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# LHT1, A Lysine- and Histidine-Specific Amino Acid Transporter in Arabidopsis

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**We have identified a new amino acid transporter from the *Arabidopsis thaliana* expressed sequence tag cDNA collection by functional complementation of a yeast amino acid transport mutant. Transport analysis of the expressed protein in yeast shows that it is a high-affinity transporter for both lysine (Lys) and histidine with Michaelis constant values of 175 and 400 $\mu$M, respectively. This transporter (LHT1, *l*ysine *h*istidine *t*ransporter) has little affinity for arginine when measured directly in uptake experiments or indirectly with substrate competition. The cDNA is 1.7 kb with an open reading frame that codes for a protein with 446 amino acids and a calculated molecular mass of 50.5 kD. Hydropathy analysis shows that LHT1 is an integral membrane protein with 9 to 10 putative membrane-spanning domains. Southern-blot analysis suggests that *LHT1* is a single-copy gene in the Arabidopsis genome. RNA gel-blot analysis shows that this transporter is present in all tissues, with the strongest expression in young leaves, flowers, and siliques. Whole-mount, in situ hybridization revealed that expression is further localized on the surface of roots in young seedlings and in pollen. Overall, LHT1 belongs to a new class of amino acid transporter that is specific for Lys and histidine, and, given its substrate specificity, it has significant promise as a tool for improving the Lys content of Lys-deficient grains.**

Long-distance transport of resources and information is a fundamental process in all multicellular organisms. In higher plants sugars and amino acids are transported from the sites of primary assimilation to heterotrophic tissues via mass flow in the phloem. The import-dependent tissue systems, which include roots, young leaves, developing fruits, and specialized storage organs, carry out many essential processes required for plant growth (Pate, 1980; Gifford et al., 1984; Thorne, 1985; Schubert, 1986). Long-distance transport of organic nutrients is also an essential activity during seed germination and grain filling (Thorne, 1985; Fisher and Macnicol, 1986), in symbiotic $N_2$ assimilation (Schubert, 1986; Mylona et al., 1995), and in nutrient recycling during senescence (Thomas and Stoddart, 1980; Feller and Fischer, 1994). In all cases integral membrane proteins that mediate sugar and amino acid transport across the plasma membrane are essential components of the resource-allocation system in higher plants.

Amino acids are the predominant form of nitrogen available to the heterotrophic tissues of the plant. As such, they are the precursors of many essential molecules, including proteins, nucleic acids, chlorophyll, phytohormones, phytoalexins, phenylpropanoids, accessory pigments, and lignin. Amino acids are actively transported into plant cells by proton-coupled symporters (Bush, 1993). Bush and Langston-Unkefer (1988) used isolated membrane vesicles and imposed proton electrochemical potentials to provide the first biochemical description of proton-coupled amino acid transport in plants. Subsequently, Li and Bush (1990, 1991) provided a detailed analysis of the transport properties and bioenergetics of several amino acid symporters using highly purified plasma membrane vesicles isolated from sugar beet leaves. They showed that these transporters are electrogenic, that they are inhibited by DEPC, and that there are at least four classes of symporters based on their preferences for groups of amino acids that share charge or structural similarities. Williams et al. (1992, 1996) and Weston et al. (1995) found similar properties of transporter activity in membrane vesicles isolated from castor bean cotyledons and roots.

Frommer et al. (1993) and Hsu et al. (1993) cloned the first plant amino acid transporter cDNA (*NAT2/AAP1*) by functional complementation of yeast amino acid transport mutants. Functional complementation in yeast has become a powerful tool for plant transport biologists because it circumvents the daunting task of purifying these extremely low-abundance membrane proteins using traditional biochemical strategies (Bush, 1993; Frommer et al., 1994; Frommer and Ninneman, 1995; Tanner and Caspari, 1996). Frommer's group went on to identify five additional transporter cDNAs that are closely related to *NAT2/AAP1*, providing good evidence that they represent a family of translocator genes (Kwart et al., 1993; Fischer et al., 1995; Rentsch et al., 1996). In addition to the *NAT2/AAP1* gene family, a cationic amino acid transporter cDNA, AAT1, that is related to an animal amino acid transporter has been described (Frommer et al., 1995), and an aromatic amino

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Abbreviations: DEPC; diethyl pyrocarbonate; EST, expressed sequence tag; Ura, uracil.

acid transporter (Chen, 1997) and two Pro transporters have also been cloned (Rentsch et al., 1996).

Given the complexity of amino acid transport activities associated with resource allocation during all phases of plant growth, we have continued to look for new amino acid transporter genes that encode additional participants of this fundamental physiological process. One approach we have used to achieve this goal has been to survey the *Arabidopsis thaliana* EST cDNA database (Newman et al., 1994) for sequences that exhibit some similarity to NAT2/AAP1 using a BLAST search protocol (Altschul et al., 1990). Although several of the ESTs identified using this strategy included previously described clones, others represented novel genes that have only small regions of sequence similarity (Chen, 1997). We requested the novel EST cDNAs from the stock center, ligated them into yeast expression vectors, and scored them for amino acid transport activity by testing their ability to complement yeast amino acid transport mutants. In the results reported here we describe a cDNA initially identified in our EST survey that encodes a new class of amino acid transporter found in Arabidopsis.

## MATERIALS AND METHODS

### Molecular Cloning

EST cDNAs were obtained from the *Arabidopsis thaliana* EST cDNA library (Ohio State University Arabidopsis Stock Center, Columbus). Inserts were analyzed by restriction digestion and subcloned into the expression vector λYES (Elledge et al., 1991) for complementation of a yeast amino acid transport mutant. λYES contains the *URA3* gene as a selectable marker for positive transformants. The EST cDNAs were double-digested with *Xba*I and *Eco*RI and then they were directionally cloned into λYES. Standard procedures for ligation and transformation into *Escherichia coli* followed the methods of Sambrook et al. (1989) or Ausubel et al. (1994).

### Yeast Transformation and Complementation

The His auxotroph and transport-deficient mutant of *Saccharomyces cerevisiae* used in this study (JT16; *MAT***a** *hip 1–614 his4–401 ura3–52 ino1 can1*) (Tanaka and Fink, 1985) was a gift from G. Fink (Whitehead Institute, Massachusetts Institute of Technology, Cambridge). JT16 was maintained on S1 medium supplemented with 0.2% Ura and without sorbitol (Hsu et al., 1993). Yeast were transformed by electroporation and/or lithium acetate as described by Becker and Guarente (1991) or Gietz et al. (1992), respectively. Complementation of His transport was scored as previously described (Hsu et al., 1993). $Ura^+$ transformants were selected with S1 medium containing 1 M sorbitol (Hsu et al., 1993). For transport complementation, positive transformants were then plated on a His-limiting medium containing 4% Gal, 0.17% yeast-nitrogen base without amino acids and ammonium salts, 0.5% ammonium sulfate, 0.002% inosine, 0.1% Arg, and 130 μM His. In all cases, insert-free λYES and NAT2/λYES were run in parallel as negative and positive controls, respectively.

### Transport Measurements

Yeast transport activity was measured as described by Hsu et al. (1993). Cells were grown to the midlogarithmic phase and then collected by centrifugation. They were resuspended at 200 to 300 mg cells $mL^{-1}$ in transport buffer that contained His-limiting medium without His and Arg (the pH was adjusted to 5.0 with KOH). Ten microliters of cells was used in a 500-μL transport reaction that contained 0.2 to 1.0 μCi of $^{14}C$-labeled and unlabeled amino acid to the desired final concentration. At predetermined times, 200 μL was removed from the reaction buffer and the cells were collected and washed on a micropore filter. JT16 cells transformed with insert-free λYES were used to measure background transport activity in all transport experiments, and results are reported as net transport (i.e. LHT1-expressing cells accumulation minus accumulation by insert-free vector controls).

### Molecular Techniques

Yeast colony PCR was performed according to the method of Schneider et al. (1995), and plasmid DNA extraction from yeast was adapted from the method of Ward (1990). Two milliliters of saturated yeast culture was centrifuged and cells were resuspended in 200 μL of 2.5 M LiCl, 50 mM Tris HCl, pH 8.0, 4% Triton X-100, and 62.5 mM EDTA, and then 200 μL of phenol-chloroform (1:1, w/v) and 2.0 g of acid-washed glass beads (0.5 mm) were added. This mixture was immediately vortexed in a minibeadbeater (Biospec, Bartlesville, OK) for 2 min and then centrifuged at 14,000 rpm for 5 min. The upper layer was collected and 600 μL of ethanol was added to precipitate the DNA. Yeast RNA was isolated using a kit according to the manufacturer's instructions (Qiagen, Chatsworth, CA). Dideoxynucleotide sequencing (Sanger et al., 1977) was performed on double-strand DNA templates using Sequenase version 2.0 according to the manufacturer's instructions (Amersham). Plant DNA was isolated according to the method of Dellaporta et al. (1983). Plant RNA was isolated using guanidinium as described by Kiedrowski et al. (1992). Whole-mount, in situ hybridization of Arabidopsis seedlings and flowers was as described by Bennett (1994).

For Southern-blot hybridizations plant genomic DNA was digested with different restriction enzymes, separated on a 0.8% agarose gel in TAE buffer (40 mM Tris-acetate and 1 mM EDTA), and depurinated with a 10-min treatment in 0.25 M HCl. DNA was transferred onto Hybond $N^+$ membranes (Amersham) in 0.4 M NaOH, and then cross-linked to the membrane using a UV cross-linker (Stratagene). $\alpha$-$^{32}P$-Labeled probes were synthesized using random hexamers (Boehringer Mannheim) or the Megaprimed system (Amersham). Probes were purified with either a Bio-Spin column (Bio-Rad) or a nucleotide-removal kit (Qiaquick, Qiagen). [$\alpha$-$^{32}P$]Cytosine was purchased from Amersham. The hybridization and wash conditions followed the suggestions of the membrane manufacturer (Amersham). Low-stringency washes consisted of two 10-min washes with 2× SSPE (Sambrook et al., 1989) and 0.1%

SDS at room temperature. The high-stringency wash included an additional wash with 1× SSPE and 0.1% SDS at 65°C for 15 min, and two washes of 0.1× SSPE and 0.1% SDS at 65°C for 30 min.

Twenty micrograms of total RNA was separated on a formaldehyde gel (Zielinski, 1987) and transferred onto nylon membranes (Nytran, Schleicher & Schuell) for RNA gel-blot analysis. $\alpha$-$^{32}$P-Labeled probe was prepared as for the Southern-blot hybridization method. The hybridization and wash conditions followed the membrane manufacturer's instruction (Schleicher & Schuell).

## RESULTS

### An EST cDNA Complements a Yeast Amino Acid Transport Mutant

One approach we have taken to identify additional amino acid transporters has been to survey the EST cDNA database for clones that exhibit low levels of deduced amino acid sequence similarity to NAT2. Several EST cDNAs were obtained from the collection at the Ohio State University Arabidopsis Stock Center because each possessed modest levels of similarity with the amino terminus of NAT2. Each EST cDNA that was sufficiently long to potentially contain a complete open reading frame was subcloned into a yeast expression vector (Elledge et al., 1991) and then transformed into a yeast strain auxotrophic for His and in which the high-affinity His transporter was deleted. Transformants that acquired the expression vector were selected on a high-His medium without Ura (Fig. 1A). The vector allows for growth in the absence of Ura because it contains the *URA3* gene as a selectable marker.

Ura$^+$ transformants were subsequently scored for their ability to complement the His-transport deficiency of the yeast mutant by growing them on a low-His medium that does not support growth of the deletion mutant (Hsu et al., 1993). Of several EST cDNAs tested, only *LHT1* allowed for growth under His-limiting conditions (Fig. 1B). Although this result suggests that *LHT1* encodes a protein with His-transport activity, there are alternative explanations for this observation. For example, a suppressor mutation in the yeast genome could allow for His uptake or the EST cDNA could encode a homolog to the mutated His biosynthesis gene (*HIS4*). We tested for suppressor mutants by substituting Glc for Gal in the selection medium. Because Glc represses the *GAL1* promotor engineered into the expression vector, shifting the C source to Glc eliminated expression of the plant protein. *LHT1*-containing transformants transferred to the Glc-based medium lost their ability to complement His-limited growth, suggesting that complementation is attributable to the encoded plant protein rather than to a suppressor mutation (Fig. 1C). The *LHT1* transformants also failed to grow on a His-free Gal medium, suggesting that the expressed plant protein is not a *HIS4* homolog in the biosynthetic pathway (Fig. 1D). These data suggest that *LHT1* is a plant amino acid transporter.

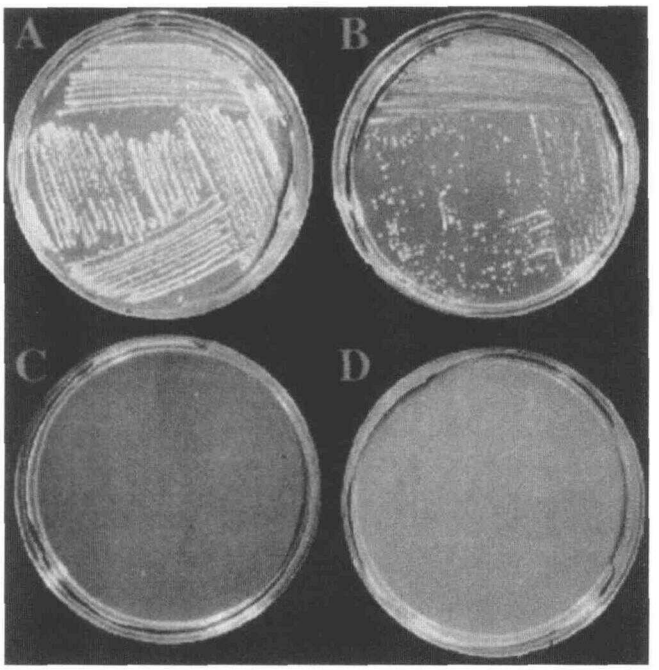


**Figure 1.** Complementation of JT16 by LHT1/λYES. The *LHT1* cDNA was subcloned into λYES and transformed into JT16 to test for functional complementation of the transport-deficient phenotype. Transformants were selected on Ura-free plates in the presence of high His (A). Ura$^+$ cells were transferred to low-His plates to test their ability to complement the His-transport mutation (B). Dependence of the suppressed phenotype on the expressed plant protein was demonstrated by testing transformants on Glc plates. This C source represses the GAL1 promotor of the expression vector (C). Verification that complementation was the result of newly acquired transport activity versus His biosynthesis was confirmed by transferring cells to a His-free medium (D). *NAT2* (a previously cloned amino acid transporter) and insert-free λYES were run in parallel as positive and negative controls, respectively (data not shown).

### LHT1 Is a Lys- and His-Specific Transporter

Amino acid transport activity of LHT1 was investigated using 13 different amino acids as potential substrates. Lys and His transport were the most active among all of the substrates examined (Fig. 2). Although LHT1 exhibited some transport capacity for a few other amino acids, such as Leu, it was most effective at transporting Lys and His, hence the name *L*ys and *H*is *T*ransporter 1, LHT1.

Lys and His transport by LHT1 displayed saturable, concentration-dependent uptake kinetics that were consistent with carrier-mediated transport, and the double-reciprocal plots of those data gave $K_m$ values of 175 and 400 $\mu$M, respectively (Fig. 3). The apparent $K_m$ for Leu was 11 mM (data not shown), which is higher than typical physiological ranges. The substrate specificity of the transporter was further demonstrated when Arg (at a 10-fold excess) did not decrease Lys transport in a substrate competition experiment (Fig. 4). Lys transport was sensitive to carbonyl cyanide *m*-chlorophenylhydrazone, which is consistent with transport coupled to the proton motive force (Fig. 4). Transport was also inhibited by DEPC, although an indirect effect on the yeast cell cannot be ruled out (Fig. 4).

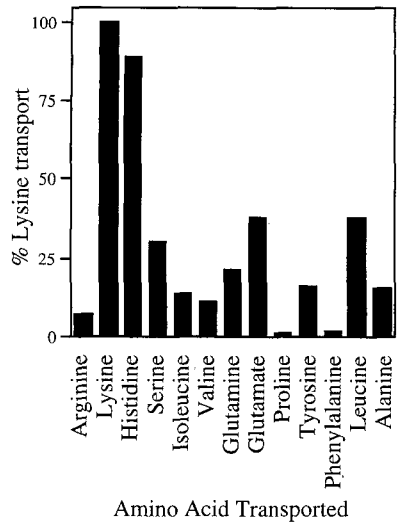


**Figure 2.** Amino acid transport in LHT1-expressing cells. Thirteen amino acids were tested as potential substrates for LHT1. Each amino acid was tested at 100 $\mu$M. Transport activity in cells containing insert-free vector was also determined for each amino acid and results are reported as LHT1-dependent transport (i.e. LHT1-expressing cell accumulation minus insert-free cell accumulation). Lys transport was 0.39 pmol $mg^{-1}$ fresh weight $min^{-1}$.

Earlier biochemical descriptions of amino acid transport into purified plasma membrane vesicles implicated a DEPC-sensitive His residue(s) in the reaction mechanism (Li and Bush, 1992).

### LHT1 Belongs to a New Class of Transport Protein

The cDNA insert of positive transformants was 1664 bp long. It contained an open reading frame encoding a protein of 446 amino acids with a calculated molecular mass of

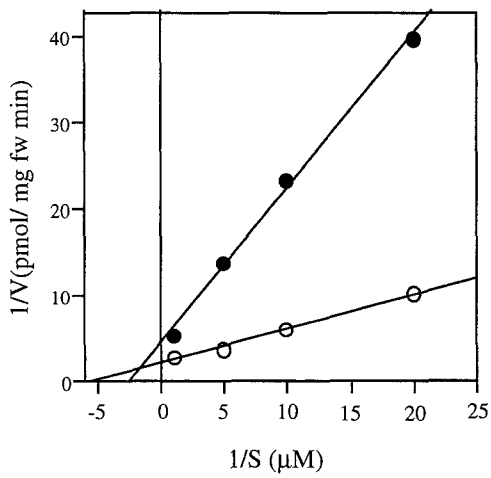


**Figure 3.** Double-reciprocal plot of Lys and His transport kinetics by LHT1-expressing cells. Transport activity in cells containing insert-free vector was also determined for each amino acid, and results are reported as LHT1-dependent transport (i.e. LHT1-expressing cell accumulation minus insert-free cell accumulation). The apparent $K_m$ for Lys was 175 $\mu$M and for His it was 400 $\mu$M.

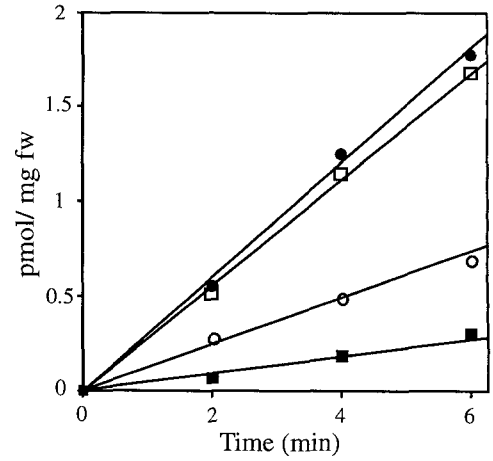


**Figure 4.** Effect of Arg and chemical inhibition on Lys transport by LHT1. Lys transport (●) was not affected by a 10-fold excess concentration of Arg (□). DEPC (○) and carbonyl cyanide *m*-chlorophenylhydrazone (■) inhibited Lys uptake. Transport solutions included 100 $\mu$M Lys in the presence or absence of 1 mM Arg, 2 mM DEPC, or 10 $\mu$M carbonyl cyanide *m*-chlorophenylhydrazone.

50.5 kD (Fig. 5A). Hydropathy analysis of the deduced sequence (Kyte and Doolittle, 1982) suggests that LHT1 is a hydrophobic integral membrane protein, with 9 to 10 putative transmembrane domains (Fig. 5B). The proposed topology of LHT1 is different from that predicted for other plant amino acid transporters, suggesting that it represents a new class of carrier. The NAT2/AAP family, the Pro transporters, and a cationic amino acid transporter all have 10 or more putative transmembrane domains (Frommer et al., 1993, 1995; Hsu et al., 1993; Kwart et al., 1993; Fischer et al., 1995; Rentsch et al., 1996). A phylogenetic comparison of LHT1 with amino acid transporters from a variety of organisms also suggests that LHT1 is not part of a previously described family of transporters (Fig. 6). It should be noted, however, that LHT1 is closely related to a putative amino acid transporter gene isolated from tobacco (Lalanne et al., 1995). Our analysis suggests that LHT1 represents a new class of plant amino acid transporters, although it does share some similarity with amino acid transporters identified in *Caenorhabditis elegans*, yeast, and plants.

### *LHT1* Is a Single-Copy Gene in the Arabidopsis Genome and Is Preferentially Expressed on the Root Surface and in Siliques

Southern-blot hybridization suggests that *LHT1* is a single-copy gene in the Arabidopsis genome (Fig. 7). RNA gel-blot analysis showed that *LHT1* is transcribed into a 1.7-kb message. Moreover, the pattern of tissue-specific expression suggests that *LHT1* is preferentially expressed in flowers, young leaves, and siliques, although it is also present in older leaves, stems, and roots (Fig. 8). Whole-mount, in situ analysis showed that expression is further localized to the surface of the root in young seedlings and in anthers (data not shown).

A

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1   MVAQAPHDDH QDDEKLAAAR QKEIEDWLPI  30
31  TSSRNAKWWY SAFHNVTAMV GAGVLGLPYA  60
61  MSQLGWGPGI AVLVLSWVIT LYTLWQMVEM  90
91  HEMVPGKRFD RYHELGQHAF GEKLGLYIVV 120
121 PQQLIVEIGV CIVYMVIGGK SLKKFHELVC 150
151 DDCKPIKLTY FIMIFASVHF VLSHLPNFNS 180
181 ISGSFSCCCR YVSQLLNNRM GIISKQRCSR 210
211 RRSIRLQSEN NSRYVFNFFS GLGDVAFAYA 240
241 GHNVVLEIQA TIPSTPEKPS KGPMWRGVIV 270
271 AYIVVALCYF PVALVGYYIF GNGVEDNILM 300
301 SLKKPAWLIA TANIFVVIHV IGSYQIYAMP 330
331 VFDMMEILLV KKLNFRPTTT LRFFVRNFYV 360
361 AAIMFVGMIF PFFGGLLAFF GGFAFAPTTY 390
391 FLPCVIWLAI YKPKKYSLSW WANWVCIVFG 420
421 LFLMVLSPIG GLRTIVIQAK GYKFYS     446
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B

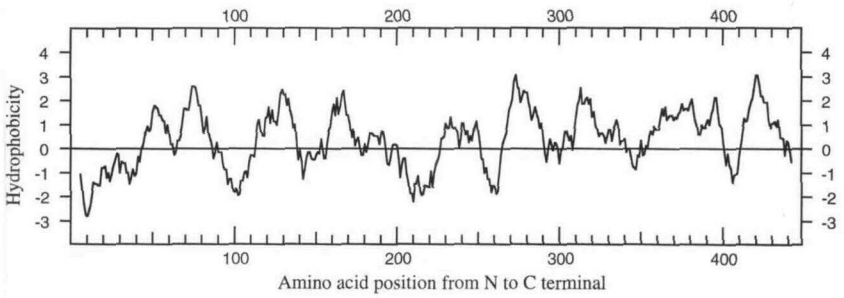


**Figure 5.** Deduced amino acid sequence and hydropathy plot of LHT1. The *LHT1* cDNA encodes a protein containing 446 amino acids (A). The Kyte and Doolittle (1982) hydropathy analysis shows that LHT1 is a hydrophobic membrane protein with 9 to 10 putative transmembrane helices (B).

## DISCUSSION

LHT1 represents a new class of amino acid transporters in plants because it has limited similarity to previously described transporters and because its predicted membrane topology differs from that of the other transporters. The similarity of LHT1 to the NAT2/AAP gene family is strongest around the first transmembrane domain, and that is why this EST cDNA showed up in our initial BLAST search of the EST database (Altschul et al., 1990). We have observed that this is a highly conserved domain shared by many eukaryotic amino acid transporters (Hoffmann, 1985; Ahmad and Bussey, 1986; Frommer et al., 1993; Hsu et al., 1993; Fischer et al., 1995; Rentsch et al., 1996) and an Arabidopsis aromatic amino acid transporter (Chen, 1997). We believe that future investigations will uncover the functional significance of this region.

LHT1 is easily differentiated from a functionally related cationic amino acid transporter, AAT1, which was recently described in Arabidopsis (Frommer et al., 1995). AAT1 has

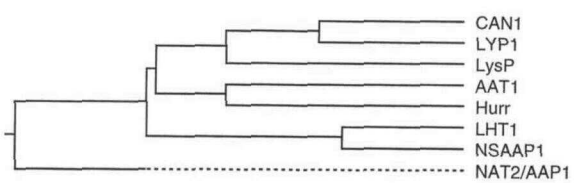


**Figure 6.** Phylogeny comparison of LHT1 with related amino acid transporters. LYP1 is the Lys-specific transporter in yeast (Sychrova and Chevallier, 1993); CAN1 is the cationic amino acid transporter in yeast (Hoffmann, 1985; Ahmad and Bussey, 1986); LysP is the Lys-specific permease in *E. coli* (Steffes et al.,1992); NSAAP1 is a putative amino acid transporter in tobacco (Lalanne et al., 1995); NAT2/AAP1 is a neutral amino acid transporter in Arabidopsis (Frommer et al., 1993; Hsu et al., 1993); Hurr is the human cationic amino acid transporter (Yoshimoto et al., 1991; Christensen, 1992); and AAT1 is an Arabidopsis cationic amino acid transporter (Frommer et al., 1995). This phylogeny was generated with MegAlign version 1.05 (DNASTAR, Madison, WI) using the Clustal method with a PAM250 residue weight table and it is rooted assuming a biological clock.

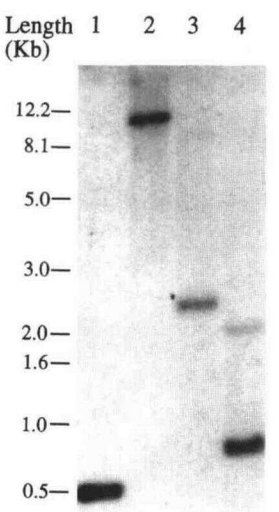


**Figure 7.** Southern-blot analysis of *LHT1*. Arabidopsis genomic DNA was digested with *Bam*HI (lane 2), *Eco*RI (lane 3), and *Hin*dIII (lane 4). Lane 1 was a 1-kb ladder. The *LHT1* cDNA has an internal *Hin*dIII site. The results shown are for a high-stringency wash. Low-stringency washes gave a similar pattern.

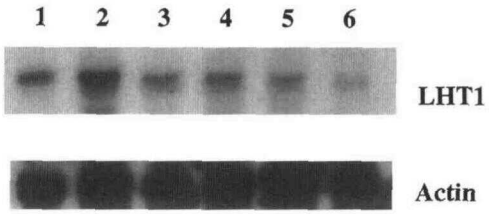


**Figure 8.** Expression pattern of *LHT1*. RNA gel-blot analysis of *LHT1* gave a single band at 1.75 kb. The actin gene was used as a loading control. RNA was isolated from siliques (lane 1), flowers (lane 2), old and young leaves (lanes 3 and 4, respectively), stems (lane 5), and roots (lane 6). The intensity of the *LHT1* signal was weak relative to actin (2-d exposure versus 2 h).

533 amino acid residues, which is much larger than LHT1 (446 amino acids). In addition, AAT1 contains 14 putative transmembrane domains (Frommer et al., 1995), whereas our analysis suggests that LHT1 is composed of 9 to 10 putative transmembrane helices (Fig. 5B). This is also less than the 11 transmembrane domains we have mapped in the *NAT2/AAP1* gene family (Chang and Bush, 1997). We believe that LHT1 belongs to a unique class of amino acid transporters in Arabidopsis. We realize, however, that more detailed analysis of the evolutionary relationships among the plant amino acid transporters may uncover a common ancestral progenitor. A putative amino acid transporter recently cloned in tobacco appears to be a *LHT1* ortholog (Lalanne et al., 1995).

Two basic amino acid transporters, CAN1 (Hoffmann, 1985; Ahmad and Bussey, 1986) and LYP1 (Sychrova and Chevallier, 1993), have been identified in budding yeast. CAN1 encodes a peptide containing 590 amino acids and LYP1 encodes one with 611 amino acids. Although both were predicted to contain 12 transmembrane domains, gene-fusion experiments with CAN1 suggest that it contains only 10 membrane-spanning regions (Ahmad and Bussey, 1988). The Lys transporter (LysP) in *E. coli* is also longer than LHT1 (489 residues; Steffes et al., 1992), and gene-fusion experiments have identified 12 transmembrane domains (Ellis et al., 1995). LHT1 is more closely related to the yeast and *E. coli* transporters than to AAT1 (Fig. 6).

An intriguing feature of the basic amino acid transporters we have examined is that the amino-terminal domains are highly acidic regions that contain many Glu and Asp residues. The acidic amino acid content for the amino terminus of these porters is 25% for LHT1, 19% for LYP1, 24% for CAN1, and 15% for LysP. Steffes et al. (1992) proposed that a glutamate residue, E-16, in LysP may play a role in substrate binding. LHT1 contains a glutamate residue at the E-25 position that may correspond to the E-16 position in LysP. Mutations in this position of the transporter will help define the role of these acidic residues in basic amino acid transporters. In addition to this acidic region, LHT1 also shares two conserved His's found in the NAT2/AAP gene family. These His residues have been mutated and shown to be essential for NAT2/AAP1 function (Chen, 1997).

Many of the plant amino acid transporters identified so far have wide substrate specificity, although they often favor a subgroup of related amino acids with lower $K_m$ values and higher rates of transport. LHT1 is somewhat intermediate in this characteristic in that it is a particularly active Lys and His transporter. The other amino acids tested had less than 35% of the Lys transport activity, and even the $K_m$ for the third most-active substrate, Leu, was 25-fold higher than that for His (11 mM, data not shown). Although Leu clearly moves through LHT1, we do not think it is a major substrate for this transporter, because plant cells generally contain low levels ($\leq$1 mM) of this amino acid (Winter et al., 1992).

The substrate specificity of LHT1 distinguishes it from AAT1, the functionally related Arabidopsis cationic amino acid transporter, and from basic amino acid transport activities previously described in barley, castor bean, and tobacco (Soldal and Nissen, 1978; Berry et al., 1981; Harrington and Henke, 1981; Bright et al., 1983; Weston et al., 1995). More recently, two transport mutants were described in Arabidopsis (*rlt11* and *raec1*) that have reduced Lys transport in the substrate concentration range in which LHT1 is active. Thus, it is possible that one of them could be a LHT1 mutant (Heremans et al., 1997). AAT1 transports both Lys and Arg, as did the amino acid transport activities described in barley and tobacco. In contrast, LHT1 is not an effective Arg transporter. This was demonstrated by the low rates of transport over a range of pH values (pH 4.0–7.0; Fig. 2 and data not shown). In addition, Arg was not a competitive inhibitor of Lys transport by LHT1, even when it was present at 10 times the concentration of Lys (Fig. 4). AAT1 substrate competition analysis showed that Arg was an excellent competitor for Lys transport in that transporter (Frommer et al., 1995). Although LHT1 and AAT1 are both basic amino acid transporters in Arabidopsis, their transport properties clearly differentiate them as unique amino acid transporters. This observation supports our earlier investigations with purified membrane vesicles, which suggested the presence of two cationic amino acid transport systems based on biphasic transport kinetics (Li and Bush, 1990).

Both RNA gel-blot analysis and whole-mount, in situ hybridization indicate that LHT1 is most strongly expressed in pollen, siliques, and on the root surface (Fig. 8). We infer from this expression pattern that LHT1 may be involved in Lys transport into heterotrophic tissue systems. This conclusion is supported by the observation that the related tobacco ortholog was isolated from a pollen-specific library (Lalanne et al., 1995). The functionally related cationic amino acid transporter (AAT1) identified in Arabidopsis is most strongly expressed in vascular tissue and flowers (Frommer et al., 1995). The expression patterns of LHT1 and AAT1 complement one another, suggesting that LHT1 may be involved in nutrient uptake in sink tissues, whereas AAT1 may contribute to long-distance transport.

LHT1 is an excellent candidate for using biotechnology to modify the nutritional value of harvested tissues (Bush, 1998). For example, many cereals are poor sources of protein for humans and animals because they are low in Lys (Kriz and Larkins, 1991; Galili, 1995). One strategy for modifying the nutritional value of these deficient crops

would be to use targeted expression of LHT1 to enhance the Lys content of harvested seed. Targeted expression of *LHT1* in the phloem of mature leaf tissue may increase the amount of Lys and His transported per unit of C. This would increase the Lys and His content of developing seeds when the Lys-enriched translocate is released from the phloem. The success of this approach is dependent on several unknown variables. For example, reciprocal increases in mesophyll synthesis and release must occur as these amino acids are actively transported into the phloem, and sink tissues must be capable of storing the excess free amino acids. There is a precedent for increased rates of synthesis under increased demand; Heldt's work (Riens et al., 1991; Winter et al., 1992) suggests that the mesophyll amino acid pools are dynamically linked to phloem loading and, thus, active loading by a high-affinity transporter may shift the equation to increased synthesis. Likewise, many cells store excess amino acids in the vacuole. We are currently generating transgenic plants to test this approach.

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