The Oligopeptide Transporters: A Small Gene Family with a Diverse Group of Substrates and Functions?

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ABSTRACT Genes in the Oligopeptide Transport family encode integral membrane proteins that are believed to translocate their substrates from either the extracellular environment or an organelle into the cytosol. Phylogenetic analyses of plant transporters have revealed two distant clades: the Yellow Stripe-Like (YSL) proteins and the so-called Oligopeptide Transporters (OPTs), for which the family was named. Three categories of substrates have been identified for this family: small peptides, secondary amino acids bound to metals, and glutathione. Notably, the YSL transporters are involved in metal homeostasis through the translocation of metal-chelates, indicating a level of conservation both in biological function as well as substrates. In contrast, the functions of OPT proteins seem to be less defined and, in this review, I will examine the supporting and contradictory evidence for the proposed roles of OPTs in such diverse functions as long-distance sulfur distribution, nitrogen mobilization, metal homeostasis, and heavy metal sequestration through the transport of glutathione, metal-chelates, and peptides.

Key words: Molecular transport; nutrient and metal transport; transporters; Oligopeptide Transporters.

INTRODUCTION

In plants, the Oligopeptide Transporters (OPTs) comprise a small gene family (pFAM designation: PF03169) whose products transport substrates synthesized from amino acids including small peptides, secondary amino acids that can complex with metals, and the modified tripeptide glutathione (Koh et al., 2002; Curie et al., 2001; Bogs et al., 2003). These integral membrane proteins are predicted to have 12 transmembrane domains and are characterized by several signature motifs (Wiles et al., 2006). It is widely accepted that these transporters are proton-coupled symporters that translocate their substrates in the cytosolic direction (Hauser et al., 2000; Bogs et al., 2003; Schaaf et al., 2004; Osawa et al., 2006). Phylogenetically they can be divided into two groups—the Yellow Stripe-Like (YSL) and Oligopeptide Transporter (PT) clades (Figure 1)—and in the A. thaliana genome, there are eight and nine of each, respectively (DiDonato et al., 2004; Koh et al., 2002). Notably, plant genes in the PT clade are closer in homology to their fungal orthologs than they are to YSL genes in the same organism (Yen et al., 2001).

The YSL genes have been found in archaea, eubacteria, plants, and fungi but not animals and their function in plants appears to be the transport of metal-chelates (Curie et al., 2001; DiDonato et al., 2004; Roberts et al., 2004; Koike et al., 2004; Murata et al., 2006) consisting of mugeneic acids or nicotianamine, both of which are synthesized from three S-adenosyl methionines. On the other hand, PT genes have only been found in plants and fungi and have been implicated in the transport of small peptides, glutathione, and metal-chelates (Koh et al., 2002; Bogs et al., 2003; Vasconcelos et al., 2008; Table 1). What is striking about the OPT family is the level of function and substrate conservation in the YSL transporters and the level of divergence in the PT clade. The YSL proteins appear to be involved in metal-chelate translocation at all possible levels including intracellular transport, long distance mobilization, and soil scavenging. The PT transporters, on the other hand, seem not to have a common biological function but rather this group may be involved in four different processes: long-distance metal distribution, nitrogen mobilization, heavy metal sequestration, and glutathione transport. This review will focus on the evidence for and
against the PT proteins contributing to these four biological functions.

Even though the gene family is named the OPT family, PT-type genes are usually named and referred to as OPTs while yellow stripe-like genes are typically called YSLs. Furthermore, in animals, the acronym OPT has been used to describe members of the Peptide Transport (PTR) superfamily (also known as the Proton-coupled Oligopeptide Transporter (POT) family). In this review, the use of OPT will refer exclusively to genes from the PT branch of the Oligopeptide Transporter family and not YSL or PTR genes.

SEVERAL OPTs EXHIBIT CHARACTERISTICS OF IRON TRANSPORTERS

Transporters from several different families have been shown to play a role in iron (Fe) trafficking and there is evidence from both a model monocot and dicot that a subset of the OPTs are involved in iron and possibly other metal transport (Wintz et al., 2003; Vasconcelos et al., 2008). Iron-transporting OPTs have been described in A. thaliana and Oryza sativa, with the A. thaliana transporter AtOPT3 being the best characterized of these. While expression and mutant analyses all suggest that AtOPT3 transports iron, actual translocation of an iron-chelate by this protein has not been demonstrated.

Several expression studies have revealed a regulatory regime for AtOPT3 that is consistent with long-distance iron transport and partitioning. First, four studies found that AtOPT3 expression was induced by iron deficiency and the increase in expression was shown to be more than an order of magnitude in roots and twofold in leaves (Wintz et al., 2003; Stacey et al., 2006, 2008; Buckhout et al., 2009). Second, loss-of-function mutants in POPEYE (PYE1), which encodes a key bHLH transcription factor that regulates iron homeostasis, had increased AtOPT3 expression regardless of iron availability (Long et al., 2010). Additionally, the same study found that PYE1 and AtOPT3 are co-regulated. Third, a meta-analysis of 58 microarray datasets showed that AtOPT3 is in the same regulatory network as other iron-partitioning genes, including the iron transporter encoded by AtNRAMP4 and the iron reductase encoded by AtFRO3 (Long et al., 2010). Finally,

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**Table 1. Summary of Proposed Functions and Substrates of Characterized OPTs.**

<table>
<thead>
<tr>
<th>Transporter</th>
<th>GenBank accession number</th>
<th>Proposed functions</th>
<th>Proposed* or demonstrated substrates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AtOPT3</td>
<td>AT4G16370</td>
<td>Long-distance iron transport or signaling</td>
<td>Fe-chelate*</td>
<td>Stacey et al., 2008</td>
</tr>
<tr>
<td>AtOPT4</td>
<td>AT5G64410</td>
<td>Loading vasculature with peptides at source tissues</td>
<td>Broad range of peptides</td>
<td>Koh et al., 2002; Stacey et al., 2006</td>
</tr>
<tr>
<td>AtOPT5</td>
<td>AT4G26590</td>
<td>Possibly mediates heavy metal phytotoxicity</td>
<td>Unknown</td>
<td>B. Ahner, personal communication</td>
</tr>
<tr>
<td>AtOPT6</td>
<td>AT4G27730</td>
<td>Long-distance GSH mobilization through loading of asculature and mediating heavy metal phytotoxicity</td>
<td>GSH, GSH-Cd, PCn-metal*, and peptides of 4–13 amino acids</td>
<td>Pike et al., 2009; Cagnac et al., 2004</td>
</tr>
<tr>
<td>BjGT1</td>
<td>CAD91127</td>
<td>GSH transport from leaves and hypothesized role in mediating heavy metal phytotoxicity</td>
<td>GSH, GSH-Cd*, PCn-metal*</td>
<td>Bogs et al., 2003</td>
</tr>
<tr>
<td>OsOPT1</td>
<td>BAB89477</td>
<td>Undetermined role in iron homeostasis</td>
<td>Fe–NA</td>
<td>Vasconcelos et al., 2008</td>
</tr>
<tr>
<td>OsGT1 (OsOPT3)</td>
<td>NP_001056651.1</td>
<td>Long-distance GSH transport</td>
<td>GSH</td>
<td>Zhang et al., 2004; Wang et al., 2007</td>
</tr>
<tr>
<td>OSOPT4</td>
<td>NP_001056665.1</td>
<td>Undetermined role in iron homeostasis</td>
<td>Fe–NA</td>
<td>Vasconcelos et al., 2008</td>
</tr>
<tr>
<td>OsOPT7</td>
<td>AAO32313</td>
<td>Undetermined role in iron homeostasis</td>
<td>Fe–NA</td>
<td>Vasconcelos et al., 2008</td>
</tr>
</tbody>
</table>

Figure 1. Phylogenetic Tree Illustrating the YSL and PT Clades Found in the OPT Family in Plants.
The tree was constructed from a ClustalW alignment comprising all 17 A. thaliana OPT family members, all nine PT O. sativa transporters, and BjGT1 from Brassica juncea.
promoter–GUS lines revealed that AtOPT3 is expressed in vascular tissue, in some organs, but not in dermal tissue, thereby precluding a role in scavenging the environment for nutrients (Stacey et al., 2006).

Two A. thaliana lines harboring AtOPT3 mutant alleles have been characterized (Stacey et al., 2002, 2008). opt3-2 is a partial loss-of-function allele caused by a T-DNA insertion in the 5′ UTR, and homozygous opt3-2 plants were not compromised in their ability to scavenge the soil for iron, and these plants were not chlorotic, although the leaf palisade cells were altered and more closely resembled the spongy mesophyll (Stacey et al., 2008). Furthermore, opt3-2 plants hyper-accumulated iron in some regions such as leaves and siliques but hypo-accumulated iron in other places such as seeds (Stacey et al., 2008). Two mechanisms have been proposed to explain the hyper- and hypo-accumulation phenotype observed in opt3-2 plants. The simple explanation is that opt3-2 plants miss partitioned iron in planta leading to regions of hyper- and hypo-accumulation. This model is further substantiated by opt3-1 plants. The complete loss-of-function opt3-1 allele exhibited an embryo-lethal phenotype (Stacey et al., 2002), suggesting that AtOPT3 provides the embryo with iron possibly by unloading the phloem, the primary pathway for this metal to reach developing seeds (Grusak, 1994). Consistent with a role in partitioning iron was the observation that AtOPT3 expression was induced in the embryo sac following fertilization and in the endosperm during embryogenesis (Stacey et al., 2002).

Two genes (FRO2 and IRT1) with a well-established role in root iron acquisition were up-regulated in opt3-2 mutants, suggesting a second model for the mutant phenotype (Stacey et al., 2008). FRO2 encodes a root-based Fe(III)-chelate reductase that reduces Fe(III) to Fe(II) before transport across the plasma membrane by the iron transporter IRT1. Both FRO2 and IRT1 are recognized as key components in root iron acquisition and are themselves up-regulated by lowIron conditions (Eide et al., 1996; Robinson et al., 1999; Vert et al., 2002). The induction of these two genes in opt3-2 mutants suggests that these plants perceived low-iron conditions in their roots, even when adequate iron was present in the plant. In this model, AtOPT3 mediates long-distance signaling between shoots and roots, and in the mutant, signaling was disrupted, leading to increased root iron export and subsequent accumulation in other tissues (Stacey et al., 2008). Whether AtOPT3 coordinates shoot-root iron levels, partitions iron in planta, or both, what is clear is that a decrease in AtOPT3 levels disrupts long-distance iron transport, although it does not affect the plant’s ability to scavenge iron from the growth medium (Stacey et al., 2008).

Whereas expression and mutant analyses strongly suggest that AtOPT3 is involved in iron transport, actual transport studies remain less convincing. Expression of AtOPT3 did slightly increase the growth rate of a yeast strain deficient in iron transport (fet3fet4) in liquid minimal Synthetic Dextrose medium (Wintz et al., 2003). What is surprising about this finding is that there was an increase in growth at all, since OPTs (and YSLs) have only been found to transport metal-chelates, peptides, or modified peptides, not metal directly. Even more surprising, the addition of the iron-chelating secondary amino acid nicotianamine (NA) to the medium did not increase the growth rate, suggesting that Fe–NA is not a substrate for AtOPT3. This is particularly disturbing because it is thought that NA is one of the primary metal carriers in the phloem (von Wieren et al., 1999), Nicotianamine synthase1 and 4 are up-regulated by iron deficiency (Wintz et al., 2003; Long et al., 2010), and localization studies as well as mutant characterization place AtOPT3 functioning in the phloem (Stacey et al., 2006, 2008). It should be noted that the yeast growth medium did contain histidine, which can form metal complexes (Cagnac et al., 2004), and it is conceivable that an iron–histidine complex was transported by AtOPT3, albeit poorly, in this heterologous system. Unfortunately, at the time of the experiment, YSL NA–Fe transporters had yet to be identified and, as a result, there was no positive control available that could be used to establish a baseline of robust transport in this heterologous system.

The only other A. thaliana OPT implicated in iron transport is AtOPT2, which was found in one early microarray study to be up-regulated by iron deficiency (Wintz et al., 2003). This was the same study that complemented a fet3fet4 yeast mutant with AtOPT3 but, interestingly, there was no mention of AtOPT2’s ability to complement a fet3fet4 yeast strain. Additionally, no subsequent works have found that AtOPT2 is regulated by iron or have placed AtOPT2 in any of the iron regulatory networks determined by microarray analyses. Based on these observations, it seems likely that AtOPT2 is not involved in iron transport, although the possibility remains that it is involved in transport of other metals (Wintz et al., 2003).

Growth experiments designed to measure Fe–NA transport in yeast fet3fet4 mutants suggest that several rice OPTs can transport Fe–NA chelates, although not equally well (Vasconcelos et al., 2008). Specifically, yeast harboring OsOPT1 or OsOPT4 exhibited robust growth on a medium containing Fe(II)–NA and Fe(III)–NA at concentrations as low as 5 and 10 μM, respectively. Yeast with OsOPT5 or OsOPT7 grew weakly on a Fe(II)–NA-containing medium and not at all on Fe(II)–NA. However, OsOPT7 expression was induced 60-fold in both shoots and roots of plants grown under iron deficiency (Zheng et al., 2009), and OsOPT7 is the closest ortholog to AtOPT3 (Figure 1), suggesting that the transport observed in yeast may be physiologically relevant. Finally, yeast transformed with OsOPT3 were able to grow on Fe(II)–NA and Fe(III)–NA, although weakly. The conditions for measuring growth were identical to the work done with AtOPT3 except the medium was solid as opposed to liquid, and consistent with OPTs transporting metal-chelates, there was no growth of transformants when FeSO₄ was the sole iron source (Vasconcelos et al., 2008; Wintz et al., 2003).

Yeast growth assays on a solid medium occur over 3–5 d and are not necessarily reflective of high levels of transport. In other words, growth by a transformant simply means that the minimum level of transport needed to sustain growth was
met. More revealing are actual uptake assays with radiolabeled substrates. From the data reported by Vasconcelos et al. (2008), at 5-μM concentrations, OsOPT4 transported Fe(II)–NA at a rate of 2.7 pmol min⁻¹ 10⁻⁶ cells, which is comparable to transport levels exhibited by the Zea mays transporter ZmYS1 (Roberts et al., 2004). On the other hand, OsOPT1 and OsOPT3 transported this same substrate at less than 1 pmol min⁻¹ 10⁻⁶ cells, although, as explained below, this is consistent with OsOPT3 (OsGT1) most likely encoding a glutathione transporter and not an iron transporter per se. In summary, it appears that a subset of OPTs in rice are able to transport Fe–NA, although definitive proof awaits mutant characterization and in planta localization of individual OPTs.

Taken together, the work from these two model plants makes a strong argument for the role of some OPTs in iron transport. However, two key questions remain unanswered. First, which chelate(s) are the substrate for these transporters? It is tantalizing to propose that NA is the carrier molecule, given all the prima facie evidence in A. thaliana and the observed transport by rice OPTs in a heterologous system; however, it is difficult to explain why AtOPT3 failed to reveal robust transport of Fe–NA in yeast when this same system worked quite well with Y5 transporters from A. thaliana, Z. mays, Thlaspi caerulescens, and Hordeum vulgare (Chu et al., 2010; Roberts et al., 2004; Schaal et al., 2004; Murata et al., 2006; Gendre et al., 2007). The three most likely explanations are that AtOPT3 failed to function properly in yeast, NA is not the substrate, or AtOPT3 encodes an iron sensor. The former could be explained by something as simple as codon usage; for example, the rice OPTs contain several codons that are rarely used in yeast (M. Hauser, personal communication). Alternatively, it is possible that NA is not the chelate, although the list of potential candidates is quite small and the high levels of NA in the phloem sap and xylem stream (130 and 20 μM, respectively) (Pich and Scholz, 1996; Schmidke and Stephan, 1995) suggest that this molecule plays a significant role in long-distance metal transport. Finally, there are several examples of transport families in which some members act as a sensor or as a sensor and transporter of the substrate (Barker et al., 2000; Ho et al., 2009). The second key question is: if these genes are not scavenging the soil for iron, then where within the vasculature are they loading or unloading the presumed iron-chelates and what is their role in iron homeostasis?

CAN AtOPT3 TRANSPORT OTHER METAL CHELATES?

In addition to mis-partitioned Fe, opt3-2 plants also hyperaccumulated zinc, manganese, and copper in certain tissues (Stacey et al., 2008). This altered distribution of other metals could be caused by the opt3-2 mutation or by the increased expression of IRT1, which can transport Zn and Mn in addition to Fe (Stacey et al., 2008; Vert et al., 2002) as suggested by altered IRT1 expression and metal accumulation in AtNRAMP3 iron transport mutants (Thomine et al., 2003). On the other hand, using Northern blots, Wintz et al. (2003) showed that AtOPT3 expression in roots could be induced by lack of manganese and copper, but not zinc. Furthermore, AtOPT3 was able to complement yeast deficient in both manganese (smf1) and copper transport (crtl), although, once again, this is surprising given the lack of a carrier molecule in the growth medium (Wintz et al., 2003). While not conclusive, these studies certainly bring to our attention the necessity for future studies that examine metals other than iron.

SEVERAL OPTs TRANSPORT GLUTATHIONE AND POSSIBLY GLUTATHIONE METAL COMPLEXES

Glutathione (γ-Glu-Cys-Gly, GSH) is a highly nucleophilic, thiol-containing, modified tripeptide that serves many cellular functions in plants, such as a key role in mediating redox potential as well as mitigating biotic and abiotic stress, including heavy metal toxicity (Cobett and Goldsbrough, 2002). Additionally, GSH is the primary form of organic sulfur that is mobilized in plants. Two OPTs from fungi, ScOpt1p from Saccharomyces cerevisiae and SpPt1 from Schizosaccharomyces pombe, have been shown to translocate glutathione (Bourbouloux et al., 2000; Thakur et al., 2008), and four plant OPTs from three different organisms have been implicated in transporting glutathione: BjGT1 from Brassica juncea (Bogs et al., 2003), OsGT1 (OsOPT3) from O. sativa (Zhang et al., 2004; Wang et al., 2007), and AtOPT6 and AtOPT5 from A. thaliana (Cagnac et al., 2004; Pike et al., 2009; B. Ahner, personal communication). Three of these—BjGT1, OsGT1, and AtOPT6—were found to complement an OPT yeast mutant deficient in GSH transport and exhibited biphasic kinetics in this system characterized by a high-affinity Kₘ in the μM concentration and a low-affinity Kₘ in the mM range (Bogs et al., 2003; Cagnac et al., 2004; Zhang et al., 2004). Further experimentation in a system other than yeast is needed to determine whether these kinetic characteristics were a function of the assay conditions or truly reflected transport in planta.

Given that GSH is synthesized in the leaves and that multiple OPTs are expressed in leaf vascular tissue, several research groups have proposed that one function of these transporters is to load the phloem sap with GSH (Bogs et al., 2003; Cagnac et al., 2004; Pike et al., 2009), which has been reported to be as high as 4–5 mM in rice (Kuzuhara et al., 2000) and 200 μM in Brassica napus (Mendoza-Cozatl et al., 2008). A second proposed function for these genes originated from the observation that BJGT1 expression was altered by the toxic metal cadmium (Cd) in the growth medium (Bogs et al., 2003). Increased GSH leads to increased Cd tolerance, possibly because this peptide is the starting molecule for the biosynthesis of phytochelatins and GSH itself can complex with Cd (Zhu et al., 1999). Phytochelatins (PCₙ: (γ-Glu-Cys)ₙ-Gly, where n = 2–11) are modified peptides that play a key role in metal detoxification by sequestering metals, which are transported
as a PC₅ complex, to the vacuole or exported to the apoplast (Ortiz et al., 1995; Cobbett and Goldsbrough, 2002; Kim et al., 2007). Expression levels of BjGT1 initially decreased in leaves of plants exposed to cadmium and then returned to steady-state levels over several days, and two mechanisms have been proposed to explain this change (Bogs et al., 2003). The first postulates that BjGT1 encodes a GSH transporter and when cadmium enters the leaf, expression is reduced to maintain high levels of GSH for PC₅ synthesis. In this model, BjGT1 is playing an indirect role in mediating Cd toxicity simply by reducing the export of GSH from the leaf. The second model proposes that BjGT1 encodes a glutathione transporter that is able to translocate GSH-Cd and its expression is down-regulated to prevent Cd loading into the phloem sap. A third possibility, that BjGT1 imports its substrate into the vacuole, seems unlikely given that four independent proteomic analyses of the A. thaliana tonoplast failed to find a single OPT in this membrane (Shimaoka et al., 2004; Szponarski et al., 2004; Carter et al., 2004), although two yellow stripe-like transporters, AtYSL6 and AtYSL4, were identified (Jaquinod et al., 2007). Furthermore, all OPTs that have been characterized move their substrates in a cytosolic direction, which would be out of the vacuole. Surprisingly, no one has directly tested whether PC₅-Cd can be transported by BjGT1.

AtOPT6 may be serving the same function in Arabidopsis as BjGT1 serves in B. juncea. When expressed in oocytes, AtOPT6 transports GSH with low affinity (Kₘ > 500 μM) and PC₅ at very low rates (Pike et al., 2009), while in yeast, AtOPT6 can transport GSH as well as GSH-Cd (Cagnac et al., 2004). Promoter–GUS fusions have shown that AtOPT6 is expressed in the leaf vasculature but, unlike BjGT1, is not induced by Cd in the growth medium (Cagnac et al., 2004; Stacey et al., 2006). Similarly, in rice, OsGT1(OsOPT3) mRNA levels were not altered by Cd (Wang et al., 2007). Taken together, these studies suggest that AtOPT6 is functioning as a high-capacity low-affinity GSH transporter that loads the phloem in leaves presumably through companion cells and is directly or indirectly involved in heavy metal-chelate transport.

Somewhat contradictory to this model was the finding that AtOPT6 in oocytes can also transport a diverse group of tetra- and pentapeptides (Pike et al., 2009). Even more surprising, the Kₘ for one of these substrates (KLLLG) was 10 μM as opposed to 500 μM for GSH (Pike et al., 2009). Similarly, the pentapeptide KLLLG competed for the uptake of radiolabeled GSH by yeast harboring OsGT1(OsOPT3) (Zhang et al., 2004). If AtOPT6 and OsGT1 encode GSH transporters, it is not clear why they also transported peptides and, in the case of AtOPT6, why substrates that varied dramatically in sequence and length were transported. One possible explanation is that AtOPT6 encodes a PC₅ transporter and therefore must be able to accommodate substrates of varying lengths (G. Stacey, personal communication).

The idea that some OPTs transport GSH-metals or PC₅-metals is further supported by work from Beth Ahner’s research group. Lead (Pb) levels were found to be altered in shoots and roots of AtOPT7 and AtOPT5 T-DNA insertion lines grown hydroponically for 5 d in a medium containing Pb and GSH. Surprisingly, the AtOPT7 mutant line showed approximately a twofold increase in root Pb levels compared to wild-type (B. Ahner, personal communication). On the other hand, the AtOPT5 mutant line accumulated Pb at levels similar to wild-type but the distribution within the plant was quite different. Specifically, AtOPT5 mutants had low root but high shoot concentrations of Pb when compared to wild-type plants that had roughly twice the concentration of Pb in their roots (B. Ahner, personal communication). This phenotype of miss partitioning metal in planta is strikingly similar to the phenotype of opt3-2 plants, suggesting that GSH-Pb or PC₅-Pb is a substrate for AtOPT5 and that this transporter, in an analogous manner to AtOPT3, helps coordinate root-shoot metal distribution in a GSH-dependent manner. Furthermore, AtOPT5 expression has only been detected during pollen development (Bock et al., 2006; Stacey et al., 2006), leading to the speculation that metals induce expression. Finally, the phenotype of the AtOPT7 mutant certainly warrants further investigation into whether or not this gene is also involved in GSH or PC₅ translocation.

In summary, these data indicate that a subset of the OPTs are most likely involved in GSH transport and given the many functions of this modified tripeptide, it seems likely that these glutathione transporters play direct as well as indirect roles in many GSH-mediated processes. For example, BjGT1 expression is altered by environmental Cd levels as explained above as well as by changes in cellular redox state (Srivastava et al., 2010). For those OPTs implicated in lessening Cd or Pb phytotoxicity, what is yet to be resolved is whether their role is simply to partition GSH or to transport all or some combination of GSH, GSH-metal, or phytochelatin-metal complexes.

**OPTs AS PEPTIDE TRANSPORTERS**

Nitrogen mobilization in plants takes many forms including, but not limited to, amino acid and peptide transport (Tegeder and Rentzsch, 2010). Peptide transport is defined as the translocation of small peptides across membranes. For the purpose of this review, peptide transport refers to the translocation of small peptides comprising the naturally occurring amino acids and not to the transport of the modified peptides GSH and phytochelatins or secondary amino acids such as NA, which are synthesized from three S-adenosyl methionines. This distinction is being drawn to separate those peptides that serve primarily as a nitrogen source from GSH, PC₅, and secondary amino acids that, as described earlier, play many distinct roles in plants. Genes from two families, the Peptide Transporter (PTR) superfamily and the OPT family, have been shown to encode proteins that transport peptides in plants (Steiner et al., 1994; Koh et al., 2002). Furthermore, the presence of OPTs and PTRs in plants has led to the widespread assumption that small peptides are transported in planta. Whereas PTR mediated di- and tripeptide transport has been shown to occur in
A. thaliana (Komarova et al., 2008), OPT-mediated peptide transport has only been observed in heterologous systems. Furthermore, the modified tripeptide glutathione is the only peptide found thus far that is both a substrate in heterologous systems and known to actually occur in plants. As explained in this final section, the lack of biologically relevant substrates has made it difficult to determine whether or not OPTs truly transport peptides for the purpose of nitrogen mobilization in plants.

FOUR OPTs IN A. THALIANA TRANSPORT PEPTIDES IN HETEROLOGOUS SYSTEMS

The only OPTs shown to transport peptides besides GSH are all from A. thaliana, although one peptide did compete with GSH uptake when the rice transporter OsGT1 was expressed in yeast (Zhang et al., 2004). Of the nine OPTs in A. thaliana, only AtOPT4 has exhibited characteristics consistent with a broad substrate transporter. AtOPT4 was able to transport a diverse range of tetra- and pentapeptides but not GSH, suggesting that this protein is specific for peptides yet has a broad range of substrates (Osawa et al., 2006; Koh et al., 2002). Given the myriad of cellular roles that GSH plays, it seems reasonable that GSH transporters and transporters involved in nitrogen mobilization do not overlap in substrates. Promotor–GUS fusions indicated that AtOPT4 is highly expressed in the vascular cylinder of stems, roots, leaves, and the inflorescence stem but not in developing seeds or flowers and has only low expression in germinating seeds (Stacey et al., 2006). This expression pattern is consistent with a transporter that is involved in loading the vascular system with nitrogen in the form of peptides at source tissues presumably by importation into phloem parenchyma or companion cells. The lack of expression or weak expression during germination, seed formation, and floral development is telling, since these three events are accompanied by nitrogen mobilization. Therefore, AtOPT4 is not loading the seed, flower, or embryo with peptides from the apoplast that arrived via the vasculature. Consistent with this model was the finding that amino acid transporters and PTR-type transporters played a significant role in loading seeds with amino acids (Schmidt et al., 2007; Hunt et al., 2010; Sanders et al., 2009). These studies suggest that AtOPT4 may encode a broad substrate transporter involved in long-distance nitrogen mobilization by loading the vasculature with peptides at source tissues. Indirectly, this model also predicts that there should be a corresponding transporter, presumably an OPT, that loads these same peptides into sink tissues.

Yeast expressing AtOPT1, AtOPT5, and AtOPT7 was able to grow on a medium containing the pentapeptide KLLLG but was not able to grow on media supplemented with KLLG, KLGL, or YGGFL (Koh et al., 2002). Furthermore, AtOPT1 and AtOPT7 had distinct but overlapping spatial and developmental expression patterns in vascular tissue with AtOPT4, suggesting that each of these three transporters has specific as well as redundant functions (Stacey et al., 2006). The finding that the transporters encoded by AtOPT1, AtOPT4, and AtOPT7 overlapped in a substrate, specifically KLLLG, and in expression patterns makes it tempting to speculate that they constitute a system for long-distance nitrogen mobilization; however, several key pieces of evidence are inconsistent.

Not consistent with a role in nitrogen mobilization is the observation that AtOPT1 and AtOPT7 failed to transport most of the peptides tested. Additionally, the hyper-accumulation of GSH-Pb in roots of an AtOPT7 mutant (B. Anher, personal communication) is hard to rectify with a transporter whose function is nitrogen distribution. Furthermore, there is no reason to believe that KLLLG, the common peptide transported, occurs in A. thaliana. This substrate was assayed because several OPTs in fungi, namely Candida albicans and S. pombe, transported this peptide (Lubkowitz et al., 1997, 1998). Finally, Gary Stacey’s laboratory has found no observable phenotype in double and triple OPT mutants and only a slight reduction in growth of a quadruple OPT mutant under normal growth conditions, suggesting a specialized rather than generalized function, such as nitrogen allocation, for these genes (G. Stacey, personal communication). One can easily imagine such a function consisting of GSH or other metal-chelate translocation that would manifest under certain growth conditions but not others.

Nitrogen mobilization also occurs during leaf senescence and it is possible that some OPTs may be involved in recovering protein hydrolysis products, but several observations suggest otherwise. Most notably, none of the peptide sequences shown to be substrates for AtOPTs, namely KLLG, KLGL, and KLLLG, is found within the large or small subunits of rubisco, the prominent protein in leaves, although KLGL is found in the subunit Rubisco LSMT (Lubkowitz, unpublished observation). Furthermore, a subtractive hybridization screen failed to detect the expression of any OPTs in senescing A. thaliana leaves (Gepstein et al., 2003). While a role for the OPTs in recovering hydrolyzed proteins during senescence cannot be completely ruled out, these studies suggest it is unlikely.

As explained earlier, AtOPT6 can transport GSH and there is evidence that indicates that AtOPT6 also plays a role in mediating phytotoxicity caused by heavy metals such as cadmium. Surprisingly, Pike et al. (2009) found that AtOPT6, when expressed in oocytes, could also transport a large and diverse group of tetra- and pentapeptides with high affinity but not the signaling molecule phytosulfokinin, which is 5 amino acids in length. Furthermore, this study found that several peptide-based signal molecules ranging in length from 5 to 13 amino acids from animals and plants were substrates for AtOPT6. In short, AtOPT6 in oocytes can transport peptides of varying length and amino acid composition as well as GSH. It is possible that AtOPT6 has dual functions in that it participates in GSH homeostasis as well as peptide translocation for nitrogen mobilization. Alternatively, the range of peptide length could be because AtOPT6 transports not only GSH, but also phytochelatins, which vary in length.
Little is known about the roles of AtOPT2, AtOPT5, AtOPT8, and AtOPT9 and what we do know comes from a small number of expression studies. AtOPT5 expression has only been detected in pollen (Bock et al., 2006; Stacey et al., 2006), where AtOPT1, AtOPT2, AtOPT8, and AtOPT9 were also found to be expressed (Bock et al., 2006). Furthermore, AtOPT9 is co-regulated with STP2, a monosaccharide transporter known to be involved in microspore development (Bock et al., 2006; Truernit et al., 1999). Considerable work needs to be done before any conclusions can be drawn about the biological role of these genes.

THREE FUNDAMENTAL QUESTIONS THAT WILL HELP US UNDERSTAND THE BIOLOGICAL FUNCTION OF OPTs IN PLANTS

Are the OPTs Really GSH, Peptide, and Metal-Chelate Transporters or Do They Share a More Restricted and Common Biological Function?

As explained in the introduction, the OPT family is not large; for example, A. thaliana has only 17 genes between the two distinct clades (Figure 1). Transports in the YSL group appear to share a common function in metal-chelate transport while the second clade, the so-called OPT genes, have been implicated in GSH, metal-chelate, and peptide transport (Table 1). Is it likely that one group would be so conserved and the other so divergent in substrates and function? Possibly, since the three groups of known substrates for OPTs share a mutual origin, they are synthesized from three or more amino acids. This common feature leaves open the possibility that the OPTs do indeed translocate three distinct substrate classes.

Ironically, the function first proposed for OPTs in plants—nitrogen distribution—is the least supported. The hypothesis that OPTs are involved in nitrogen mobilization was partially based on the observation that OPTs in fungi do transport oligopeptides, which is consistent with a lifestyle of a saprophyte or pathogen that grows in an environment with a diverse array of peptides. On the other hand, the problem with suggesting that the plant OPTs function in bulk amino acid movement is the lack of widespread observed peptides in the apoplast or in planta outside of germination. In a heterologous system, rice OPTs that were expressed during germination did not transport peptides produced from endosperm storage proteins, further suggesting that OPTs are not involved in bulk nitrogen movement (Lubkowitz, unpublished observation). Finally, all observed mutant phenotypes have affected metal distribution in planta. Although it is premature to conclude that none of the OPTs is involved in nitrogen mobilization in plants, it is clear that many are not, but rather are affecting long-distance metal transport either directly through GSH–metal, PCn–metal, or NA–metal complexes or indirectly by controlling GSH distribution.

What Are the Metal-Chelates that Serve as Substrates for OPTs?

There is strong evidence from mutant analyses and expression studies that a subset of the OPTs are involved in long-distance metal transport by loading the vascular system with metal-chelates but determining the nature of the chelating agent(s) has proven to be difficult. For example, four rice OPTs were able to transport NA–Fe with varying success but AtOPT3 failed to transport this substrate, and PC synthase1 is not regulated by iron, suggesting that Fe–PC is not a substrate (Wintz et al., 2003). On the other hand, GSH–metal transport in a heterologous system does leave the possibility that GSH is a chelating agent in planta but this does not preclude the possibility that PCn–metal complexes are also substrates. Furthermore, the transport of PCn–metal complexes where N > 2 have not been examined directly and is obviously one of the highest priorities for determining the role of this gene family. Finally, the broad substrate specificity exhibited in heterologous systems by some of these transporters needs to be rectified with the proposed role in metal transport. Ultimately, functional redundancy makes measuring transport in plants difficult, and heterologous systems are suggestive but not conclusive, thereby underscoring the importance of complementary expression, localization, and mutant studies.

What Are the Cellular and Sub-Cellular Locations of OPTs?

Promoter–GUS fusions have demonstrated that the OPTs are heavily expressed in the vasculature but a comprehensive model requires localization within the vascular cylinder to determine specifically which cells are being loaded. Furthermore, given that two YSL transporters have been found in the tonoplast (Jaquinod et al., 2007) and that ScOpt2p from S. cerevisiae has been localized to an internal membrane (Aouida et al., 2009), it would be prudent to determine the sub-cellular locale of plant OPTs. This is important because nitrogen mobilization, GSH homeostasis, and metal distribution all involve a substantial number of genes from many different families, and determining the specific contributions of the OPTs in this complex system requires that we know the cellular and sub-cellular location of each OPT, since that information illuminates the possible roles that any given transporter may play in the system.

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