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Molecular Mechanisms of Urea Transport in Plants

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Abstract. Urea is a soil nitrogen form available to plant roots and a secondary nitrogen metabolite liberated in plant cells. Based on growth complementation of yeast mutants and "*in-silico* analysis", two plant families have been identified and partially characterized that mediate membrane transport of urea in heterologous expression systems. AtDUR3 is a single *Arabidopsis* gene belonging to the sodium solute symporter family that cotransports urea with protons at high affinity, while members of the tonoplast intrinsic protein (TIP) subfamily of aquaporins transport urea in a channel-like manner. The following review summarizes current knowledge on the membrane localization, energetization and regulation of these two types of urea transporters and discusses their possible physiological roles *in planta*.

Key words: Water channel — Sodium solute symporter — Plasma membrane — Tonoplast transport — Urease — Ornithine cycle — *Arabidopsis thaliana*

Urea as an External Nitrogen Source for Plants

Modern agricultural plant production relies on high nitrogen fertilizer inputs to successfully exploit crop yield potentials and maintain elevated protein contents. Since high chemical inputs of nitrogen fertilizers have a negative environmental impact, affecting biodiversity, air and water quality, and the global climate, approaches enhancing fertilizer use efficiency have become a major concern. With respect to these partially opposing requirements urea fertilizers combine the advantages of rapid availability to plants and of a retarded microbial transformation into nitrate, which is the nitrogen form most prone to leaching. The microbial degradation process of urea can even be further slowed down by the co-application of urease inhibitors that have been demonstrated to further reduce nitrogen losses from urea-fertilized plots (Xu et al., 2000). Due to its high nitrogen content and cheaper production costs, urea fertilizers make up more than 50% of the total nitrogen fertilizers used worldwide (http://www.fertilizer.org/ifa/statistics/STATSIND/).

In soils, urea is rapidly degraded to ammonium and CO_2 by urease, a nickel-dependent enzyme, which amongst others is synthesized and secreted by microorganisms (Watson et al., 1994). Therefore, the concentration of urea in lakes or natural soils is usually low and ranges between 0.1 - 3 μM (Cho et al., 1996; Mitamura, Kawashima and Maeda, 2000a; Mitamura et al., 2000b), but up to 70 μM in fertilized crop-planted soils (Gaudin, Dupuyu & Bournat, 1987). With regard to this very low concentration it was believed that plants take up urea-derived nitrogen mainly in the form of ammonium (Polacco & Holland, 1993; Marschner, 1995).

Due to its low molecular weight and neutral character urea was believed for a long time to enter plant cells via diffusion through plant membranes (Galluci, Micelle & Lippe, 1971). Only a very limited number of physiological studies indicated that urea uptake may be plant-regulated, for example, since ammonium and nitrate exerted adverse effects on urea uptake (Bradley, Morgan & O'Toole, 1989), but all in planta-studies lacked a verification whether urea itself or its degradation product ammonium had been transported across plant membranes. The first reliable experimental evidence for protein-mediated urea uptake by plant cells has been obtained by Wilson, O'Donoghue and Walker (1988), who reported that short-term influx of ^{14}C-labeled urea in algal cells was dependent on the ATPase inhibitor DCCP or the protonophore CCCP and therefore appeared to be coupled to the proton gradient across the plasma membrane. Since concentration-depen-

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dent uptake followed bi- or even multiphase kinetics, the authors suggested the combined action of a high- and a low-affinity urea transport system *in planta*. Kinetically and energetically, these transport systems are clearly set apart from a passive, diffusion-like transport mechanism (Wilson & Walker, 1988). Recently, CCCP-sensitive urea uptake was also confirmed in *Arabidopsis* suspension cells (Liu et al., 2003a).

Urea as an Intermediate in Secondary Nitrogen Metabolism

Besides acquisition from the environment, urea can also accumulate in plant cells as a consequence of secondary nitrogen metabolism. Two major biochemical pathways may lead to urea synthesis. In the first pathway, also described as the ornithine or urea cycle, urea is produced in mitochondria, when arginine is digested into ornithine and urea by the action of arginase (Polacco & Holland, 1993). In the second pathway, urea is liberated during the catabolism of purins or ureides, like allantoin and allantoate. In particular, leguminous plant species employ ureides for long-distance translocation of nitrogen, and thus shuttle potentially larger amounts of nitrogen through urea in their sink tissues (Stebbins & Polacco, 1995). Since urea accumulation is particularly high in source leaves of older plants and in germinating seeds (Zonia, Stebbins & Polacco, 1995), it was proposed that urea accumulation mainly reflects nitrogen recycling as a consequence of protein and in particular arginine catabolism, which is emphasized by an increase in arginase protein levels relative to total seed protein (Bailey & Boulter, 1971, Zonia et al., 1995). These stages of enhanced nitrogen re-mobilization are further characterized by increased activities of cytosolic glutamine synthetase (GS1), probably as a prerequisite for the generation and re-fixation of urea-derived nitrogen (Masclaux et al., 2000; Witte et al., 2002). Although tissue aging and seed germination are accompanied by massive increases in urea concentrations, the routes of intracellular urea synthesis and the size of intracellular urea pools in different plant compartments have not yet been determined quantitatively. Thus, it is currently unclear to what extent urea is transported across intracellular membranes and to what extent it requires transport systems to do so.

A Role of ScDUR3 in Urea Uptake in Yeast

Short-term uptake experiments using ^{14}C-labeled urea identified two major pathways for urea uptake into yeast cells: one is an active, energy-dependent transport system with a rather low K_m of 14 μM, while the other is a passive transport system that operates at concentrations above 0.5 mM (Cooper & Sumrada, 1975). Screening of EMS-mutagenized yeast cells on media with different nitrogen sources allowed identification of a yeast strain that did not or poorly grew on urea or ureidoglycolate (Sumrada, Gorski & Cooper, 1976). Transformation of this mutant by a genomic library from yeast and screening of transformants with complemented urea uptake led to the identification of *ScDUR3* (ElBerry et al., 1993). Supply of ^{14}C-labeled urea to a liquid culture of the *ScDUR3*-complemented yeast mutant and trapping of liberated $^{14}CO_2$ indicated that the reintroduced gene, which encodes a hydrophobic protein with 15 putative trans-membrane domains (Turk & Wright, 1997; Saier, 2000), mediated or at least assisted in urea uptake. Under adequate nitrogen supply, *ScDUR3* expression was repressed in a manner similar to that of other genes in the allantoin pathway, suggesting a regulatory link between *ScDUR3* and other genes involved in the urea-releasing nitrogen catabolism (ElBerry et al. 1993).

Plant Aquaporins Can Act as Urea Transporters

To isolate genes encoding urea transporters in plants, the *ScDUR3* gene was disrupted in a *ura*$^-$ yeast background and transformed with a cDNA library from *Arabidopsis* seedlings. Subsequent screening of the resulting transformants on < 5 mM urea as a sole nitrogen source allowed the isolation of four genes, *AtTIP1;1*, *AtTIP1;2*, *AtTIP2;1* and *AtTIP4;1*, that all encoded members of the tonoplast intrinsic protein (TIP) subfamily of aquaporins (Liu et al., 2003b). Growth complementation of the *ura*$^-$ *dur3*$^-$ mutant by the isolated TIPs from *Arabidopsis* was insensitive to pH but inhibited in the presence of phloretin [3-(4 hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-1-propanone], which is commonly used as a transport inhibitor for various classes of urea transporters including aquaporins (You et al., 1993; Ishibashi et al., 1994; Tsukaguchi et al., 1998). In a parallel approach, *AtTIP2;1* was heterologously expressed in *Xenopus laevis* oocytes where it enhanced the accumulation of radio-labeled urea in oocytes independent of external pH in a range from pH 5 to 8, confirming the pH-independent growth complementation in yeast (Liu et al., 2003b). Urea accumulation in *AtTIP2;1*-expressing oocytes increased linearly with external urea supply even when raising urea concentrations from 100 μM to 30 mM. Such linear concentration-dependent kinetics is typical for channel-mediated substrate transport and commonly observed for low-affinity transporters, although discrete substrate affinities cannot be calculated.

A function of TIPs in urea transport had been previously observed, when a TIP homologue from

tobacco, NtTIPa, that in regard to sequence is close to AtTIP2;1, was expressed in *Xenopus laevis* oocytes, where it was permeable to radiolabeled glycerol and urea besides water (Gerbeau et al., 1999). Moreover, oocyte expression also allowed the demonstration that plasma membrane intrinsic proteins (PIPs), too, may be permeable to urea. Functional expression of NtAQP1 and, to a lesser extent, of *Arabidopsis* PIP2;1, facilitated significantly the accumulation of radiolabeled urea relative to water-injected oocytes (Eckert et al., 1999). In another yeast complementation approach, which used an expression library from zucchini for complementation of the *dur3* mutant, CpNIP1, an NOD26-like intrinsic protein was isolated (Klebl, Wolf & Sauer, 2003). This emphasized that not only members of the TIP subfamily but also PIP- and NIP-like aquaporins may permeate urea, thus suggesting a comparable situation to that in mammals, where 4 out of 11 AQPs transport urea besides water (King, Kozono & Agre, 2004).

Are there Common Structural Elements in Urea-transporting TIPs?

A phylogenetic analysis of human aquaporins allowed the sorting of aquaporin homologues into two subgroups that also differ in substrate permeability. AQP homologues for which only water transport activity was found were subsumed as aquaporins in a narrow sense, while the other group of aquaglyceroporins may transport also small solutes like glycerol and urea besides water (King et al., 2004). In the genome of the model plant *Arabidopsis*, 35 major intrinsic protein (MIP) genes were identified, which have been divided into four subfamilies: the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), the nodulin 26-like intrinsic membrane proteins (NIPs), and the small basic intrinsic proteins (SIPs) (Weig, Deswarte & Chrispeels, 1997; Johansson et al., 2001; Quigley et al., 2001). An extended analysis of 450 MIP sequences showed that TIP and PIP proteins cluster in close vicinity to the human aquaporins but not to the aquaglyceroporins (Zardoya, 2005). Selecting just human urea-permeable and non-permeable AQPs for a more focused phylogenetic analysis together with *Arabidopsis* MIPs confirmed that urea-permeable TIPs or even PIPs do not cluster with human aquaglyceroporins (Fig. 1). Thus, urea permeability in plant MIPs can obviously not be predicted from their phylogenetic relations as it seems possible for mammalian AQPs (King et al., 2004).

An X-ray structural analysis revealed that AQP1, a water-specific aquaporin, forms at its narrowest region a pore size of 2.8 Å, whereas the *E. coli* GlpF glycerol channel is approximately 1 Å wider than AQP1, which was taken as one presumption to allow GlpF to be permeable to glycerol (King et al., 2004). This information was then used for structural modeling and sequence alignments to subdivide *Arabidopsis* MIPs into eight subclasses according to their amino acid signature in the predicted selectivity filter, which is represented by the narrowest constriction region in the pore of these proteins (Wallace & Roberts, 2004). This constriction region is gated by a tetrad formed by amino acid residues from opposing helices and the third outer loop, forming an aromatic/arginine signature that has been proposed to create the main selectivity filter of AQP1 and GlpF (Fu et al., 2000; Sui et al., 2001; Thomas et al., 2002). Interestingly, all four urea-transporting TIPs belong to group I or group II that are characterized by a conserved hydrophilic histidine residue in the second and an aliphatic isoleucine residue in the fifth transmembrane-spanning domain adjacent to the constriction region. In most other MIPs, this histidine residue is substituted by a hydrophobic aromatic residue. According to Wallace and Roberts (2004), however, an altered amino acid signature can lead to a reversed polarity that in turn might affect substrate selectivity. Since the urea-permeable channels NtAQP1 and CpNIP1 deviate in this position, however, the hydrophobicity of the pore region alone is unlikely to determine urea permeability in plant MIPs. Indeed, recent substrate permeability studies on AQP1 mutants confirmed a strong influence of the pore size. Urea permeability was achieved as soon as the urea molecule matched the constriction diameter (Beitz et al., 2006). Future approaches to define protein domains of substrate selectivity should therefore include an extended analysis of amino acid-substituted MIPs tested for the transport of urea and water in parallel.

Urea Transport across Tonoplast and Plasma Membranes

The high abundance of TIPs that were found to mediate urea transport suggested that their host membrane, the tonoplast, should exhibit high urea permeabilities. Indeed, recording volume changes in membrane vesicles from wheat roots in response to external urea revealed an up to 3-fold higher and mercury-sensitive permeability of vesicles enriched with endosomal membranes compared to the lower, mercury-insensitive urea permeability of plasma membrane-enriched vesicles (Tyerman et al., 1999). By stopped-flow spectrofluometry tonoplast vesicle preparations from tobacco were reported to be permeable to urea at a 75-fold higher rate than plasma membrane vesicles (Gerbeau et al., 1999). Whether these differences are caused by a higher activation state and/or density of urea-transporting TIPs in tonoplast membranes or by a higher ratio of urea-

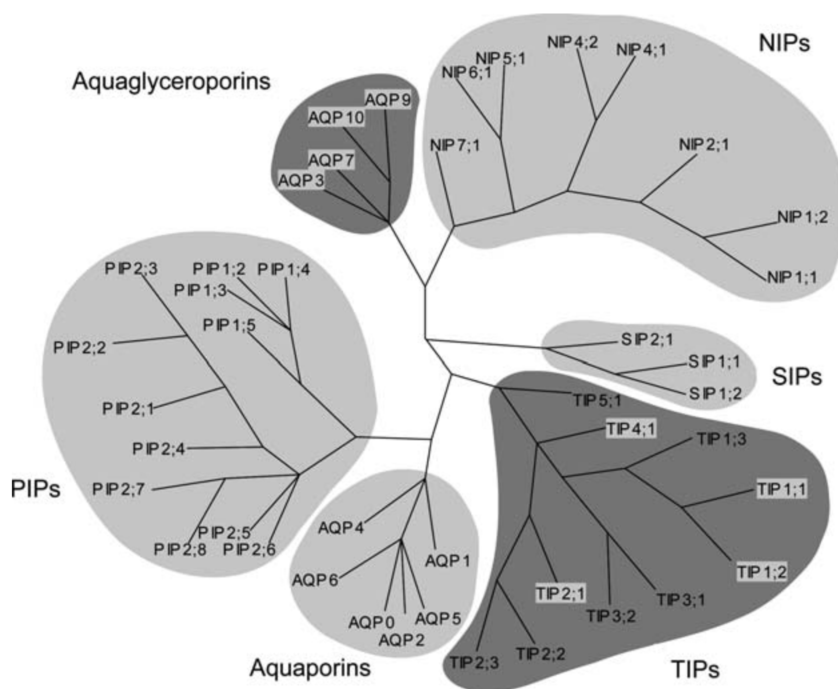

Fig. 1. Phylogenetic tree of aquaporins and aquaglyceroporins from humans together with major intrinsic proteins from *Arabidopsis thaliana*.

transporting versus urea-impermeable aquaporins is currently unresolved. A prerequisite for tackling this question is a reliable membrane localization of the diverse urea-transporting aquaporins. A membrane assignment based on the sequence-dependent classification into TIPs and PIPs, at least, appears not reliable, because some aquaporins can be found in both types of membranes, as put in evidence by Western analysis with membrane fractions from the ice plant *Mesembryanthemum crystallium* (Barkla et al., 1999), by GFP-tagging of TIP proteins and expression in *Arabidopsis* protoplasts (Liu et al., 2003b), or by systematic sequencing of membrane proteins (Marmagne et al., 2004). Nevertheless, for certain TIPs, such as AtTIP2;1, a major localization in the tonoplast has been reported by several independent approaches (Daniels et al., 1996; Saito et al., 2002; Carter et al., 2004) and thus appears very reliable. Taken together, these observations suggest that urea transport across the tonoplast is of greater physiological significance for a plant cell than that across the plasma membrane, supposing that urea transport is not just a non-specific side activity of TIPs.

Possible Physiological Roles of TIPs in Urea Transport in Plants

Although some TIPs show distinct organ- and cell type-specific expression patterns (Ludevid et al., 1992; Daniels et al., 1996; Ma et al., 2004), systematic gene expression analyses in different *Arabidopsis* organs indicated that all plant organs express a certain individual subset of TIPs (AtGenExpress; Schmid et al., 2005), per se not allowing to derive information on their possible function in urea transport.

Vacuolar compartmentalization mediated by TIPs could be beneficial to transiently store or to detoxify an excess amount of urea that otherwise would accumulate in the cytoplasm. Under natural conditions this might be a rare event, but it is expected to become relevant in leaf-fertilized crop plants. An addition of the urease inhibitor phenylphosphorodiamidate (PPD) to foliar-applied urea increased leaf tip necrosis and increased the urea content but decreased ammonia levels and urease activity in soybean leaves (Krogmeier, McCarty & Bremner, 1989). Moreover, enhanced urea levels in necrotic areas indicated that leaf tip necrosis in response to leaf fertilization resulted from accumulation of excess urea rather than from the formation of excess ammonia. It will certainly be interesting to test whether an increased expression level of urea-transporting TIPs in urea-fertilized leaves of transgenic lines will diminish or delay leaf damage via an enhanced vacuolar loading capacity for urea.

To further elucidate a physiological role of urea-transporting TIPs in different plant tissues, a separate determination of urea concentrations in different intracellular compartments seems required, since it is still unclear whether urea can accumulate in the vacuole or in other organelles. Theoretically, TIPs will mediate urea transport independently of the proton gradient across the membrane, just following the direction of the substrate concentration gradient.

Due to a rather constitutive expression and activity of urease in almost any cell type (Holland et al., 1987; Polacco & Holland, 1993), cytoplasmic urea concentrations should be low. With respect to micromolar K_m values of plant ureases (Kerr et al., 1983), urea-transporting TIPs will rather move low amounts of urea *in planta*, while their urea-transporting function would increase whenever leaves are sprayed with urea fertilizers or whenever urease activity is lost, i.e., under nickel deficiency (Gerendás & Sattelmacher, 1997).

The *Arabidopsis* Urea Transporter AtDUR3 Belongs to the Sodium Solute Symporter Protein Family

A genome-wide *in silico* search indicated that the *Arabidopsis* gene At5g45380 showed 41% sequence identity to the putative yeast urea transporter gene *ScDUR3*. Homologous EST clones were also found in maize, rice, soybean, barley, wheat, and oilseed rape. It is interesting to note, however, that in all plant species investigated so far only one *DUR3* homolog could be identified on the basis of database search in EST collections and genomic DNA. An *Arabidopsis* EST clone matching the genomic sequence of *AtDUR3* was then used for functional complementation of the urea uptake-defective yeast mutant *ura⁻ dur3⁻*. Heterologous expression of *AtDUR3* conferred yeast growth on 2 mM urea but only at a medium pH of 6 or lower, indicating that protons might stimulate AtDUR3-dependent urea transport (Liu et al., 2003a). AtDUR3 was predicted to encode an integral membrane protein with 14 transmembrane-spanning domains with its N and C termini protruding into the apoplasmic space (Schwacke et al., 2003). A phylogenetic analysis of AtDUR3 and the 22 most homologous and best characterized amino acid sequences derived from a BLAST search revealed a relatively high similarity among DUR3 proteins from plants and yeast and that all these sequences belong to the superfamily of sodium solute symporters (SSS) (Liu et al., 2003a). The SSS family includes currently more than one hundred members of pro- and eukaryotic origin (Jung, 2002) and some of them have been described to transport sugars, amino acids, nucleosides, myo-inositols, vitamins, ions, phenyl acetate, water and urea (Reizer, Reizer & Saier, 1994; Turk & Wright, 1997; Saier, 2000). Among all SSS proteins, DUR3 members showed closest relation to bacterial sodium pantethonate or sodium proline symporters (Fig. 2). Since all these substrates appear to be structurally quite different, it will be exciting to uncover how substrate selectivity is determined in this transporter class. Leung et al. (2000) reported that SSS transporters such as the low-affinity sodium-glucose transporters from pig (pSGLT3) or rabbit (rbSGLT1), the sodium-iodide transporter from rat (rNIS), or the human sodium-chloride-GABA transporter can act as urea channels in the absence of substrate but as urea cotransporters in the presence of substrate. This raises the possibility that in SSS transporters urea is just an alternative substrate for water that usually maintains the bulk flow of the substrates.

Substrate Specificity and Transport Mechanism of AtDUR3

To study its transport mechanism in more detail, AtDUR3 was expressed in *X. laevis* oocytes and substrate transport was investigated by two-electrode voltage clamp. Although urea itself is neutral, the presence of urea induced a weak inward current of positive charge, indicating the cotransport of cations (Liu et al., 2003a). To increase sensitivity of urea transport measurements in oocytes the accumulation rate of ^{14}C-labeled urea was determined. It was found that low pH strongly stimulated urea import. This observation was in good agreement with an improved yeast complementation at acidic pH and suggested that AtDUR3 co-transported mainly protons. Even though urea accumulation was not altered in the presence of 3 mM sodium in the bathing solution (Liu et al., 2003a), it might be too early to exclude that also alternative cation gradients might drive uphill transport of urea. For example, in the SSS member OpuE, a sodium/proline cotransporter, substrate transport strongly depended on the external sodium concentration in the medium (von Blohn et al., 1997).

Since SSS family members have been described to mediate the transport of a large variety of solutes, substrate specificity of AtDUR3 was tested, but no current was observed in response to glucose, galactose, myo-inositol or proline. Only in the presence of thio-urea an even weaker current was observed than for urea, suggesting a rather high urea specificity of AtDUR3. In most of the cases, SSS proteins were suggested to preferentially transport one substrate or a structurally closely related substrate group (Turk & Wright, 1997), whereas SSS proteins with a broad range of different substrates seem to be the exception (Leung et al., 2000).

Similar to urea-induced currents, radio-labeled urea uptake into AtDUR3-expressing oocytes was concentration-dependent and saturated around 50 μM urea (Liu et al., 2003a). Both transport assays allowed to calculate a K_m value of approx. 3 μM, indicating that AtDUR3 mediates high-affinity transport of urea. Thus, AtDUR3 exhibited a relatively high affinity compared to most other urea transporters that saturate at millimolar concentrations (Leung et al., 2000). As pointed out by Liu et al.

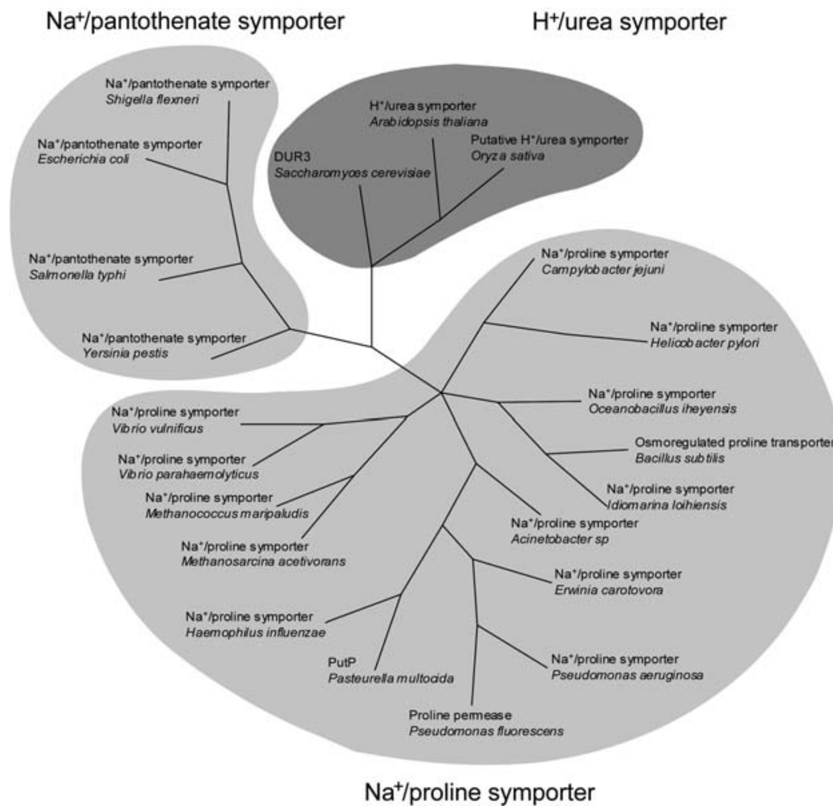

Fig. 2. Phylogenetic tree of characterized SSS family proteins with highest sequence similarity to AtDUR3.

(2003a), uptake studies with AtDUR3 in yeast cells were difficult to perform due to the rapid degradation of urea by endogenous yeast urease. Acid trapping and determination of $^{14}CO_2$ released after the supply of radiolabeled urea, as described in ElBerry et al. (1993), does not allow determination of short-term substrate influx like it is required for the biochemical characterization of membrane transporters.

Studies in cyanobacteria showed that substrate degradation can be successfully avoided when high-affinity urea influx was investigated in the absence of any urease activity (Valladares et al., 2002). Only after disruption of the UreG gene, which encodes an accessory protein required for the synthesis of active urease, high-affinity urea influx could be reliably determined in *Synechocystis*. The corresponding ABC-type urea transporter Urt was then shown to transport urea against a concentration gradient at a substrate affinity of 1 μM (Valladares et al., 2002). Thus, at least in plants, fungi and cyanobacteria measurements of high-affinity urea import can be easily perturbed if urease-dependent urea degradation remains uncontrolled.

Regulation of *AtDUR3* Gene Expression in *Arabidopsis*

To collect evidence for the physiological function of AtDUR3-mediated urea transport *in planta*, changes of *AtDUR3* mRNA levels were examined under different conditions (Liu et al., 2003a). When *Arabidopsis* plants were cultured hydroponically and then subjected to nitrogen deficiency for 3 days *AtDUR3* mRNA levels strongly increased in roots but not in shoots. This transcriptional response was reminiscent of the nitrogen deficiency-induced derepression of high-affinity ammonium (AMT) and nitrate (NRT) transporters in *Arabidopsis* roots (Gazzarrini et al., 1999; Lejay et al., 1999), both of which are considered to contribute to nitrogen uptake under nitrogen-deficient growth conditions.

Gene expression levels of *AtDUR3* were further examined in mature leaves and germinating seeds of *Arabidopsis* (Liu et al., 2003a), since it is known that in both of these developmental stages high amounts of urea are liberated via the degradation of storage proteins. Indeed, *AtDUR3* mRNA was abundant in germinating seeds peaking five days after germination, suggesting a direct and/or indirect role of AtDUR3 in the transport of endogenously synthesized urea. Since arginase appeared to be localized in mitochondria, whereas urease is localized in the cytoplasm (Faye, Greenwood & Chrispeels, 1986; Goldraij & Polacco, 2000), urea is supposed to pass the mitochondrial membrane on its way into the cytoplasm by a yet unknown transport mechanism (Fig. 3). As AtDUR3-mediated transport of urea depends on a proton gradient, it is difficult to imagine that AtDUR3 participates in this transport step.

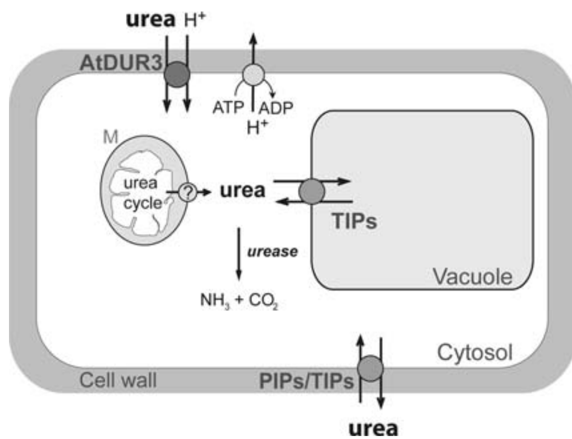

Fig. 3. Model for protein-mediated urea transport pathways in plant cells. AtDUR3 mediates secondary active, high-affinity urea transport across the plasma membrane, while aquaporins of the PIP or TIP subfamilies mediate low-affinity urea transport. In particular TIPs might further transport urea across the tonoplast for transient storage in the vacuole or remobilization. *M*, mitochondrion.

Presuming a more likely localization in the plasma membrane, however, also doesn't point to an obvious role of AtDUR3 in mature leaves. Thus, it remains to be noted that *AtDUR3* gene expression in seedlings coincides with developmental stages, in which urea might accumulate that has been liberated from internal nitrogen sources.

Physiological Roles of High- and Low-Affinity Urea Transporters in Plants

As a proton/urea symporter AtDUR3 is able to transport urea into root cells even when external concentrations are low. With regard to the almost ubiquitous occurrence of ureases in soil substrates and their millimolar affinity constants (Dalal, 1985) AtDUR3 might find its role in the lower micromolar concentration range of urea that might be left over in the soil solution after microbial degradation. The particularly low K_m of AtDUR3 might therefore reflect an evolutionary adaptation directed to use this diluted but valuable nitrogen source. Future studies on transgenic *Arabidopsis* plants that lack or overexpress AtDUR3 are therefore required to demonstrate to what extent high-affinity urea uptake in roots of wild-type plants will depend on AtDUR3.

Plasma membrane-localized aquaporins of the PIP or, depending on their membrane localization, even of other MIP families might increasingly contribute to urea import with increasing concentrations of external urea. Their linear concentration-dependent transport kinetics makes it likely that this transporter class serves in low-affinity uptake of urea. Under agricultural conditions, this transport system might become relevant in roots after urea application to soils or in leaves after foliar spray with liquid urea fertilizers. With respect to the tonoplast localization of certain or even most TIPs, a low-affinity transport pathway would also allow plant cells to load urea into the vacuole for transient storage of this nitrogen source (Fig. 3). To what extent this transport path depends on cytoplasmic urease requires further studies determining urea in different subcellular compartments of plant lines with altered urease activities.

In conclusion, plants possess different types of urea transporters for passive and secondary active transport of urea across different cellular membranes. Together with a large number of transport systems for other nitrogen forms these membrane transporters appear to enable plants optimizing their nitrogen intake and compartmentation in dependence of the nitrogen forms being available in the medium and being synthesized endogenously. A deeper understanding of urea transport processes in plants and their regulation by nitrogen will not only allow a better understanding of the importance of urea for plant nitrogen nutrition but also an improvement of its utilization as nitrogen fertilizer for soil and foliar application in agricultural crop production.

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