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INVITED REVIEW

The physiology and molecular biology of peptide transport in seeds

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Abstract

Peptide transport plays a major role in the nitrogen nutrition of the cereal embryo during germination. During germination, enzymatic hydrolysis of storage proteins in the cereal grain endosperm forms a reservoir of small peptides and amino acids which are translocated across the scutellum to supply organic nitrogen to the growing embryo. Uptake of these solutes by the scutellum, a modified cotyledon which functions in nutrient transport, is mediated by carriers localized to the plasma membrane of the scutellar epithelium. To date the peptide transporter HvPTR1 is by far the best characterized example of a nutrient transporter involved in reserve mobilization during germination. Peptide transport in the barley scutellum has been relatively well-characterized biochemically, and in recent years the barley scutellar peptide transporter HvPTR1 has been cloned and characterized at the molecular level. Here, we review the physiological role and importance of peptide transport in germination, focusing on recent characterization of the barley peptide transporter HvPTR1. In barley, the uptake of small peptides by the scutellum appears to be mediated by a proton-coupled peptide transport system capable of handling peptides 2–4 amino acid residues in length.

Keywords: germination, *Hordeum vulgare*, peptide transport, reserve mobilization, scutellum

Introduction

The transport of organic nitrogen in the form of peptides plays an important role in the nutrition of living organisms, from bacteria to humans. In plants, the study of peptide transport has focused mainly on germinating cereal grains, although an increasing awareness of macromolecular transport of proteins and peptides between source and sink tissues is now becoming apparent (Thompson and Schulz, 1999).

The onset of seed germination is associated with the rapid resumption of cellular RNA and protein synthesis, whereas the replication of DNA is often delayed for several hours (Bray, 1979). This delay allows completion of any repair processes prior to resumption of cell cycle activity and subsequent growth processes (Osborne, 1983). In barley grains, the scutellum, a modified cotyledon and specialized absorptive tissue which functions in nutrient transport, accounts for almost all the protein synthesis in barley embryos after 4.5 h imbibition, with much lower levels found in the root and coleoptile (Stoddart and Thomas, 1973). In the immediate post-imbibition period, the scutellum synthesizes a variety of specific carrier proteins that are thought to localize subsequently to the plasmalemma of scutellar epithelial cells adjoining the endosperm. Carrier proteins transport degradation products arising from mobilization of cereal endosperm reserves (e.g. glucose, peptides and amino acids) across the scutellum. These degradation products are then translocated into the embryo, either prior to or after further metabolism (Bewley and Black, 1994).

During the germination of cereal grains, storage proteins in the endosperm are hydrolysed to form a reservoir of small peptides (2–6 residues) and amino acids (Higgins and Payne, 1981). These hydrolysis products are translocated across the scutellum by a number of secondary active carrier systems. Peptides are then rapidly hydrolysed to amino acids by peptidases in scutellar cells, and these free amino acids are subsequently transported into the embryo, where they represent a major supply of organic nitrogen used to support growth processes during

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germination and early seedling establishment. Amino acids and peptides are translocated across the scutellar epithelium by separate distinct transporter systems that are present in the scutellum of several cereals during germination (Salmenkallio and Sopanen, 1989). The flux of solutes across the scutellum is a short-term event in the plant's life cycle. Nevertheless, these carriers have a crucial, albeit transient, role to play in the processes leading to successful seed germination and establishment of an autotrophic young plant.

The physiology of peptide transport in the germinating barley grain

Protein constitutes approximately 10% of the dry weight of the ungerminated barley grain and is localized mostly in the starchy endosperm as storage protein reserves that are mobilized during germination (Kreis and Shewry, 1992; Shewry, 1995). Prior to imbibition, large pools of peptides and amino acids are detectable in both embryo and endosperm tissues of quiescent barley grains (Higgins and Payne, 1981). The peptide pool size decreases during the first 24 h following grain imbibition, coincident with the development of peptide uptake activity in barley scutellum that becomes detectable by 6 h imbibition and increases rapidly during the following 24 h (Walker-Smith and Payne, 1985; West *et al*., 1998) (Fig. 1). This early development of peptide transport activity in barley grain germination challenges the suggestion that endosperm reserve mobilization is entirely a post-germinative event. Initiation of endosperm storage protein degradation by endopeptidase and carboxypeptidase actions produces a pool of peptides over a period of 1–3 d, which is coincident with visible signs of barley grain germination and development of peptide transporter activity. Although it is plausible that peptides may be hydrolysed to amino acids in the endosperm, peptidase activities are low in the barley endosperm compared to the scutellum, suggesting an important role for peptide transport in nitrogen mobilization. Peptide transport peaks around 24 h after hydration and remains high for the next 4 d during hydrolytic mobilization of endosperm protein. A decline in the size of the peptide pool occurs over a period of 3–6 d. Amino acid transport activity develops later than peptide transport, becoming significant by 2–3 d after hydration, and remaining high for 6 d onwards (Sopanen, 1979a; Walker-Smith and Payne, 1984a, 1985; West *et al*., 1998). The temporal development of peptide uptake varies to some extent between studies, and this could be attributable to differences in the seed lots (i.e. cultivar, age, storage conditions) or germination conditions used.

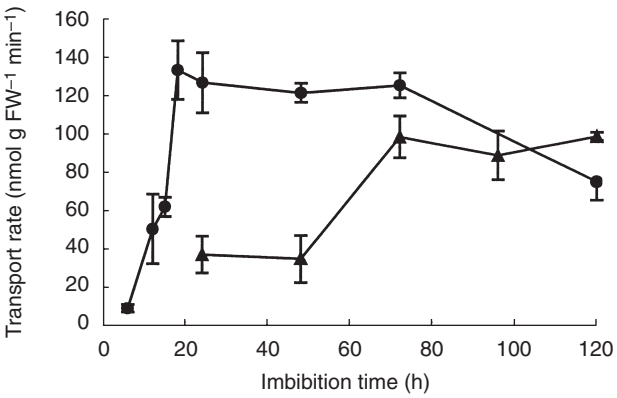

Figure 1. Development of peptide and amino acid transport by isolated scutella during germination. Scutella were isolated from barley grains imbibed for the time indicated, and assayed for either Ala-[^{14}C]Phe (●) or [^{14}C]Ala (▲) uptake. Values are the mean ± SE of 2–4 independent experiments.

During germination, the size of the peptide pool in the cereal endosperm is determined by a balance between three processes: (1) the transport of peptides from endosperm to embryo across the scutellar epithelium; (2) the appearance of peptides in the endosperm arising from endopeptidase activity on storage proteins; and (3) degradation of peptides to amino acids by exopeptidase activities appearing in the endosperm as germination proceeds. The peptide pool in barley endosperm reaches its maximum size before the free amino acid pool, as might be expected if free amino acids are produced from peptides by hydrolysis. Analysis of the amino acid composition of the peptides in the peptide pool indicates that all major classes of endosperm storage proteins are contributing degradation products to this pool (Higgins and Payne, 1981).

Peptide levels, arising from storage protein hydrolysis in the barley endosperm, peak at 2–3 d after the onset of imbibition, with amino acid concentrations reaching a maximum 1–2 d later. At 1 d following imbibition, when the amount of nitrogen available in amino acid and peptide pools is approximately equal in the germinating barley endosperm, the transport of alanine and glycine occurs preferentially in the form of peptides rather than free amino acids (Higgins and Payne, 1981). This argues for an important role for peptide transport in the nitrogen nutrition of the germinating barley grain, especially in the first few days of reserve mobilization.

Calculations of peptide transport rates from endosperm to embryo during the germination of barley grains serve to reinforce the importance of peptide transport in the nitrogen nutrition of the

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heterotrophic cereal embryo. Peptide transport has been estimated to proceed at rates up to 12 μmol peptide grain^{-1} day^{-1} (Higgins and Payne, 1981), which is more than adequate to account for the complete transfer of nitrogen from endosperm to embryo during germination. For rates of amino acid transport across the scutellum during germination and seedling growth, similar calculations show that amino acid transport alone cannot account for the required rates of nitrogen transfer. In practice, both peptide and amino acid transport across the scutellum can occur simultaneously, but peptide transport predominates early in the germination process. Concentrations of peptides in the barley endosperm reach 2–4 mM during germination. Since it is unlikely that the peptide pool is evenly distributed across the endosperm *in vivo*, there is a distinct possibility that even higher concentrations of peptides will be found in the region adjacent to the scutellar epithelium facing the endosperm. The barley scutellar peptide transporter is a low-affinity transporter (see later), and these observations on peptide pool sizes confirm that peptides are present in the endosperm at concentrations adequate for the efficient operation of this low-affinity transporter in organic nitrogen redistribution between endosperm and embryo during germination.

The breakdown of cereal grain storage proteins during germination and the early stages of seedling establishment is effected by the concerted action of endo- and exoproteases. More than 40 different endopeptidase activities, representing all proteinase classes, can be found in germinated barley grains and green malt (Zhang and Jones, 1996). Although the role of these different proteases and their isoenzymes is unclear, their number is a reflection of the complexity of the germination process *per se*. Cysteine endopeptidases are the most abundant proteases in the germinating barley grain (Zhang and Jones, 1995). The endopeptidases are active under the acidic conditions found in the endosperm of the germinating grain and are the rate-limiting enzymes for hydrolysis of the hordein storage proteins. Specific cysteine endopeptidases rapidly cleave hordein proteins to small peptides of fewer than six amino acid residues, which can either be transported across the scutellum or degraded to amino acids by barley exopeptidases. This rapid hydrolysis of hordeins into small peptides accounts for the absence of any build-up of intermediate-size hordein protein peptides during the hydrolysis of endosperm storage proteins (Zhang and Jones, 1996). The major exopeptidases found in cereal seeds are the serine carboxypeptidases (Dal Degan *et al.*, 1994; Ranki *et al.*, 1994), *de novo* synthesis of which accompanies germination, either in the embryo or the aleurone layer from which they are secreted into the endosperm.

Several studies indicate that there are significant differences between peptide hydrolase activities in the endosperm of germinating cereals from phylogenetically unrelated species (Winspear *et al.*, 1984; Saarelainen and Mikola, 1987). At equivalent stages of germination, endopeptidase activity is the major proteolytic activity in the endosperm of a number of temperate-zone cereals originating from Central America or Asia (maize, rice, sorghum), whereas carboxypeptidase activity is the major proteolytic activity in species originating from Asia Minor (wheat, barley, oats, rye). These observations suggest that, at least during the initial germination stages, proportionally less amino acid may be liberated from storage protein digestion in the starchy endosperm of maize and rice than in barley and wheat. Consequently, it is possible that the uptake of peptides could play an even more important role in the mobilization of storage protein digestion products in maize and rice than in wheat or barley. However, this expectation is not supported by measurements of peptide and amino acid transport rates across the scutellum in these different cereals. The rates of peptide transport across the scutellum were similar, at least on a gram-fresh-weight basis, when compared in wheat, barley, rice and maize, although maize scutellar tissue exhibited considerably reduced rates of amino acid uptake compared to the other cereals (Salmenkallio and Sopanen, 1989). The conundrum concerning proteolytic activities in the endosperm of germinating cereals and preferred modes of transport of organic nitrogen across the scutellum still awaits a definitive answer.

The kinetics of peptide transport

The transport of nitrogen in the form of peptides is energetically more favourable than in the form of individual amino acids. The discovery of peptide transport systems in bacteria, yeast and animals was followed by their characterization in plants. In the late 1970s the role and characteristics of peptide transport in the germinating barley grain were established. Active uptake of the hydrolysis-resistant dipeptide glycylsarcosine (GlySar) by the scutellum of the germinating barley grain was demonstrated independently by two research groups (Higgins and Payne, 1977a; Sopanen *et al.*, 1977), establishing that products of storage protein hydrolysis were not only transported in the form of amino acids but also as small peptides. Subsequent studies established the characteristics of peptide transport in the germinating barley grain. Dipeptide transport displays saturation kinetics with a K_m of 2–4 mM (Higgins and Payne, 1977b; Sopanen *et al.*, 1978) and a maximum rate of transport of 41 μmol g^{-1} h^{-1} for GlyGly. The pH

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optimum of peptide transport in the barley scutellum was low, at pH 3.8, reflecting the acidic environment of the cereal endosperm during germination (Higgins and Payne, 1977b).

Peptide uptake is inhibited by anoxia, azide, cyanide and uncouplers of the transmembrane proton motive force, including dinitrophenol (DNP) and carbonyl-*m*-chlorophenylhydrazone (CCCP), suggesting that peptide transport is both active and dependent on a H^+ gradient across the plasma membrane (Higgins and Payne, 1977b; Walker-Smith and Payne, 1984a). Dipeptides and tripeptides, but not glycyl-tetrapeptides, are substrates for peptide transport (Higgins and Payne, 1978a, b, 1980). In these early studies, the amounts of peptides in barley embryo extracts were quantified by chromatography of 5-dimethylaminonaphthalene-1-sulphonyl (dansyl) derivatives against standards. Measurement of peptide uptake by this method is relatively insensitive and requires long incubations of embryos in peptide-containing medium (6 h), during which time hydrolysis of peptide in the medium will complicate analysis of results. In contrast, Sopanen *et al*. (1978) found that tetraglycine competed with [^{14}C]GlySar for uptake by the barley scutellum whereas [^{14}C]GlySarSarSar was ineffective in competition experiments**.** Thus, some tetrapeptides may possibly be transported by the barley scutellum. The reported length of peptides transported by small peptide transporters in other systems varies (reviewed by Meredith and Boyd, 2000). Only di-/tripeptides are directly transported by *Saccharomyces cerevisiae* (Nisbet and Payne, 1979a, b; Parker *et al*., 1980), whereas peptides of 2–4 residues in length are transported in the mammalian intestine (reviewed by Meredith and Boyd, 1995). Small peptides containing 4–5 amino acids are transported by the OPT1 class of peptide transporters recently identified in *Candida albicans*, *Schizosaccharomyces pombe* and *S. cerevisiae* (Saier, 2000). Homologues are present in the *Arabidopsis* genome (Saier, 2000). In *S. cerevisiae* enkephalins are transported by OPT1, but OPT1 is expressed only during sporulation and the G_2/M phase of the cell cycle; tetra- or pentapeptides cannot satisfy auxotrophic requirements under normal growth conditions (Hauser *et al*., 2000).

Peptide uptake is largely specific for transport of peptides containing L-isomers (Higgins and Payne, 1978c). However, DL-stereoisomers are transported, albeit at a reduced rate than the corresponding LL-isomers, only if the D-isomer is located at the N-terminus. However, peptides with a D-isomer at the C-terminus exhibit negligible active transport. Although transported at over 50% the rate of the LL-isomers, the DL-isomers display little competition for AlaAla uptake when present to a ninefold excess, consistent with the presence of multiple peptide transporters with differing substrate specificities in the barley scutellum.

A number of studies have been performed to determine the number of peptide carriers present in the scutellar epithelium, but have reached differing conclusions. Higgins and Payne (1978b) examined the competitive inhibition of GlyIle and di/tri-alanine by various peptides, and concluded that di- and tripeptides are either transported by a common carrier, or by multiple transporters with overlapping specificities. Di- and tri-alanine completely inhibit transport of either GlySar and GlySarSar. The effects of various competitor dipeptides at fixed concentrations on the uptake of GlySar at different concentrations were subsequently investigated, and a mixture of competitive and non-competitive inhibition (a decrease in both K_m and V_{max}) was reported. Sopanen *et al.* (1985a) argued that this type of inhibition is often associated with allosteric (non-competitive) inhibition, possibly resulting from a phenomena known as transinhibition. In this situation, inhibitor peptides bind to sites on the cytoplasmic face of the plasma membrane and reduce uptake of external peptide, even when present at an infinite concentration. Sopanen *et al.* (1985a) suggested that these data argue indirectly for the existence of multiple peptide transport mechanisms within the barley scutellar epithelium.

Peptide transport is thiol-reagent sensitive

Peptide transport in the barley scutellum is sensitive to chemical reagents that modify thiol groups, possibly implying that cysteine residues may be important in substrate binding or the transport mechanism (Walker-Smith and Payne, 1983a, b, 1984b). The thiol reagents *p*-chloromercuribenzenesulphonic acid (*p*CMBS) and *N*-ethylmaleimide (NEM) specifically inhibited peptide transport, and *p*CMBS modification could be prevented in the presence of di- and tri-peptides. NEM is membrane permeant and so may modify transporter thiol groups located on the cytoplasmic membrane face that are not substrate protectable (Waterworth *et al*., 2000a). Phenylarsine oxide (PAO), a reagent which forms stable derivatives only with two thiol groups in close proximity (vicinal dithiols), also inhibits peptide transport (Walker-Smith and Payne, 1983a, b, 1984b).

Affinity-labelling studies of peptide transport proteins in the barley scutellum

A number of affinity-labelling approaches have been used to identify and characterize putative protein components of the peptide uptake system in the

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barley scutellum. Because the inhibition of peptide transport in the barley scutellum by the membrane impermeant thiol-reagent *p*C[^{203}Hg]MBS is substrate-protectable, this reagent can be used to radiolabel specifically peptide-protectable thiols on the outer face of the plasma membrane (Payne and Walker-Smith, 1987). Two substrate-protectable proteins of approximately 42 kDa and 66 kDa were identified when membrane proteins were extracted from *p*C[^{203}Hg]MBS-derivatized tissue and analysed by SDS–PAGE under non-reducing conditions and autoradiography. Comparable proteins were labelled in similar experiments designed to label peptide-protectable vicinal dithiols. The derivatized proteins were only identifiable in scutellum imbibed for 20 h and 3 d but not in tissue imbibed for 5 h, which is consistent with the developmental appearance of peptide transport activity in the barley scutellum during germination (see above).

In a subsequent study, the photoactivatable peptide analogue [U-^{14}C]-alanyl-4-azido-2-nitrophenylalanine was used to affinity label peptide-binding proteins in the barley scutellum (Hardy and Payne, 1991). SDS–PAGE and autoradiography of membrane proteins isolated from derivatized scutellar epithelial tissue showed that two polypeptides, of 42 and 54 kDa, were photoaffinity-labelled in tissue imbibed for 24–30 h, but not in scutella imbibed for 4 h. β-Lactam antibiotics contain a peptide bond and are well-characterized substrates for mammalian peptide transporters. The photoactivatable derivative [*phenyl*-4(n)-^{3}H]benzylpenicillin has been used to derivatize barley scutellar epithelial tissue, and membrane polypeptides of 96 kDa and 63 kDa were radiolabelled after 30 h, but not 4 h (Waterworth *et al*., 1995). Collectively, a number of affinity-labelling studies have therefore identified two putative peptide transporter proteins. Discrepancies in the molecular masses of these candidate peptide transporter proteins could be attributable to differences in size estimation, or that different proteins were derivatized by each affinity-labelling approach. However, it must be emphasized that these affinity-labelling studies only identified peptide-binding proteins, which could also be enzymes, receptors or sensor proteins and not necessarily peptide transporter proteins.

In further studies, barley scutellar membrane proteins of 42 kDa and 66 kDa were also specifically labelled in a strategy designed to label peptide-protectable proteins with [^{14}C]NEM (Waterworth *et al*., 2000a). The subcellular localization of these proteins to the plasma membrane, the anticipated site of peptide transporters involved in nutrient mobilization, was demonstrated by thiol-affinity-labelling of plasma membrane vesicles isolated from barley scutellar tissue. A number of approaches were used to confirm that the 66 kDa protein identified in barley scutellar tissue by thiol-affinity-labelling studies was the barley scutellar peptide transporter HvPTR1. Antiserum raised to the cloned barley scutellar peptide transporter HvPTR1 recognized a scutellar membrane protein of 66 kDa. A protein of comparable size, similar to the predicted molecular mass of HvPTR1, was identified by [^{14}C]NEM labelling studies of *Xenopus laevis* oocytes expressing HvPTR1. The identity of the 42 kDa peptide-binding protein labelled in scutellar tissue remains to be determined; the possibility that it is a second peptide transport protein cannot be excluded, but it could also be a receptor, enzyme or sensor protein.

Cloning and functional expression of the barley scutellar peptide transporter HvPTR1

Although the biochemistry of peptide transport in the barley scutellum has been relatively well characterized, an understanding of this transport system at the molecular level remained elusive until recently. Over the past few years a number of peptide transporters from yeast and animal sources have been cloned and sequenced and, on the basis of sequence identity, designated as the PTR (peptide transporter) family of oligopeptide transporters (Steiner *et al*., 1995), also referred to as the POT family of transporters (Paulsen and Skurray, 1994). A reverse transcriptase–polymerase chain reaction (RT-PCR) approach using degenerate primers, designed to target regions of high sequence identity between PTR family members, enabled the barley scutellar peptide transport gene *HvPTR1* to be cloned (West *et al*., 1998). The predicted protein sequence of *HvPTR1* showed 58% amino acid identity to the *Arabidopsis thaliana* peptide transporter AtPTR2-B (Fig. 2). Hydropathy analysis indicated that HvPTR1 contains 12 putative transmembrane domains, typical of this family of transporter proteins. HvPTR1 was demonstrated to be a functional peptide transporter by heterologous expression in *Xenopus* oocytes (West *et al*., 1998).

Northern analysis showed that *HvPTR1* transcripts were detectable 6–9 h into germination and rapidly increased, to peak at 24 h, after which time mRNA levels remained high for the next few days of seedling growth (West *et al*., 1998). Therefore, *HvPTR1* expression is coincident with the development of peptide uptake activity by the scutellum. The expression of *HvPTR1* was confined to the germinating barley scutellum, although in a later study HvPTR1 was also detected in the developing barley grain (W.M. Waterworth, unpublished results). *HvPTR1* is one of the few genes identified that is induced early in germination. This early expression of *HvPTR1* during germination also challenges the hypothesis that nutrient reserve mobilization is

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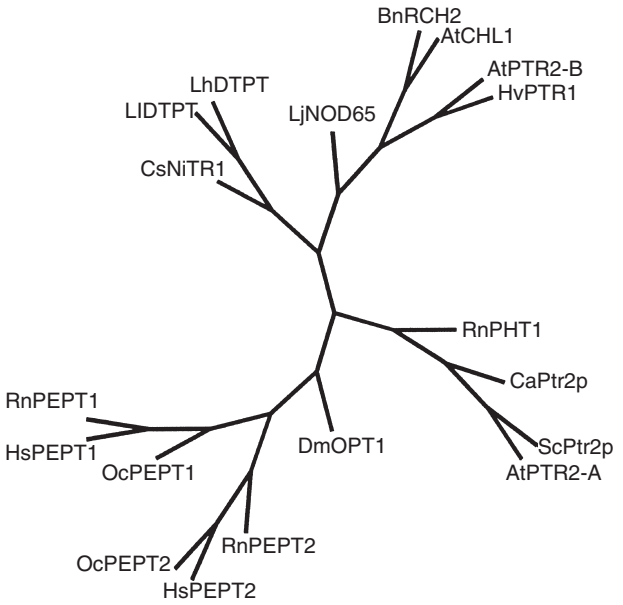

Figure 2. Phylogenetic analysis of PTR and related transport proteins. An alignment of protein sequences with homology to HvPTR1 (ClustalW, Thompson *et al*., 1994) was analysed to determine the probable phylogeny of the proteins by the parsimony method (Phylip). Proteins shown include peptide transporters (PTR) from yeast and plants, peptide transporters from the mammalian intestine (PEPT1) and kidney (PEPT2), a *Drosophila melanogaster* oligopeptide transporter (OPT1), a peptide/histidine transporter from *Rattus norvegicus* brain (PHT1), plant nitrate (chlorate) transporters (CH2, CHL1), chloroplast nitrite transporter (NiTR1), bacterial H^+-coupled di-/tripeptide transporters (DTPT) and a peptide transporter expressed in *Lotus japonicus* root nodules (NOD65).

exclusively a post–germinative event. Promoter analysis of *HvPTR1* is under way in our laboratory and will hopefully identify DNA regulatory elements important in the induction of transcription of *HvPTR1* during early germination. No related transporters have been identified in barley scutellar tissue by RT-PCR using degenerate primers or Southern analysis under high stringency (W. Waterworth, unpublished observations). However, we still cannot exclude the possibility that a distantly related or unrelated transporter protein exists in the barley scutellar epithelial plasma membrane.

Regulation of peptide transport in the germinating barley grain

Peptide transport activity in a viable grain becomes detectable by 6–12 h of imbibition and increases rapidly to a peak activity at 24 h (West *et al*., 1998). The development of peptide transport activity is inhibited by both cycloheximide and cordycepin, inhibitors of protein and mRNA synthesis, respectively, consistent with *de novo* synthesis of peptide transporter proteins and mRNA transcripts during germination (Sopanen, 1979a; Walker-Smith and Payne, 1984a, 1985). However, by 3 d of germination of barley grains when peptide uptake remains high, transport activities become cycloheximide insensitive. Conversely, embryos treated with *p*CMBS at 15 h developed peptide transport activity normally, whereas transport was abolished in 3-d embryos. This suggests that protein components of the peptide transport system exhibit low turnover in the plasma membrane of the scutellar epithelium during the immediate post-germinative phase when seedling growth becomes apparent.

Little is known as yet about the factors that induce expression of the peptide transporter in the scutellum of the germinating barley grain, consistent with our poor understanding of the factors that control gene expression in the embryo during germination (Bewley, 1997). The development of peptide transport in the first 12 h of germination is unaffected by removal of the endosperm from the embryo axis (Sopanen, 1979b), suggesting that the initial development of peptide transport activity in the scutellum is independent of external signals from the endosperm. Additionally, development of peptide transport was insensitive to gibberellin, abscisic acid and an inhibitor of gibberellic acid (GA) biosynthesis, 2-chloroethyltrimethylammonium chloride. An understanding of *HvPTR1* expression may provide insight into the factors that control gene expression in the germinating cereal embryo.

Peptide transport in the barley scutellum is an excellent system with which to study the mechanisms that regulate nutrient transport, because the barley embryo can be isolated easily from the endosperm and peptide transport into the scutellum will remain high for the subsequent 24 h. The effects of endosperm nutrient reserve hydrolysis products on peptide transport have been investigated. In these studies isolated barley embryos were placed scutellum-face-down on to agar containing appropriate additives at concentrations representative of those present in the germinating barley grain (Waterworth *et al*., 2000b). Amino acids at such concentrations (5 mM) inhibited peptide transport by 65% within 4 h. Glucose, the major hydrolytic product of starch degradation in the endosperm and the form in which most carbohydrate reserves are transported across the scutellum, had a stimulatory effect on peptide transport. Recent studies in our laboratory have shown that HvPTR1 mRNA and protein levels are not affected by amino acid concentration, suggesting that regulation of peptide transport activity by metabolites is mediated at the post-

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translational level (W.M. Waterworth, unpublished results). Furthermore, we have shown that the protein phosphatase inhibitor, okadaic acid, inhibits peptide transport and that HvPTR1 is heavily phosphorylated in the presence of amino acids. This is consistent with the decrease in peptide transport activities observed a few days into germination, which also coincides with the increasing availability of amino acids arising from extensive storage protein degradation in the barley endosperm at this time.

Molecular characterization of peptide transporters in *Arabidopsis*

Peptide transporters, members of the PTR or POT family of oligopeptide transporters, have been cloned from *Arabidopsis thaliana* by complementation of a yeast (*S. cerevisiae*) mutant defective in peptide transport with an *Arabidopsis* cDNA library. *AtPTR2-A* was the first peptide transporter reported to be isolated from higher plants (Steiner *et al*., 1994). *AtPTR2-A* mRNA could only be detected in roots by RT–PCR but displayed the highest sequence homology to yeast and fungal peptide transporters. Subsequently, *AtPTR2-A* was reported to be of fungal origin and may represent a fungal contaminant in the original plant sources analysed (Steiner *et al*., 2000).

An *Arabidopsis* peptide transporter was identified independently by two laboratories as the low-affinity *NTR1* histidine transporter (Rentsch *et al*., 1995) and the oligopeptide transporter *AtPTR2-B* (Song *et al*., 1996). *NTR1* expression was high in the developing seed-pod, intermediate in source leaves, with low transcript levels in root, stem, flower and sink leaf (Rentsch *et al*., 1995). *In situ* hybridization showed that *NTR1* expression was highest in the embryo, with lower levels in the seed-coat. *AtPTR2-B* appeared to be expressed constitutively in all plant organs, including germinating seeds, flowers and siliques (Song *et al*., 1996). Differences in seed-pod expression levels could reflect developmental regulation of this peptide transporter during silique maturation. The physiological role of AtPTR2-B was further investigated by the generation of transgenic plants expressing *AtPTR2-B* in sense and antisense orientations (Song *et al*., 1997). Antisense plants showed a 30–80% decrease in mRNA levels in leaves and flowers; these plants flowered later than control plants and showed a reduction in seed number per silique, although individual seed weights increased. Aborted seeds did not develop beyond the heart or torpedo stage. The exact role of AtPTR2-B remains obscure, although it may play a role in seed development. A significant number of sequences encoding genes with substantial sequence homologies to the PTR family of oligopeptide transporters generated by the *Arabidopsis* genome sequencing project are now present in the database. The functions and expression patterns of these 48 putative peptide transporter genes in *Arabidopsis*, now await further analysis.

Peptide transport in other plant systems

There are few well-documented studies of peptide transport in plants, with the exception of the germinating barley grain. Analysis of the recently completed genome sequence indicates that *Arabidopsis* possesses ten times more predicted peptide transporters compared to other organisms sequenced to date, and that these largely display homology to the H^+-dependent PTR family (Kaul *et al*., 2000). This may indicate that peptide transport plays a more central role in higher plant physiology than previously thought. Peptides are often considered to be involved only in short-distance translocation of nitrogen in plants, or protein reserve mobilization during germination and senescence. This may be an erroneous assumption when the paucity of research in this area is considered. Although peptides have not been considered to be involved in long-distance transport of nitrogen, this conclusion may be based on studies in which the method of analysis of assimilates could have caused decomposition of any peptides to constituent amino acids. There is now an increasing awareness of the extent and importance of the transport of macromolecules, including peptides and proteins, between source and sink tissues (Thompson and Schulz, 1999), and a reappraisal of peptide transport in long-distance transport of nitrogen around the plant may now be timely. Peptide transport is both energetically more favourable than that of free amino acids and avoids the competition that may occur between free amino acids (Hardy and Payne, 1992).

Peptide transport also participates in the assimilation of degraded proteins in a number of carnivorous plants (reviewed in Hardy and Payne, 1992). In a recent study, the distribution of transporters involved in absorption of nitrogenous compounds was investigated in the insect-trapping organs of the carnivorous plant *Nepenthes* (Schulze *et al*., 1999). The peptide transporter gene *NaNTR1* was detected in phloem cells of the vascular tissue within pitchers, indicating that NaNTR1 may function in phloem loading of nitrogen exported from the pitcher. Peptide uptake in roots of aquatic plants has also been demonstrated (reviewed in Hardy and Payne, 1992).

H^+-coupled [^{14}C]Gly–Gly transport was detected in leaf mesophyll cells of broad bean (*Vicia faba*) (Jamai *et al*., 1994). This transport system was competitively inhibited by di- and tripeptides but not tetrapeptides,

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displayed a pH optimum of 6.0, and appeared to be of low affinity (K_m of 16 mM). Further studies identified three other systems for dileucine transport: two of high affinity (K_m values of 20 and 350 μM, respectively) and a low-affinity transporter (K_m = 43 mM), which displayed a ten- to 100-fold greater maximal rate of peptide transport, compared to the former two (Jamai *et al*., 1996). All these transporters were H^+-coupled and sensitive to thiol reagents (Jamai *et al*., 1994, 1996), as is the barley scutellar peptide transporter. A low-affinity ATP-dependent peptide transport system with a K_m of approximately 50 mM was also characterized in the tonoplast of barley leaf mesophyll cells (Jamai *et al*., 1995). The physiological role of these transporters has not been ascertained.

A potential role for peptide transport in the uptake of nitrogen from soil, particularly from soils rich in decomposing organic matter, is also worthy of investigation. Peptides have also been implicated as signalling molecules in host–pathogen interactions. Peptide transporters might also facilitate the uptake of peptide-like bacterial toxins (Steiner *et al*., 1995) and facilitate translocation of signalling peptides around the plant. A peptide transporter homologue was expressed in response to adhesion of *Pseudomonas putida* to seeds (Espinosa-Urgel *et al*., 2000).

A role for peptide transport in seed development?

The developing grain is supplied with nutrients from the vegetative tissues of the mother plant. In the absence of a direct vascular connection between maternal and filial (endosperm/embryo) tissue, it is believed that assimilates are delivered to the extracellular space, i.e. the apoplast, dividing these tissues and preceding uptake into the developing grain. Nutrient uptake into developing seeds has previously been reviewed by Wolswinkel (1992) and Weber *et al*. (1998), but the uptake of nitrogen has been poorly characterized compared to that of carbon. Amino acid transport into legume cotyledons is passive during early seed development, but additional active transport coincides with seed storage protein synthesis. The expression patterns of a small number of amino acid permeases (AAPs) have now been examined at the molecular level. However, the possibility that peptide transport may play a role in seed development has been poorly addressed, in contrast to the established importance of peptide transport in cereal grain germination. Evidence for a physiological role for peptide transport in *Arabidopsis* seed development has arisen from studies with transgenic *Arabidopsis* plants expressing AtPTR2-B (Song *et al*., 1996, 1997) (see above). Active peptide transport to the embryo of the developing barley grain has been demonstrated, although not across the transfer region *per se* (Sopanen *et al*., 1985b). Rates of [^{14}C]GlySar uptake increased during the early and middle stages of embryo development (between 20 and 28 d after anthesis) and decreased with grain maturation. Developing barley embryos showed a peptide transport rate of 1.5 μmol g^{-1} h^{-1} for GlySar, with a pH optimum of 4.5. Recent studies demonstrated that the barley peptide transporter HvPTR1 is expressed in the developing barley grain (W.M. Waterworth, unpublished results). HvPTR1 tissue distribution and developmental expression during barley grain filling suggests that HvPTR1 could play an important role in the nitrogen nutrition of the developing grain (W.M. Waterworth, unpublished results).

Biotechnological applications of peptide transport

How important is reserve mobilization for the vigour and viability of seeds? Transporters localized to the scutellum play a crucial role in the delivery of the products of mobilized storage reserves to the germinating embryo, indicative of a vital function in growth and establishment of the young seedling. The levels of peptide transport activity in the scutellum of imbibing barley grains decrease in parallel with loss of viability of a seed lot and are an early and sensitive indicator of the viability of barley grain (Waterworth *et al*., 2000b). This indicates that peptide transport could be a potential marker in germination tests for cereal species, in particular as a possible replacement of the cold test for germination of maize. A greater understanding of peptide transport in cereal grain germination should have implications for seed quality in terms of our ability to control the onset of germination, which encompasses seed vigour, malting and pre-harvest sprouting. Manipulation of peptide transporter levels could result in enhanced reserve mobilization during germination and consequently improved seed vigour and viability. The establishment of a role for peptide transport in grain filling may possibly lead to improvements in grain yield and nutrition.

Summary

The role and importance of peptide transport in cereal grain germination is now well established. Peptide transport in the germinating barley grain has been characterized physiologically, biochemically and, more recently, at the molecular level. However, the involvement of peptide transport in germination of dicotyledonous seeds remains to be established. The early expression of the peptide transporter *HvPTR1* in scutellar tissue suggests a more central role for peptide

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transport in the onset of growth processes during germination than previously considered (West *et al*., 1998). Analysis of nutrient transporters in the scutellum during cereal grain germination is a neglected area of research, particularly at the molecular level. To date, peptide transporters are the most thoroughly characterized examples of these transporter systems and the only type to be specifically characterized at the molecular level. Utilizing the barley scutellar peptide transporter HvPTR1 as a model plasma membrane protein should contribute to our knowledge of structure–function relationships and regulatory mechanisms of plant transporter proteins.

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