerance in *Arabidopsis*. Acta Bot Sin 44: 956–962

Stitt M (1999) Nitrate regulation of metabolism and growth. Curr Opin Plant Biol 2: 178–186

Stitt M, Muller C, Matt P, Gibon Y, Carillo P, Morcuende R, Scheible WR, Krapp A (2002) Steps towards an integrated view of nitrogen metabolism. J Exp Bot 53: 959–970

Stockinger EJ, Gilmour SJ, Thomashow MF (1997) *Arabidopsis thaliana CBF1* encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a *cis*-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc Natl Acad Sci USA 94: 1035–1040

Su YH, Frommer WB, Ludewig U (2004) Molecular and functional characterization of a family of amino acid transporters from *Arabidopsis*. Plant Physiol 136: 3104–3131

Tully RE, Hanson AD, Nelsen CE (1979) Proline accumulation in water-stressed barley leaves in relation to translocation and the nitrogen budget. Plant Physiol 63: 518–523

Ueda A, Shi W, Sanmiya K, Shono M, Takabe T (2001) Functional analysis of salt-induced proline transporter of barley roots. Plant Cell Physiol 42: 1282–1289

Vaughn MW, Harrington GN, Bush DR (2002) Sucrose-mediated transcriptional regulation of sucrose symporter activity in the phloem. Proc Natl Acad Sci USA 99: 10876–10880

Voetberg GS, Sharp RE (1991) Growth of the maize primary root tip at low water potentials. III. Role of increased proline deposition in osmotic adjustment. Plant Physiol 96: 1125–1130

Wang R, Okamoto M, Xing X, Crawford NM (2003) Microarray analysis of the nitrate response in *Arabidopsis* roots and shoots reveals over 1,000 rapidly responding genes and new linkages to glucose, trehalose-6-phosphate, iron, and sulfate metabolism. Plant Physiol 132: 556–567

Yamaguchi-Shinozaki K, Shinozaki K (1994) A novel *cis*-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6: 251–264

Yamauchi D (2001) A TGACGT motif in the 5′-upstream region of alpha-amylase gene from *Vigna mungo* is a *cis*-element for expression in cotyledons of germinated seeds. Plant Cell Physiol 42: 635–641

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