FEBS Letters 581 (2007) 2281–2289

Minireview

# Transporters for uptake and allocation of organic nitrogen compounds in plants

Doris Rentsch[a,*], Susanne Schmidt[b,1], Mechthild Tegeder[c,2]

[a] University of Bern, Institute of Plant Sciences, Altenbergrain 21, 3011 Bern, Switzerland
[b] University of Queensland, School of Integrative Biology, Brisbane Qld 4072, Australia
[c] Washington State University, School of Biological Sciences, Pullman, WA 99164-4236, USA

Received 20 February 2007; revised 10 April 2007; accepted 11 April 2007

Available online 18 April 2007

Edited by Ulf-Ingo Flügge and Julian Schroeder

**Abstract Nitrogen is an essential macronutrient for plant growth. Following uptake from the soil or assimilation within the plant, organic nitrogen compounds are transported between organelles, from cell to cell and over long distances in support of plant metabolism and development. These translocation processes require the function of integral membrane transporters. The review summarizes our current understanding of the molecular mechanisms of organic nitrogen transport processes, with a focus on amino acid, ureide and peptide transporters.**


*Keywords:* Transport; Organic nitrogen; Amino acid; Peptide; Ureide; Assimilate partitioning

## 1. Introduction

Nitrogen (N) is quantitatively the most important nutrient for plant growth and productivity. Plants have evolved diverse and complex strategies for acquisition, assimilation and partitioning of nitrogen. Uptake of inorganic or organic N from the soil, and transport of newly synthesized organic N from so-called source organs (e.g. leaves and roots) to sinks (developing leaves, flowers and seeds) are integral to the physiology of plants and essential for plant development. In addition, reallocation of N stored as protein reserves is important during leaf senescence, seed germination, spring growth of perennials and development of new generations from tubers and other reproductive propagules. Biological membranes provide natural barriers for movement of assimilates within the plant, but the presence and tightly regulated activity of transport proteins allow for controlled distribution within cells as well as between cells and over short and long distances. Identification and characterization of transport systems that direct the flow of N metabolites between cellular compartments, in tissues and organs, and throughout the plant, is therefore crucial to understanding N transport and allocation.

Research on organic N uptake and distribution has focused on amino acids (including amides) that represent in most plants the principal transport form for organic N in both phloem and xylem. In addition to amino acids, some plant species use ureides for long distance N transport. It is generally assumed that translocation of small peptides contributes to N allocation in plants, but concentrations of small peptides in the transport path have not been analyzed in detail. One exception is glutathione, a tripeptide that is important for transporting organic sulphur in plants.

This review focuses on membrane proteins that are involved in transport of amino acids, ureides and peptides. Translocation processes of other N-containing metabolites such as hormones, nucleobases, nucelosides or nucleotides, as well as secondary plant metabolites, are not discussed here. Similarly, transport of peptide-conjugates, i.e. glutathione conjugates by members of the ABC transporter family, and urea via DUR3 and aquaporins is described elsewhere [1,2] (Maurel, this issue). Here, the role of amino acid, ureide and peptide transporters is presented in the context of their biochemical properties and *in planta* function.

## 2. Transporters for organic nitrogen compounds in plants

While much of the initial research on molecular aspects of organic N transport was performed using *Arabidopsis thaliana*, some transporter homologs have been identified and analyzed in other model or crop plants, including tomato, potato, broad and castor bean, pea, barley, and rice [3–6]. Many of the Arabidopsis N transporters were isolated using heterologous complementation of yeast (*Saccharomyces cerevisiae*) mutants deficient in the uptake of the respective metabolites. Heterologous expression in *S. cerevisieae* and/or *Xenopus laevis* oocytes allowed determination of substrate selectivity and affinity of the transporters, which most likely reflects their biochemical properties *in planta*. Naming of the first organic N transporter families was based on the substrate selectivity originally determined or on already-characterized homologs in other kingdoms. Thus, names of N transporter families do not always mirror the complete or correct substrate selectivity of a specific transporter. For example, the first analyzed member of the ProT (proline transporter) family, AtProT1, not only

*Corresponding author. Fax: +41 31 631 4942.
*E-mail addresses:* doris.rentsch@ips.unibe.ch (D. Rentsch), susanne.schmidt@uq.edu.au (S. Schmidt), tegeder@wsu.edu (M. Tegeder).

[1]Fax: +61 7 3365 1699.
[2]Fax: +1 509 335 3184.

0014-5793/$32.00 
doi:10.1016/j.febslet.2007.04.013

transports proline, but also other compatible solutes, as demonstrated by recent studies [7]. Nevertheless, the analyses showed that most of the transporters of the same clade (subfamily) share similar substrate selectivity and affinity (see below). The gene families that have been functionally characterized are briefly described here, but for a detailed description of individual gene families and the phylogenetic relationship of the transporters, for analysis of characteristic motifs, as well as other information such as an overview of transporter expression and regulation of expression, we refer to recent reviews [3–5,8–11].

### 2.1. Amino acid transporters

Physiological studies on N transport processes in plants using plasma membrane vesicles indicate that multiple systems exist for uptake and transport of amino acids [12–15]. So far, amino acid transporters have been identified as members of at least five gene families and these transport proteins display different substrate selectivities and affinities as well as distinct subcellular localization (Table 1).

*2.1.1. Amino acid transporters of the ATF family.* The ATF (amino acid transporter family) also referred to as AAAP (amino acid/auxin permease)-family was first described in plants and comprises 46 members in Arabidopsis (http://aramemnon.botanik.uni-koeln.de; [16]). Within the ATF, 6 subfamilies exist: (i) AAPs (amino acid permeases) with eight members; AtAAP1 to AtAAP6 and AtAAP8 have been characterized in more detail using heterologous expression systems, and they preferentially transport neutral amino acids and glutamate [17,18]. While AtAAP6 has a high affinity for these amino acids, the other AAPs recognize their substrates with moderate or low affinity.[3] Recent studies using Arabidopsis *ataap1* mutants demonstrated that the substrate selectivity of AtAAP1 *in planta* is consistent with that resolved by heterologous expression analyses [19]. Kinetics determined in oocytes revealed for AtAAP1 random binding and simultaneous transport of amino acids and protons [20] and a general co-transport of one proton per neutral, basic and uncharged acidic amino acid for all AAPs analyzed [17,20,21]. With respect to protein structure, up to now there is little direct evidence for the structure of organic nitrogen transporters and information on topology is mainly based on predictions. However, using epitope-tagged proteins expressed in animal tissue culture cells, 11 transmembrane spanning domains could be determined for AtAAP1/NAT2 [22]. (ii) LHTs ('lysine/histidine' transporters) were named according to the substrate selectivity originally determined for AtLHT1 [23]. However, more detailed studies on the biochemical properties of AtLHT1 and AtLHT2 in *S. cerevisiae* revealed, that compared to basic amino acids, their affinities for neutral and acidic amino acids are much higher [24,25]. Consequently, the LHTs are now classified as high affinity transporters for these groups of amino acids. (iii) ProTs transport the imino acid proline, glycine betaine and related quaternary ammonium compounds, as well as the amino acid α-aminobutyric acid (GABA) with moderate or low affinity [7]. (iv) GATs (γ-aminobutyric acid transporters) transport GABA and GABA-related compounds. At least AtGAT1 displays a high affinity towards its substrates [26]. (v) ANT1 (aromatic and neutral amino acid transporter 1)-like proteins; so far only AtANT1 has been characterized as a moderate affinity transporter for aromatic and neutral amino acids, whereas the biochemical properties of the remaining 18 members are unknown [27]. Considering the phylogenetic relationship of ANT1-like proteins, this family might be subdivided into groups with different substrate selectivity and sub-cellular localization (see below). (vi) The AUX (auxin-resistant) subgroup. Based on the phenotype of an Arabidopsis *aux1* mutant, a role of AtAUX1 in transport of the phytohormone auxin, which is structurally related to the amino acid tryptophan, has long been postulated, but AtAUX1-mediated indole-3-acetic acid (IAA) transport has only recently been demonstrated [28].

While the members of the different Arabidopsis ATF subfamilies might have similar substrate selectivity and affinity, expression analyses revealed that individual transporters show highly specific expression patterns, indicating that they fulfil specific roles *in planta* [3]. Amino acid transporters isolated from other plant species exhibited biochemical properties comparable to Arabidopsis homologs when analyzed in *S. cerevisiae* or *Xenopus* oocytes. This suggests that the function of amino acid transporters is conserved among higher plants. However, differences in tissue specificity and expression levels of homologous transporters were observed between species [3]. For example, RcAAP3 from castor bean has a substrate selectivity similar to AtAAP3, the most closely related transporter in Arabidopsis, but while *AtAAP3* is primarily expressed in roots, *RcAAP3* is expressed in various source and sink tissues [29].

ATF homologs from other kingdoms show highest similarity to members of the ANT1-subgroup. In animals, these transporters include a vesicular inhibitory amino acid transporter (VIAAT), responsible for GABA uptake into synaptic vesicles, and sodium coupled neutral amino acid (system N/A) transporters, mediating amino acid import (and export), as well as PAT1 and related transporters responsible for import or export of amino acids into the cell and out of the lysosome, respectively [30–32]. Also associated with the ANT1-subgroup are *S. cerevisiae* amino acid transporters for vacuolar import or export [33]. In addition, several amino acid transporters from parasitic protozoa are distantly related to members of the ATF [34].

*2.1.2. Amino acid transporters of the APC family.* The second large gene family of amino acid transporters is the plant APC (amino acid–polyamine–choline) family. Homologs of this family are present in prokaryotes (bacteria, archaea) and eukaryotes (parasitic protozoa, fungi, animals). The plant transporters of the APC transporter family fall into two subgroups. (i) CATs (cationic amino acid transporters) show highest homology to mammalian CATs, and Arabidopsis AtCAT1 and AtCAT5 have been described as high affinity transporters for cationic amino acids [35–37]. Furthermore, AtCAT6 has been recently demonstrated to transport both essential neutral amino acids and the basic amino acid lysine with moderate affinity [38]. This transport is likely to be energized by protons [38]. (ii) LATs (L-type amino acid transporters) were named after a subgroup found in mammalian systems, representing the light chain of the hetero(di)meric amino acid transporters (HATs) (LATs are also called gpaAT for glycoprotein

[3] In this review, high affinity refers to $K_{0.5}$ values for at least one amino acid (or other organic nitrogen compounds) of smaller than 50 μM, while $K_{0.5}$ values for moderate and low affinity transporters are between 50 and 500 μM and higher than 500 μM, respectively (see also Table 1).

Table 1
Gene families of Arabidopsis transporters for major organic nitrogen compounds

| Substrate | Gene family | Subfamily | No. genes | Selectivity[a] | Affinity[a,b] | Subcelluar localization[c] | Reference |
|---|---|---|---|---|---|---|---|
| Amino acids | ATF (or AAAP) *amino acid transporter family* (or *amino acid/auxin permease*) | AAP *amino acid permease* | 8 | neutral amino acids (aa), glutamate | moderate (AtAAPl-5, 8), high (AtAAP6) | PM(AtAAPl,GFP), PM/EM (AtAAP3, GFP, c-Myc) | [17–19,86] |
| | | LHT '*lysine/histidine*' *transporter* | 10 | neutral aa, acidic aa | high (AtLHTl,AtLHT2) | PM (AtLHTl, proteome), PM (AtLHT4, proteome), PM (Atlg48640, proteome) | [24,25,78,95] |
| | | ProT *proline transporter* | 3 | proline, quaternary ammonium compounds | moderate/low (all AtProTs) | PM (AtProTl, 2, 3, GFP) | [7] |
| | | GAT *γ-aminobutyric acid transporter* | 2 | γ-aminobutyric acid, and related compounds | high (AtGATl) | PM (AtGATl, GFP), PM (AtGAT2, proteome, GFP[e]) | [26,78] |
| | | ANT1-like *aromatic and neutral amino acid transporter* | 19 | neutral aa, aromatic aa | moderate (AtANT1) | TP (At3g30390, proteome); Cpl (At5g02180, proteome) | [27,70,75] |
| | | AUX *auxin restistant* | 4 | auxines | high (AtAUXl) | PM (AUX1, HA-tag, proteome), PM (AtLAXl, proteome) | [28,78,95–97] |
| | APC *amino acid–polyamine–choline* | CAT *cationic amino acid transporter* | 9 | neutral aa | high (AtCATl, AtCAT5), moderate (AtCAT6) | PM (AtCAT5, GFP), PM/EM (AtCAT6 and 8, GFP), TP (AtCAT2, GFP), TP (AtCAT4, proteome) | [11,36,38,70] |
| | | LAT *L-type amino acid transporter* | 5 | – | – | – | [11] |
| | MCF *mitochondrial carrier family* | BAC *(mitochondrial) basic amino acid carrier* | 2 | Arg, Lys, Orn, His | moderate (exchange, AtmBACl, AtmBAC2) | Mitochondria | [49–51] |
| | OEP16 or PRAT[d] *plastid outer envelope porin of 16 kDa* (or *preprotein and amino acid transport*) | OEP16 *plastid outer envelope protein of 16 kDa* | 3 | pea OEP16: amino acids and amines | – | Cpl, outer envelope | [44–46] |
| | DASS *divalent anion:$Na^+$ symporter* | DiT2-homologs *dicaboxylate transport* | 2 | DiT2.1: exchange glutamate/malate | – | Cpl, inner envelope, Cpl (DiT2.1, proteome) | [48,75,76] |
| Ureides | DMT *drug/metabolite transporter* | UPS *ureide permease* | 5 | allantoin, xanthine, uracil | high (AtUPS1, 2, 5) | – | [54,55] |
| Peptides | PTR[d] (or POT) *peptide transporter* (or *proton-coupled oligopeptide transporter*) | PTR1-branch[f] *peptide transporter* | 5[f] | di- and tripeptides | high (AtPTRl, AtPTR2) | PM (AtPTRl; GFP, proteome), TP (AtPTR2; GFP[g], proteome) | [58,59,70,72,73,95] |
| | OPT[d] *oligopeptide transporter* | OPT-subfamily *oligopeptide transporter* | 9 | tetra- and pentapeptides, glutathione | high (AtOPT4), moderate (GSH, AtOPT6) | – | [6,8,63] |
| | ABC[d] *ATP binding cassette* | TAP *transport associated with antigen processing* | 3 | larger peptides (no experimental evidence) | – | TP (AtTAP2, proteome) | [9,72] |

[a]Substrate selectivity and affinity were determined in *S. cerevisiae* and/or *Xenopus* oocytes.
[b]High affinity refers to $K_{0.5}$ values for at least one substrate of <50 μM, moderate 50–500 μM and low >500 μM.
[c]PM, plasma membrane; EM, endomembranes (endoplasmic reticulum, Golgi apparatus or small vesicles); TP, tonoplast; Cpl, chloroplast/plastids (predictions for membrane targeting using bioinformatic tools are not included).
[d]These families contain additional subfamilies, which are not listed here (see text).
[e]Meyer and Rentsch, unpublished.
[f]For further putative peptide transporters see Tsay et al. (this issue).
[g]Suter and Rentsch, unpublished.

associated amino acid transporters; [37]). So far, no data are available on the biochemical properties of plant LATs. As animal homologs require interaction with a glycoprotein for proper targeting, a second, interacting protein might be required for function in heterologous expression systems.

*2.1.3. Amino acid transporters in other gene families.* In animals, amino acid transporters have also been found among members of the MFS (major facilitator superfamily). These include, for example, the aromatic amino acid transporter TAT1, and the vesicular glutamate transporter VGLUT1 as well as members of the system-L like amino acid transporter (SLC43) family, which transport neutral amino acids [39–42]. In *S. cerevisiae*, a family of vacuolar transporters for basic amino acids (VBA) has been identified [43]. However, there is currently no experimental evidence that MFS members are involved in translocation of amino acids in plants.

Interestingly, several other gene families contain plant amino acid transporters which are localized at organellar membranes. Transporters have been identified that facilitate amino acid transport from the cytosol across the plastid envelopes. In the outer envelope, OEP16 from pea forms a cation selective high-conductance channel with a strong bias for amino acids and amines [44]. The Arabidopsis genome contains three OEP16 (outer envelope protein of 16 kDa)-genes belonging to the OEP16 (plastid outer envelope porin of 16 kDa)-family [45]. OEP16 proteins show homology to the inner mitochondrial membrane proteins Tim17, Tim22 and Tim23, and to LivH, a component of a prokaryotic amino acid permease. Thus, the OEP16 family was also named PRAT (preprotein and amino acid transport)-family [46]. In addition, although phylogenetically unrelated to OEP16, the OEP24 channel allows passage of amino acids at the outer plastid envelope membrane. However, OEP24 is rather unspecific, as it also permits flux of triosephosphate, dicarboxylic acids, sugars, ATP and inorganic phosphate [47].

So far, the only amino acid transporter identified to operate at the inner plastid envelope is DiT2.1 (dicarboxylate transport), belonging to the DASS (divalent anion:$Na^+$ symporter) family. DiT2.1 functions in glutamate/malate exchange essential in the photorespiratory pathway [48]; transport activity for its closest homolog, DiT2.2, has not been demonstrated yet.

Mitochondrial amino acid transporters have been found in plants that, like the yeast and animal mitochondrial transport systems, are members of the MCF (mitochondrial carrier family) [49,50]. In Arabidopsis, two AtmBACs (mitochondrial basic amino acid carriers) have been functionally characterized, and their substrate selectivities resemble those of the corresponding human proteins. Reconstituted in liposomes, AtmBAC1 and AtmBAC2 transport Arg, Lys, Orn and His by exchange mechanism. AtmBAC2 has a less narrow substrate selectivity than AmBAC1 since, for example, it also transports D-isomers of Arg and Lys [51]. Further, in *S. cerevisiae* and humans, mitochondrial aspartate–glutamate and glutamate transporters of the MCF are known, and homologs might be present among the plant MCF members [52].

### 2.2. Ureide transporters

In legumes of warm climates such as soybean, common bean and cowpea, the ureides allantoin and allantoic acid are the main products of $N_2$ fixation transported from the nodules to the shoot. While plant allantoic acid transporters are unknown, allantoin transporters (ureide permeases; UPS) have been identified as members of the drug/metabolite transport (DMT) family [53–56]. The plant UPS share no significant homology with known allantoin transporters from other organisms such as yeast or bacteria. Interestingly, the first ureide permease (*AtUPS11*) was isolated from Arabidopsis, which does not use ureides as a main form of N for long-distance transport [53]. Heterologous expression of *AtUPS1* in *S. cerevisiae* and *Xenopus* oocytes showed that AtUPS1 mediates transport of allantoin, but other oxo-N-heterocycles like uracil and xanthine are transported with circa 10-fold higher affinity compared to allantoin (Table 1) [53–55]. Further members of the AtUPS family transport the same substrates as AtUPS1 with highest affinity for uracil (AtUPS2) or xanthine (AtUPS5) [54,55]. The only ureide transporter thus far isolated from 'ureide-transporting' plants is *PvUPS1* from common bean [56]. PvUPS1 transports allantoin, but also recognizes uric acid and xanthine. Since in bean plants allantoin levels in the apoplast (e.g. nodule and xylem) and phloem are high in comparison to concentrations of uric acid and xanthine, and since *PvUPS1* is expressed in phloem tissues, allantoin is expected to be the physiological substrate for PvUPS1 [56,57].

### 2.3. Peptide transporters

Plant peptide transporters generally belong to three different gene families, each recognizing peptides of specific length (Table 1). Di- and tripeptides are transported by members of the PTR (peptide transporter)-family which in other kingdoms including prokaryotes, fungi and animals, comprises only few members. In plants, this gene family is much larger and consists of over 50 genes in Arabidopsis. So far, function in di- and tripeptide transport has only been demonstrated for one sub-branch of the PTR family, the AtPTR1-like proteins [58], also referred to as subgroup II [5] and representing a clade of subfamily II (Tsay et al., this issue). These AtPTR1-like proteins include functionally characterized peptide transporters from Arabidopsis (AtPTR1, AtPTR2; [58,59]), faba bean (VfPTR1; [60]) and barley (HvPTR1, [61]) as well as several non-characterized genes from Arabidopsis (three genes) and other plant species. However, recent unpublished data suggest that peptide transporters are also present in other subgroups of the PTR family (Tsay et al., this issue). The Arabidopsis PTR1 and PTR2 have been characterized in more detail showing that they recognize various di- and tripeptides with different affinity [58,59]. Chiang et al. [59] also established that AtPTR2 transports peptides and protons simultaneously by a random binding mechanism. For most plant PTR proteins the substrate selectivity has not been determined yet, but it is evident that some PTR proteins do not transport peptides; for example, Arabidopsis NRT1 mediates transport of nitrate (see Tsay et al., this issue), and AgDCAT1 from alder transports carboxylates with moderate affinity [62].

Transport of larger peptides (4–5 amino acids) is mediated by members of the OPT (oligopepide transporter)-family [8]. This family can be grouped into two subfamilies: (i) the *true* OPTs (also referred to as peptide transporter (PT) clade of OPTs) occur only in plants and fungi. There are nine members in Arabidopsis, for which so far only the uptake of selected tetra- and pentapeptides has been shown [8,63]. In addition, some OPTs from different plant species are able to transport glutathione and glutathione conjugates when expressed in *S. cerevisiae*, and AtOPT3 might transport metals [6,64,65]. (ii) The YSL (yellow stripe like) proteins are more widely distributed

than true OPTs and are found in archae, bacteria, plants and fungi. In the Arabidopsis genome 8 YSL homologs are present that are predicted to transport metal–chelating amino acids. At least for some plant YSL proteins the transport of metal–nicotianamine or metal–phytosiderophore complexes has been shown [6].

In animals, transporters for large peptides (6–59 amino acids) have been identified as members of the ABC (ATP binding cassette) transporter gene family [9]. For example, animal TAPs (transport associated with antigen processings) preferentially mediate transport of peptides of 8–12 amino acids. Hexamers and longer peptides of up to 40 amino acids are also translocated, but with lower efficiency. Further, TAPL (a TAP-like transporter) recognizes 6 to 59-mer peptides with a preference for peptides of ~23 amino acids [66]. Three members of the TAP subfamily have been identified in the Arabidopsis genome (see http://aramemnon.botanik.uni-koeln.de; [16]). Whether, like in animals, these transporters import protein degradation products into the ER lumen, is an interesting hypothesis that remains to be tested.

## 3. Intra- and intercellular transport of organic nitrogen

A large number of metabolites are synthesized in plant cells, and the pathways that the metabolites feed into are complex and often partitioned between cells and organelles. Thus, a variety of organic N transporters (importers and exporters) are expected to be present at the different membranes. The transporters of the ATF, APC, UPS, PTR, and OPT families characterized so far were all described as cellular uptake systems. Whether some of these transporters may additionally facilitate export has generally not been investigated. Up to now, plant export systems for organic N at the plasma membrane have not been identified at the molecular level. Using activation tagged mutants, Pilot et al. [67] described a plant-specific protein with one predicted transmembrane domain that, when overexpressed, led to increased levels of glutamine in the guttation droplets of leaf hydathodes. However, the mechanism by which it contributes to amino acid excretion is unclear. Depending on gradients of amino acids and co-substrates, in animals, cellular export of amino acids is mediated by exchange, facilitated diffusion, co-transport or vesicular transport [31,32,37]. Export of amino acids by vesicular excretion is also used in *S. cerevisiae* [68]. In bacteria, several families of amino acid export systems have been identified (e.g. LysE, RhtB, ThrE and BrnFE) that mediate cellular export of specific amino acids by different mechanisms [69]. Similarly, in plants export might operate by carrier-mediated efflux or by vesicular transport. Whether plant transporters that are localized to small vesicles contribute to vesicular excretion needs to be analyzed.

Based on their transport mechanisms (proton-symport) or simply based on the ability to mediate uptake of organic N when expressed in heterologous expression systems, it has been suggested that most of the transporters are plasma membrane transporters mediating uptake of N from the apoplast. However, studies using fusion proteins such as transporter-GFP or transporter-small epitope (i.e. HA or c-Myc tags) fusions, as well as information gained from proteome analyses, demonstrate localization of some of the organic N transport proteins to membranes other than the plasma membrane (Table 1). While evidence for their physiological function and mechanism of operation is still lacking, transporters have been localized to the tonoplast (AtCAT2, AtCAT4, AtPTR2) or are suggested to be present at the plasma membrane and ER or Golgi, respectively (AtAAP3, AtCAT6 and AtCAT8) (Table 1). Members of the ANT1-subgroup might function at the tonoplast since homologs from *S. cerevisiae* and mammalian systems can mediate amino acid transport across the vacuolar membrane [30,33]. This notion is consistent with proteome analyses, which found the ANT1-like protein At3g30390 and a barley homolog (gi/47497044) at the tonoplast [70,71]. In addition, proteome studies also identified AtPTR2 and a barley homolog (gi/1576661) at the vacuolar membrane [70–73]. Physiological analyses with labeled substrates showed carrier or channel-mediated uptake of amino acids and peptides across the tonoplast modulated by free ATP [74]. As the concentration of amino acids in vacuoles is usually lower than in the cytosol, energy-dependent vacuolar export systems can be postulated. So far export by proton-coupled amino acid symport has only been demonstrated for *Chara* vacuoles [74]. With respect to peptide translocation processes, AtPTR2, a proton-coupled transporter for di- and tripeptides [59], was localized to the tonoplast (Table 1), where it might be responsible for export of protein degradation products from the vacuole.

Plastids are key compartments for amino acid biosynthesis. Some amino acids appear to be synthesized exclusively within plastids (e.g. phenylalanine, tyrosine, tryptophan, lysine) whereas others are produced in the cytosol (e.g. proline and asparagine) or even in multiple compartments (e.g. glutamine, aspartate and serine), suggesting that both import and export of amino acids are important for cellular function. Proteome analyses identified DiT2.1 and the ANT1-like protein At5g02180 as components of the plastid envelope (Table 1) [75,76]. In addition, bioinformatic analyses using predictions for plastid targeting signals (and pI values) found several amino acid transporters (i.e. AtDiT2.1, AtDiT2.2, AtLHT4, AtLHT5, AtCAT6, AtCAT9, AtLAT1 and the AtANT1-like protein At2g40420) as being localized at the inner plastid envelope [76,77]. However, supporting experimental evidence is largely missing or contradicts these analyses. In fact, AtCAT6, a predicted plastid transporter, is localized to the plasma membrane, ER and Golgi [38] and according to proteome data, AtLHT4 is targeted to the plasma membrane [78]. The only organic N transporter characterized at the envelope membrane so far is the glutamate/malate exchanger DiT2.1 [48].

At the mitochondrial membrane, amino acid uptake systems are important for mitochondrial protein synthesis or amino acid degradation (e.g. arginine, proline and GABA), but so far only transporters for basic amino acids have been identified (Table 1) [49,50]. N transport systems are also expected to occur in other organelles, including peroxisomes and endoplasmic reticulum, that are locations for synthesis of a variety of organic N compounds. Proteome analyses, localization approaches and forward genetic screens are certainly excellent tools to identify the transporters for inter- and intracellular organic N transfer.

## 4. Uptake of organic N compounds from the soil

Organic N is present in considerable quantities in soils of natural ecosystems and agricultural systems, but the organic compounds have generally been considered to be a N source for soil microbes rather than plants. There is no evidence that

plants access complex N forms such as protein directly, but it is established that soil microbes including mycorrhizal fungi break down complex organic compounds. Mycorrhizal fungi take up degradation products and deliver low molecular weight N compounds to the plant host. For example, in the ectomycorrhizal fungus *Hebeloma cylindrosporum*, uptake of amino acids into hyphae is mediated by the amino acid permease HcGAP1 [79] and di- and tripeptides by HcPTR2A and B [80]. However, it has long been known that plants can acquire amino acids directly from the soil and may even prefer amino acids over inorganic N forms. It is unclear how well plant roots compete for soil amino acids compared to microbes (see reviews by [81,82]), but the ability to effectively acquire amino acids from the soil appears to characterize plants from cold climate ecosystems such as tundra where mineralization rates are generally low. Measured concentrations of amino acids in soil solution range from 0.01 to 1000 μM, and amino acids can constitute between 1% and 25% of soluble soil N compounds ([81,82] and references cited therein). For uptake of amino acids by the root, high and low affinity transport systems have been predicted. Although several amino acid and peptide transporters are expressed in roots, a direct role in organic N uptake has only been demonstrated for two amino acid transporters, AtAAP1 and AtLHT1. Using T-DNA insertion lines as well as overexpressing lines, Hirner et al. [25] showed that, in Arabidopsis, AtLHT1 is responsible for the uptake of amino acids into roots as well as into mesophyll cells. AtLHT1 was also identified in a forward genetic screen, selecting for mutants resistant to D-alanine [83]. In the *lht1* mutants, acquisition of a variety of amino acids was reduced and changes of the composition of free amino acids in leaves were observed [25,83]. Using a similar approach selecting for mutants resistant to high concentrations of amino acids, AtAAP1 was identified as an essential uptake system for amino acids in roots [19]. Transport studies with labeled N compounds established that *in planta* AtAAP1 mediates uptake of neutral, uncharged amino acids and glutamate, confirming earlier work on substrate selectivity of AtAAP1 using heterologous expression of the transporter in *S. cerevisiae* and *Xenopus* oocytes [17]. Studies with *atcat6* mutants showed failure of seedling growth on medium containing L-glutamine as sole N source. However, *AtCAT6* is not expressed in root cells involved in uptake of nutrients from the soil but in root tip cells. Therefore the observed growth inhibition of *atcat6* mutants is probably due to amino acid partitioning processes within the tip of the root rather than reduced amino acid uptake [38].

In addition to amino acids, soils contain many organic N compounds of higher molecular weight; of these, small peptides are particularly interesting. There is evidence that plants can grow with peptides as the sole N source and that putative peptide transporters are expressed in (cluster) roots of *Hakea* [84], but molecular data are lacking. Whether expression of amino acid and peptide transporters in roots characterizes species from ecosystems with different soil N characteristics is an interesting hypothesis that still awaits experimental support.

## 5. Long distance transport of organic nitrogen

Soil-derived organic N, as well as organic N compounds synthesized in roots or legume nodules, are delivered in the transpiration stream (xylem) to the source leaves, where they are either metabolized, transiently accumulated or immediately transferred to the phloem for long distance transport to sink tissues. Alternatively, the N exported by the leaf is derived from reduction of inorganic N delivered via the xylem or from hydrolysis of leaf protein. Our understanding of the mechanisms operating in mature leaves for long distance transport of organic N to sink organs is incomplete. In general, amino acids, ureides or peptides are loaded into the phloem in order to be exported from the leaf and phloem loading might be by the symplasmic and apoplastic path, respectively. In the symplasmic loading mechanism, movement of assimilates from the mesophyll cells to the sieve element-companion cell complex (SE/CC) of the phloem occurs via plasmodesmata. For apoplastic phloem loading, assimilates are released into the apoplast, followed by uptake into the SE/CCs, which requires plasma membrane-located transport proteins. Using promoter-GUS studies and RNA localization experiments, expression of transporters for amino acids (AtAAP2, AtAAP3 [85,86]), allantoin (PvUPS1 [56,57]) and peptides (AtOPTs, NaNTR1, AtPTR1 [10,58,87]) has been localized to the vasculature or phloem, suggesting a role in apoplastic phloem loading of organic N. In addition, *antisense* repression of the source leaf-specific amino acid transporter *StAAP1* from potato led to a reduction in the free amino acid content in tubers, which indicates function of StAAP1 in uptake of amino acids into the SE/CC complex for long distance transport to sinks [88].

## 6. Translocation of organic N during seed development and germination

During reproductive growth, seeds represent the major sink for organic N. In leaves, newly assimilated N, transiently stored N (e.g. as vegetative storage proteins) or N derived from protein breakdown during senescence is remobilized and loaded into the phloem for transport to the seed sinks. Since the filial parts of the seeds (embryo/cotyledons and endosperm) are largely symplasmically isolated, organic N is exported from the maternal seed coat into the seed apoplast, followed by uptake into filial cells. Imported N compounds might be needed for seed development and, dependent on the plant species, are stored as storage proteins in the endosperm or in the embryo/cotyledons. Expression of several seed amino acid transporters precedes storage protein synthesis. AtAAP1, AtAAP8 and AtCAT6 from Arabidopsis as well as the legume PsAAP1 and VfAAP1 are predicted to play a role in import of amino acids into the seed/cotyledons [18,38,85,89,90] and AtOPT3, AtOPT8, VfPTR1 and AtPTR2 seem to provide the seed with peptides [10,60,91,92].

So far, only a few organic N transporters have been shown to be essential for seed loading or development. Homozygous plants with a T-DNA insertion in *AtOPT3* exhibited arrested embryo growth around the octant stage of embryo development, which suggests a critical role in peptide transport at early developmental stages [92]. In a similar manner, using *antisense* repression Song et al. [91] demonstrated involvement of the di- and tripeptide transporter AtPTR2 in seed growth. *Atptr2 antisense* lines showed a delay in flowering and displayed arrested seed development. However, this effect could not be observed in two independent *atptr2* knock-out mutants (Dietrich and Rentsch, unpublished), indicating that

down-regulation of more than one AtPTR transporter had occurred in the *antisense* lines and was necessary to obtain the growth phenotype observed. None of the amino acid transport mutants analyzed thus far show an obvious phenotype with respect to seed development, indicating that N import deficiency might be compensated by other transporters. However, embryo-specific expression of faba bean *VfAAP1* in *Vicia narbonensis* and pea led to increased seed size and seed protein content, suggesting that *VfAAP1* expression affects sink strength and that N import is limiting for seed protein synthesis [93].

Storage proteins are remobilized during germination to support growth of the seedling. In barley, it could be shown that uptake rates of small peptides at the scutlleum were higher than amino acid transport rates. This indicates that storage protein breakdown products of the endosperm might be transported as amino acids and small peptides. Localization of the HvPTR1 peptide transporter exclusively at the plasma membrane of scutellar epithelial cells of germinating barley grains supports these physiological data and a role of peptide transport during seed germination [61,94]. Miranda et al. [60] suggest that VfPTR1 functions in a similar manner in faba bean. In addition, several Arabidopsis amino acid (AtAAPs, [3]; AtCAT6, [38]) and peptide (AtPTR1, [58]; AtOPTs, [10]) transporters have been proposed to be important for seed germination and establishment of the seedling.

## 7. Conclusions

A large number of (putative) transporters for organic N compounds have been identified in the last 15 years. While individual transporters have been characterized with respect to their expression in plants and substrate selectivity/affinity using heterologous expression systems, for most of the organic N transporters, the biochemical properties and their physiological role *in planta* remain unknown. The diversity of organic N transporters characterized from plants so far reflects their importance for complex regulation of N distribution, compartmentation and storage in support of plant growth and reproduction, as well as in response to changing environmental conditions. Only a detailed and comprehensive analysis of N transport processes using cell-biological techniques combined with molecular, genetic and physiological approaches will help discovering and understanding the integrated role of organic N transporters in overall plant function.

*Acknowledgements:* We are grateful to the Swiss National Science Foundation (SNF 3100A0-107507; NCCR Plant Survival; D.R.), the Australian Research Council (DP0557010; ARC Network for Vegetation Futures; S.S.) and the US National Science Foundation (IOB 0135344, IOB 0448506; M.T.) for financial support.

## References

[1] Liu, L.H., Ludewig, U., Frommer, W.B. and von Wiren, N. (2003) AtDUR3 encodes a new type of high-affinity urea/$H^+$ symporter in Arabidopsis. Plant Cell 15, 790–800.
[2] Klein, M., Burla, B. and Martinoia, E. (2006) The multidrug resistance-associated protein (MRP/ABCC) subfamily of ATP-binding cassette transporters in plants. FEBS Lett. 580, 1112–1122.
[3] Liu, X. and Bush, D.R. (2006) Expression and transcriptional regulation of amino acid transporters in plants. Amino Acids 30, 113–120.
[4] Tegeder, M. and Weber, A.P.M. (2006) Metabolite transporters in the control of plant primary metabolism (Plaxton, W. and McManus, M., Eds.), Annual Plant Reviews: Control of primary metabolism in plants, vol. 22, pp. 85–120, Blackwell Publishing, Oxford.
[5] Waterworth, W.M. and Bray, C.M. (2006) Enigma variations for peptides and their transporters in higher plants. Ann. Bot. 98, 1–8.
[6] Lubkowitz, M. (2006) The OPT family functions in long-distance peptide and metal transport in plants. Genet. Eng. (NY) 27, 35–55.
[7] Grallath, S., Weimar, T., Meyer, A., Gumy, C., Suter-Grotemeyer, M., Neuhaus, J.M. and Rentsch, D. (2005) The AtProT family. Compatible solute transporters with similar substrate specificity but differential expression patterns. Plant Physiol. 137, 117–126.
[8] 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.
[9] Stacey, G., Koh, S., Granger, C. and Becker, J.M. (2002) Peptide transport in plants. Trends Plant Sci. 7, 257–263.
[10] 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.
[11] Wipf, D., Ludewig, U., Tegeder, M., Rentsch, D., Koch, W. and Frommer, W.B. (2002) Conservation of amino acid transporters in fungi, plants and animals. Trends Biochem. Sci. 27, 139–147.
[12] Li, Z.C. and Bush, D.R. (1990) pH-Dependent amino acid transport into plasma membrane vesicles isolated from sugar beet (*Beta vulgaris* L.) leaves. I. Evidence for carrier-mediated, electrogenic flux through multiple transport systems. Plant Physiol. 94, 268–277.
[13] Li, Z.C. and Bush, D.R. (1991) pH-Dependent amino acid transport into plasma membrane vesicles isolated from sugar beet (*Beta vulgaris* L.) leaves. II. Evidence for multiple aliphatic, neutral amino acid symports. Plant Physiol. 96, 1338–1344.
[14] Li, Z.C. and Bush, D.R. (1992) Structural determinants in substrate recognition by proton–amino acid symports in plasma membrane vesicles isolated from sugar beet leaves. Arch. Biochem. Biophys. 294, 519–526.
[15] Weston, K., Hall, J.L. and Williams, L.E. (1995) Characterization of amino acid transport in *Ricinus communis* roots using isolated membrane vesicles. Planta 196, 166–173.
[16] Schwacke, R., Schneider, A., van der Graaff, E., Fischer, K., Catoni, E., Desimone, M., Frommer, W.B., Flügge, U.I. and Kunze, R. (2003) ARAMEMNON, a novel database for Arabidopsis integral membrane proteins. Plant Physiol. 131, 16–26.
[17] Fischer, W.N., Loo, D.D.F., Koch, W., Ludewig, U., Boorer, K.J., Tegeder, M., Rentsch, D., Wright, E.M. and Frommer, W.B. (2002) Low and high affinity amino acid $H^+$-cotransporters for cellular import of neutral and charged amino acids. Plant J. 29, 717–731.
[18] Okumoto, S., Schmidt, R., Tegeder, M., Fischer, W.N., Rentsch, D., Frommer, W.B. and Koch, W. (2002) High affinity amino acid transporters specifically expressed in xylem parenchyma and developing seeds of Arabidopsis. J. Biol. Chem. 277, 45338–45346.
[19] Lee, Y.-H., Foster, J., Chen, J., Voll, L.M., Weber, A.P.M. and Tegeder, M. (2007) AAP1 transports uncharged amino acids into roots of Arabidopsis. Plant J. 50, 305–319.
[20] Boorer, K.J., Frommer, W.B., Bush, D.R., Kreman, M., Loo, D.D.F. and Wright, E.M. (1996) Kinetics and specificity of a $H^+$ amino acid transporter from *Arabidopsis thaliana*. J. Biol. Chem. 271, 2213–2220.
[21] Boorer, K.J. and Fischer, W.N. (1997) Specificity and stoichiometry of the Arabidopsis $H^+$ amino acid transporter AAP5. J. Biol. Chem. 272, 13040–13046.
[22] Chang, H.C. and Bush, D.R. (1997) Topology of NAT2, a prototypical example of a new family of amino acid transporters. J. Biol. Chem. 272, 30552–30557.
[23] Chen, L. and Bush, D.R. (1997) LHT1, a lysine- and histidine-specific amino acid transporter in Arabidopsis. Plant Physiol. 115, 1127–1134.

[24] Lee, Y.H. and 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.
[25] Hirner, A., Ladwig, F., Stransky, H., Okumoto, S., Keinath, M., Harms, A., Frommer, W.B. and Koch, W. (2006) Arabidopsis LHT1 is a high-affinity transporter for cellular amino acid uptake in both root epidermis and leaf mesophyll. Plant Cell 18, 1931–1946.
[26] Meyer, A., Eskandari, S., Grallath, S. and Rentsch, D. (2006) AtGAT1, a high affinity transporter for gamma-aminobutyric acid in *Arabidopsis thaliana*. J. Biol. Chem. 281, 7197–7204.
[27] Chen, L.S., Ortiz-Lopez, A., Jung, A. and Bush, D.R. (2001) ANT1, an aromatic and neutral amino acid transporter in Arabidopsis. Plant Physiol. 125, 1813–1820.
[28] Yang, Y.D., Hammes, U.Z., Taylor, C.G., Schachtman, D.P. and Nielsen, E. (2006) High-affinity auxin transport by the AUX1 influx carrier protein. Curr. Biol. 16, 1123–1127.
[29] Neelam, A., Marvier, A.C., Hall, J.L. and Williams, L.E. (1999) Functional characterization and expression analysis of the amino acid permease RcAAP3 from castor bean. Plant Physiol. 120, 1049–1056.
[30] Boll, M., Daniel, H. and Gasnier, B. (2004) The SLC36 family: proton-coupled transporters for the absorption of selected amino acids from extracellular and intracellular proteolysis. Pflugers Arch. Eur. J. Physiol. 447, 776–779.
[31] Gasnier, B. (2004) The SLC32 transporter, a key protein for the synaptic release of inhibitory amino acids. Pflugers Arch. Eur. J. Physiol. 447, 756–759.
[32] Mackenzie, B. and Erickson, J.D. (2004) Sodium-coupled neutral amino acid (System N/A) transporters of the SLC38 gene family. Pflugers Arch. Eur. J. Physiol. 447, 784–795.
[33] Russnak, R., Konczal, D. and McIntire, S.L. (2001) A family of yeast proteins mediating bidirectional vacuolar amino acid transport. J. Biol. Chem. 276, 23849–23857.
[34] Akerman, M., Shaked-Mishan, P., Mazareb, S., Volpin, H. and Zilberstein, D. (2004) Novel motifs in amino acid permease genes from *Leishmania*. Biochem. Biophys. Res. Commun. 325, 353–366.
[35] Frommer, W.B., Hummel, S., Unseld, M. and 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.
[36] Su, Y.H., Frommer, W.B. and Ludewig, U. (2004) Molecular and functional characterization of a family of amino acid transporters from Arabidopsis. Plant Physiol. 136, 3104–3113.
[37] Verrey, F., Closs, E.I., Wagner, C.A., Palacin, M., Endou, H. and Kanai, Y. (2004) CATs and HATs: the SLC7 family of amino acid transporters. Pflugers Arch. Eur. J. Physiol. 447, 532–542.
[38] Hammes, U.Z., Nielsen, E., Honaas, L.A., Taylor, C.G. and Schachtman, D.P. (2006) AtCAT6, a sink-tissue-localized transporter for essential amino acids in Arabidopsis. Plant J. 48, 414–426.
[39] Babu, E., Kanai, Y., Chairoungdua, A., Kim, D.K., Iribe, Y., Tangtrongsup, S., Jutabha, P., Li, Y.W., Ahmed, N., Sakamoto, S., Anzai, N., Nagamori, S. and Endou, H. (2003) Identification of a novel system L amino acid transporter structurally distinct from heterodimeric amino acid transporters. J. Biol. Chem. 278, 43838–43845.
[40] Reimer, R.J. and Edwards, R.H. (2004) Organic anion transport is the primary function of the SLC17/type I phosphate transporter family. Pflugers Arch. Eur. J. Physiol. 447, 629–635.
[41] Halestrap, A.P. and Meredith, D. (2004) The SLC16 gene family – from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch. Eur. J. Physiol. 447, 619–628.
[42] Bodoy, S., Martin, L., Zorzano, A., Palacin, M. and Estevez, R. (2005) Identification of LAT4, a novel amino acid transporter with system L activity. J. Biol. Chem. 280, 12002–12011.
[43] Shimazu, M., Sekito, T., Akiyama, K., Ohsumi, Y. and Kakinuma, Y. (2005) A family of basic amino acid transporters of the vacuolar membrane from *Saccharomyces cerevisiae*. J. Biol. Chem. 280, 4851–4857.
[44] Pohlmeyer, K., Soll, J., Steinkamp, T., Hinnah, S. and Wagner, R. (1997) Isolation and characterization of an amino acid-selective channel protein present in the chloroplastic outer envelope membrane. Proc. Natl. Acad. Sci. USA 94, 9504–9509.
[45] Murcha, M.W., Elhafez, D., Lister, R., Tonti-Filippini, J., Baumgartner, M., Philippar, K., Carrie, C., Mokranjac, D., Soll, J. and Whelan, J. (2007) Characterization of the preprotein and amino acid transporter gene family in Arabidopsis. Plant Physiol. 143, 199–212.
[46] Rassow, J., Dekker, P.J.T., van Wilpe, S., Meijer, M. and Soll, J. (1999) The preprotein translocase of the mitochondrial inner membrane: function and evolution. J. Mol. Biol. 286, 105–120.
[47] Pohlmeyer, K., Soll, J., Grimm, R., Hill, K. and Wagner, R. (1998) A high-conductance solute channel in the chloroplastic outer envelope from pea. Plant Cell 10, 1207–1216.
[48] Renné, P., Dressen, U., Hebbeker, U., Hille, D., Flügge, U.I., Westhoff, P. and Weber, A.P.M. (2003) The Arabidopsis mutant dct is deficient in the plastidic glutamate/malate translocator DiT2. Plant J. 35, 316–331.
[49] Catoni, E., Desimone, M., Hilpert, M., Wipf, D., Kunze, R., Schneider, A., Flügge, U.I., Schumacher, K. and Frommer, W.B. (2003) Expression pattern of a nuclear encoded mitochondrial arginine–ornithine translocator gene from Arabidopsis. BMC Plant Biol. 3, 1.
[50] Hoyos, M.E., Palmieri, L., Wertin, T., Arrigoni, R., Polacco, J.C. and Palmieri, F. (2003) Identification of a mitochondrial transporter for basic amino acids in *Arabidopsis thaliana* by functional reconstitution into liposomes and complementation in yeast. Plant J. 33, 1027–1035.
[51] Palmieri, L., Todd, C.D., Arrigoni, R., Hoyos, M.E., Santoro, A., Polacco, J.C. and Palmieri, F. (2006) Arabidopsis mitochondria have two basic amino acid transporters with partially overlapping specificities and differential expression in seedling development. Biochim. Biophys. Acta 1757, 1277–1283.
[52] Picault, N., Hodges, M., Paimieri, L. and Palmieri, F. (2004) The growing family of mitochondrial carriers in Arabidopsis. Trends Plant Sci. 9, 138–146.
[53] Desimone, M., Catoni, E., Ludewig, U., Hilpert, M., Schneider, A., Kunze, R., Tegeder, M., Frommer, W.B. and Schumacher, K. (2002) A novel superfamily of transporters for allantoin and other oxo derivatives of nitrogen heterocyclic compounds in Arabidopsis. Plant Cell 14, 847–856.
[54] Schmidt, A., Su, Y.H., Kunze, R., Warner, S., Hewitt, M., Slocum, R.D., Ludewig, U., Frommer, W.B. and Desimone, M. (2004) UPS1 and UPS2 from Arabidopsis mediate high affinity transport of uracil and 5-fluorouracil. J. Biol. Chem. 279, 44817–44824.
[55] Schmidt, A., Baumann, N., Schwarzkopf, A., Frommer, W.B. and Desimone, M. (2006) Comparative studies on ureide permeases in *Arabidopsis thaliana* and analysis of two alternative splice variants of AtUPS5. Planta 224, 1329–1340.
[56] Pélissier, H.C., Frerich, A., Desimone, M., Schumacher, K. and Tegeder, M. (2004) PvUPS1, an allantoin transporter in nodulated roots of French bean. Plant Physiol. 134, 664–675.
[57] Pélissier, H.C. and Tegeder, M. (2007) PvUPS1 plays a role in source-sink transport of allantoin in French bean (*Phaseolus vulgaris* L.). Funct. Plant Biol. 34, 282–291.
[58] Dietrich, D., Hammes, U., Thor, K., Suter-Grotemeyer, M., Flückiger, 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.
[59] 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.
[60] 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.
[61] West, C.E., Waterworth, W.M., Stephens, S.M., Smith, C.P. and Bray, C.M. (1998) Cloning and functional characterisation of a peptide transporter expressed in the scutellum of barley grain during the early stages of germination. Plant J. 15, 221–229.
[62] Jeong, J.Y., Suh, S., Guan, C.H., Tsay, Y.F., Moran, N., Oh, C.J., An, C.S., Demchenko, K.N., Pawlowski, K. and Lee, Y. (2004) A nodule-specific dicarboxylate transporter from alder is a

member of the peptide transporter family. Plant Physiol. 134, 969–978.
[63] 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.
[64] Wintz, H., Fox, T., Wu, Y.Y., Feng, V., Chen, W.Q., 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.
[65] 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.
[66] Wolters, J.C., Abele, R. and Tampe, R. (2005) Selective and ATP-dependent translocation of peptides by the homodimeric ATP binding cassette transporter TAP-like (ABCB9). J. Biol. Chem. 280, 23631–23636.
[67] Pilot, G., Stransky, H., Bushey, D.F., Pratelli, R., Ludewig, U., Wingate, V.P.M. and Frommer, W.B. (2004) Overexpression of glutamine dumper1 leads to hypersecretion of glutamine from hydathodes of Arabidopsis leaves. Plant Cell 16, 1827–1840.
[68] Velasco, I., Tenreiro, S., Calderon, I.L. and Andre, B. (2004) *Saccharomyces cerevisiae* Aqr1 is an internal membrane transporter involved in excretion of amino acids. Eukar. Cell 3, 1492–1503.
[69] Eggeling, L. and Sahm, H. (2003) New ubiquitous translocators: amino acid export by Corynebacterium glutamicum and *Escherichia coli*. Arch. Microbiol. 180, 155–160.
[70] Carter, C., Pan, S.Q., Jan, Z.H., Avila, E.L., Girke, T. and Raikhel, N.V. (2004) The vegetative vacuole proteorne of *Arabidopsis thaliana* reveals predicted and unexpected proteins. Plant Cell 16, 3285–3303.
[71] Endler, A., Meyer, S., Schelbert, S., Schneider, T., Weschke, W., Peters, S.W., Keller, F., Baginsky, S., Martinoia, E. and Schmidt, U.G. (2006) Identification of a vacuolar sucrose transporter in barley and arabidopsis mesophyll cells by a tonoplast proteomic approach. Plant Physiol. 141, 196–207.
[72] Dunkley, T.P.J., Hester, S., Shadforth, I.P., Runions, J., Weimar, T., Hanton, S.L., Griffin, J.L., Bessant, C., Brandizzi, F., Hawes, C., Watson, R.B., Dupree, P. and Lilley, K.S. (2006) Mapping the Arabidopsis organelle proteome. Proc. Natl. Acad. Sci. USA 103, 6518–6523.
[73] Shimaoka, T., Ohnishi, M., Sazuka, T., Mitsuhashi, N., Hara-Nishimura, I., Shimazaki, K.I., Maeshima, M., Yokota, A., Tomizawa, K.I. and Mimura, T. (2004) Isolation of intact vacuoles and proteomic analysis of tonoplast from suspension-cultured cells of *Arabidopsis thaliana*. Plant Cell Physiol. 45, 672–683.
[74] Martinoia, E., Massonneau, A. and Frangne, N. (2000) Transport processes of solutes across the vacuolar membrane of higher plants. Plant Cell Physiol. 41, 1175–1186.
[75] Kleffmann, T., Russenberger, D., von Zychlinski, A., Christopher, W., Sjolander, K., Gruissem, W. and Baginsky, S. (2004) The *Arabidopsis thaliana* chloroplast proteome reveals pathway abundance and novel protein functions. Curr. Biol. 14, 354–362.
[76] Ferro, M., Salvi, D., Riviere-Rolland, H., Vermat, T., Seigneurin-Berny, D., Grunwald, D., Garin, J., Joyard, J. and Rolland, N. (2002) Integral membrane proteins of the chloroplast envelope: identification and subcellular localization of new transporters. Proc. Natl. Acad. Sci. USA 99, 11487–11492.
[77] Weber, A.P.M., Schwacke, R. and Flügge, U.I. (2005) Solute transporters of the plastid envelope membrane. Annu. Rev. Plant Biol. 56, 133–164.
[78] Nühse, T.S., Stensballe, A., Jensen, O.N. and Peck, S.C. (2004) Phosphoproteomics of the Arabidopsis plasma membrane and a new phosphorylation site database. Plant Cell 16, 2394–2405.
[79] Wipf, D., Benjdia, M., Tegeder, M. and Frommer, W.B. (2002) Characterization of a general amino acid permease from *Hebeloma cylindrosporum*. FEBS Lett. 528, 119–124.
[80] Benjdia, M., Rikirsch, E., Müller, T., Morel, M., Corratge, C., Zimmermann, S., Chalot, M., Frommer, W.B. and Wipf, D. (2006) Peptide uptake in the ectomycorrhizal fungus *Hebeloma cylindrosporum*: characterization of two di- and tripeptide transporters (HcPTR2A and B). New Phytol. 170, 401–410.
[81] Lipson, D. and Näsholm, T. (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologia 128, 305–316.
[82] Jones, D.L., Healey, J.R., Willett, V.B., Farrar, J.F. and Hodge, A. (2005) Dissolved organic nitrogen uptake by plants – an important N uptake pathway? Soil Biol. Biochem. 37, 413–423.
[83] Svennerstam, H., Ganeteg, U., Bellini, C. and Näsholm, T. (2007) Comprehensive screening of Arabidopsis mutants suggest the lysine histidine transporter I to be involved in plant uptake of amino acids. Plant Physiol. (in press).
[84] Schmidt, S., Mason, M.G., Sangtiean, T. and Stewart, G.R. (2003) Do cluster roots of *Hakea actities* (Proteaceae) acquire complex organic nitrogen? Plant Soil 248, 157–165.
[85] Hirner, B., Fischer, W.N., Rentsch, D., Kwart, M. and Frommer, W.B. (1998) Developmental control of $H^+$/amino acid permease gene expression during seed development of *Arabidopsis*. Plant J. 14, 535–544.
[86] Okumoto, S., Koch, W., Tegeder, M., Fischer, W.N., Biehl, A., Leister, D., Stierhof, Y.D. and Frommer, W.B. (2004) Root phloem-specific expression of the plasma membrane amino acid proton co-transporter AAP3. J. Exp. Bot. 55, 2155–2168.
[87] 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.
[88] Koch, W., Kwart, M., Laubner, M., Heineke, D., Stransky, H., Frommer, W.B. and 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.
[89] Tegeder, M., Offler, C.E., Frommer, W.B. and Patrick, J.W. (2000) Amino acid transporters are localized to transfer cells of developing pea seeds. Plant Physiol. 122, 319–326.
[90] Miranda, M., Borisjuk, L., Tewes, A., Heim, U., Sauer, N., Wobus, U. and 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.
[91] Song, W., Koh, S., Czako, M., Marton, L., Drenkard, E., Becker, J.M. and Stacey, G. (1997) Antisense expression of the peptide transport *gene AtPTR2-B* delays flowering and arrests seed development in transgenic *Arabidopsis* plants. Plant Physiol. 114, 927–935.
[92] 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.
[93] Rolletschek, H., Hosein, F., Miranda, M., Heim, U., Gotz, K.P., Schlereth, A., Borisjuk, L., Saalbach, I., Wobus, U. and 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.
[94] 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.
[95] Alexandersson, E., Saalbach, G., Larsson, C. and Kjellbom, P. (2004) Arabidopsis plasma membrane proteomics identifies components of transport, signal transduction and membrane trafficking. Plant Cell Physiol. 45, 1543–1556.
[96] Swarup, R., Friml, J., Marchant, A., Ljung, K., Sandberg, G., Palme, K. and Bennett, M. (2001) Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. Genes Dev. 15, 2648–2653.
[97] Nelson, C.J., Hegeman, A.D., Harms, A.C. and Sussman, M.R. (2006) A quantitative analysis of Arabidopsis plasma membrane using trypsin-catalyzed O-18 labeling. Mol. Cell. Proteomics 5, 1382–1395.