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Review

Molecular mechanisms of phosphate and sulphate transport in plants

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Abstract

The application of molecular techniques in recent years has advanced our understanding of phosphate and sulphate transport processes in plants. Genes encoding phosphate and sulphate transporters have been isolated from a number of plant species. The transporters encoded by these genes are related to the major facilitator superfamily of proteins. They are predicted to contain 12 membrane-spanning domains and function as $H^+/H_2PO_4^-$ or H^+/SO_4^{2-} cotransporters. Both high-affinity and low-affinity types have been identified. Most research has concentrated on genes that encode transporters expressed in roots. The expression of many of these genes is transcriptionally regulated by signals that respond to the nutrient status of the plant. Nutrient demand and the availability of precursors needed in the assimilatory pathways also regulate transcription of some of these genes. Information on the cell types in which phosphate and sulphate transporters are expressed is becoming available. These data, together with functional characterisation of the transporters, are enabling the roles of various transporters in the overall phosphate and sulphate nutrition of plants to be defined.

Keywords: Phosphate transport; Sulfate transport; Ion transporter; Mineral nutrition

1. Introduction

The uptake of inorganic nutrients by plants and their transfer between different plant organs and organelles necessitates transport across cell membranes. The most widely studied of these transport processes is the primary uptake process in plant roots by which inorganic ions are transferred from an external solution across the plasmalemma into the cell cytoplasm. The physiology and kinetics of this process has been long known. In classical experiments conducted over 40 years ago, Epstein and colleagues [1,2] demonstrated that the uptake of inorganic ions by plant roots could be described by first-order kinetics in a similar manner to many enzyme reactions. They further demonstrated that, for the major nutrients studied, two phases could be described: a high-affinity system operating at low external nutrient concentrations and a low-affinity system operating at higher external concentrations. These experiments implied that proteins embedded in plant membranes were implicated in these transport processes. However, it has only been in the past five years that some of the specific proteins involved in these ion transport processes in plants have been characterised and the genes encoding these proteins identified. This has been done by the application of molecular techniques to the study of the mechanisms of ion transport.

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Limitations to many agricultural production systems arising from phosphate or sulphate deficiencies ensured that these nutrients were among the first to be studied by the new molecular approach. Plants acquire both of these nutrients as inorganic anions from the rhizosphere surrounding their roots. The concentrations of these ions in the rhizosphere are usually very low (commonly less than 2 μM) whilst concentrations inside the cell are commonly in the millimolar range. This, together with the net negative charge on the inside of the plasmalemma, necessitates that strong electrochemical gradients need to be overcome for successful transfer of these anions into plant cells. Sulphate and phosphate transport systems are therefore providing useful models for anion transport into plant cells. Whilst studies to date have concentrated on transport systems operating in roots, it should not be forgotten that the transfer of phosphate and sulphate across plant membranes occurs in many other plant tissues as well. Notably, readsorption of nutrients delivered to shoots by the vascular system, phloem loading and unloading, transfer to chloroplasts, transfer in and out of vacuoles and transfer to storage and reproductive organs all involve transport across membranes. This paper reviews advances in the physiological understanding of phosphate and sulphate transport processes in plants that have arisen from the adoption of molecular techniques over the past 5 years.

2. Identification of plant sulphate and phosphate transporters

The first genes encoding sulphate transporters from plants were isolated in 1995 [3] using complementation of a yeast mutant in which the gene encoding the SUL1 sulphate transporter had been deleted [4]. This screening procedure identified three cDNA clones encoding sulphate transporters in a tropical legume species *Stylosanthes hamata* [3] and a cDNA clone from barley [5]. Heterologous expression in the SUL1 deficient yeast mutant has shown that two of the clones from *Stylosanthes*, *SHST*1 and *SHST*2 encode high-affinity sulphate transporters with K_ms for sulphate of 10 and 11.2 μM, respectively. The clone isolated from barley, *HVST*1, also encodes a high-affinity sulphate transporter with a K_m for sulphate of 6.8 μM. However, the other clone isolated from *Stylosanthes*, *SHST*3, encodes a transporter with a higher K_m for sulphate of 100 μM. Subsequently, there have been reports of identification of genes encoding similar sulphate transporters from other plant species [6–10]. These include seven different sulphate transporters from *Arabidopsis thaliana*.

Similarities between a partial cDNA sequence in an *Arabidopsis* EST clone and genes encoding phosphate transporters that had been isolated from yeast and fungi led to the cloning of the first reported genes encoding plant phosphate transporters [11,12]. These genes, isolated from *Arabidopsis*, now form part of a growing family of plant phosphate transporters including those isolated from potato [13], tomato [14,15], *Catharanthus* [16], *Medicago* [17], barley [18] and additional isolates from *Arabidopsis* [19]. Eight different phosphate transporters have now been identified in the barley genome [18].

3. Topology of sulphate and phosphate transporters

A characteristic of the deduced sequences of the plant phosphate transporter proteins identified to date is their high degree of similarity. Members of this family of transporter proteins are approximately 58 kDa and 520–550 amino acids in length. Hydrophobicity analyses indicate that they have 12 membrane-spanning domains (MSDs) each usually composed of 17–25 amino acids. These MSDs are arranged in a well defined 6+6 configuration (Fig. 1, upper). Extracellular and intracellular loops of more highly charged hydrophilic amino acids separate the MSDs. Computer analyses predict that the C-terminal, N-terminal and long central loop are oriented towards the inner surface of the membrane. This configuration is shared by a number of other membrane transporters in the major facilitator superfamily of proteins [20,21] including transporters involved in movement of sugars, organic acids, amino acids and inorganic ions across biological membranes. The topology of the eukaryotic phosphate transporters appears to have been well conserved during evolution since there are strong similarities

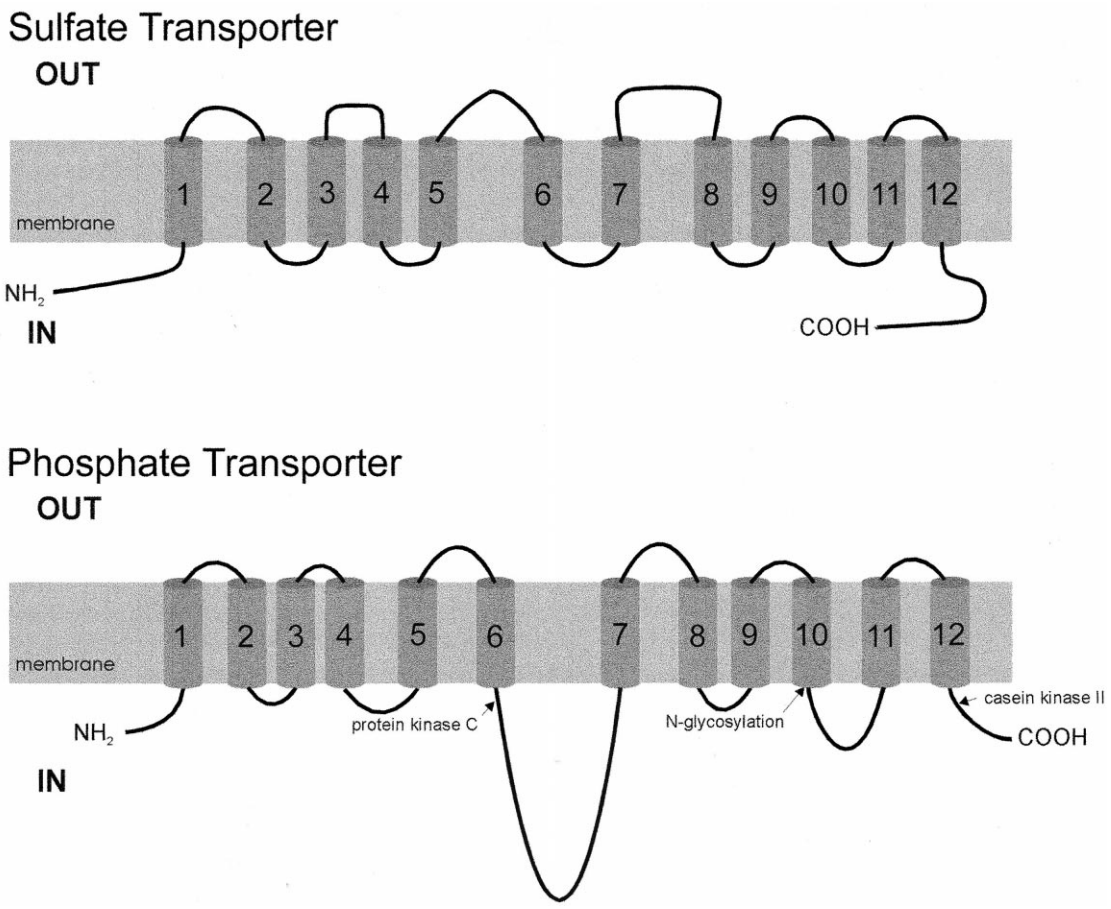

Fig. 1. (Upper) The topology of a plant phosphate transporter. Phosphate transporters typically have 12 membrane-spanning domains arranged in a '6+6' configuration. The long central loop is predicted to be intracellular. The position of potential phosphorylation and glycosylation sites that are conserved on most plant phosphate transporters are indicated. (Lower) The topology of plant sulphate transporters. Sulphate transporters are also predicted to contain 12 membrane-spanning domains. However, these lack the long intracellular loop found in many members of the major facilitator superfamily of transporter proteins.

between fungal, yeast, plant and some mammalian phosphate transporters [12].

Plant sulphate transporter proteins are larger than phosphate transporters. Those identified to date are 69 to 75 kDa and range from 635 to 685 amino acids in length. They are also predicted to contain 12 MSDs with the C-terminal and N-terminal towards the inside of the membrane (Fig. 1, lower). However, the sulphate transporters lack the long inner central loop of the phosphate transporters. Again this topology of sulphate transporter proteins has been well conserved during evolution. There are strong similarities in topology and sequence between eukaryotic sulphate transporters including those from filamentous fungi, yeast, plants, mammals and humans [3].

Regrettably, there is little detailed information available at present on the structure/function relationships of plant sulphate and phosphate transporters. Obvious questions that require researching relate to the structural characteristics of these transporters that define their specificity for a particular ion and their affinity for the transport of that ion. Amino acid residues in the hydrophylic loops between the MSDs and residues within the MSDs on the inner surface of the central pore of the transporter are likely to influence these properties. The observation that sulphate transport is sensitive to the membrane impermeable arginine antagonist hydroxyphenylglyoxal [22] suggests that one or more of the exposed arginine residues in plant sulphate transporters may be specifically involved in sulphate binding or transport. The availability of clones of phosphate and

sulphate transporters should now enable such structure/function relationships to be examined. This could be done through use of site directed mutagenesis to alter potentially important domains and using heterologous expression systems to determine the effects of these alterations on transport. Plant sulphate transporters present a useful model for such studies because they can be reliably expressed in yeast thus providing a relatively simple system for measuring sulphate transport rates.

4. Functional analysis of phosphate and sulphate transporters

The function of a number of the isolated plant sulphate transporters has been verified by heterologous expression of full-length cDNA clones in mutants of the yeast *Saccharomyces cerevisiae*. Two mutants have proven useful for this purpose. In mutant YSD1 the gene encoding the high-affinity sulphate transporter SUL1 has been deleted [4]. A more recent mutant CP154-7A has disruptions to the genes encoding both the SUL1 and SUL2 yeast sulphate transporters [23]. Complementation of these mutations by expressed plant cDNAs results in restoration of sulphate uptake by these mutants. Such studies have enabled functional sub-groups comprising high-affinity or lower-affinity sulphate transporters to be differentiated. Kinetic studies with this expression system have shown that the SHST1 and SHST2 transporters from *Stylosanthes* and the HVST1 transporter from barley are representative of a sub-group of high-affinity sulphate transporters whilst the SHST3 clone from *Stylosanthes* is representative of a sub-group of lower-affinity sulphate transporters [3,5].

A yeast mutant defective in the yeast PHO84 phosphate transporter has been available for many years [24]. However, the value of this mutant to demonstrate the function of putative plant phosphate transporters by complementation with plant cDNAs has been disappointing. Where heterologous expression in this mutant has been successful, measured K_m values have generally been much higher than expected [13,17]. This has not permitted reliable kinetic studies to be performed. As a result, there is less known about the affinity sub-groupings of plant phosphate transporters than there is about the sulphate transporters. The recent availability of a yeast double mutant, PM971, in which the genes encoding both the PHO84 phosphate transporter and a Na^+-coupled PHO89 phosphate transporter have been disrupted [25] may improve the functional analysis of plant phosphate transporters. Using this mutant an apparent K_m for phosphate of 31 μM was measured for the tomato LePT1 phosphate transporter [14]. Whilst this may still be higher than expected for a high-affinity phosphate transporter, it is an order of magnitude lower than some of the measurements on plant phosphate transporters that have been made with the single PHO84 mutant. The most reliable functional analysis of a plant phosphate transporter has been obtained by expressing the cDNA in cultured tobacco cells. The PHT1 phosphate transporter from *Arabidopsis* was proven to be a high-affinity phosphate transporter with an apparent K_m for phosphate of 3.1 μM using this expression system [26]. Some of the phosphate transporters that have been isolated from *Arabidopsis* [12] and barley [18] have almost identical sequences and expression patterns suggesting they have similar functions and roles. It appears likely that, at least in some diploid plant genomes, redundancy of genes encoding phosphate transporters critical to plant survival has developed.

Where complementation of yeast mutants with plant cDNAs has yielded reliable sulphate or phosphate uptake data, uptake rates have been shown to be sensitive to the extracellular pH. Sulphate uptake rates by the *Stylosanthes* SHST1, SHST2 and SHST3 transporters and the barley HVST1 transporter declined as the external pH increased from 5.5 to 7.5 [3,5]. Similarly, phosphate uptake rates by the potato StPT1 and StPT2 phosphate transporters were reduced when the pH was increased over the range 4.5–7.5 [13] and uptake by the tomato LePT1 phosphate transporter was dependent upon the proton gradient across the plasma membrane [14]. Physiological measurements indicate that during phosphate uptake the cytoplasmic pH decreases [27,28] and that agents that collapse the membrane potential inhibit phosphate uptake. These data provide evidence that these transporters function as proton cotransporters and their transport function relies upon a H^+ pump to maintain a proton gradient across the membrane.

5. Regulation of plant phosphate and sulphate transporters

The concentration of inorganic nutrients within plant tissues is maintained within limits that establish ion homeostasis [29,30]. This requires close regulation of the influx and efflux processes by which ions move across plant membranes. To date, little is known about the molecular mechanisms of sulphate and phosphate efflux. However, the availability of clones of genes encoding sulphate and phosphate transporters for use as probes has established that transcriptional control is an important regulatory mechanism for at least some of the genes involved in sulphate and phosphate influx. Smith et al. [3,5] showed that expression of the high-affinity SHST1, SHST2 and HVST1 sulphate transporters was controlled by the sulphur status of the plants. Restriction of the external sulphate supply resulted in a rapid increase in the steady state mRNA levels corresponding to these sulphate transporters and increases in the capacity of these plants to take up sulphate. At the same time, there were marked decreases in the internal concentrations of sulphate and soluble reduced sulphur compounds such as cysteine and glutathione [31]. Upon resupplying sulphate to such plants mRNA levels corresponding to the sulphate transporters decreased rapidly, sulphate uptake rates declined and the levels of sulphate and reduced sulphur compounds increased. Transcription of the genes encoding the sulphate transporters is therefore closely controlled by a negative feedback loop. Studies with split-root experiments have suggested that phloem-translocated glutathione plays an integral role in this negative feedback regulation [32].

Studies on the dynamics of appearance and disappearance of steady state sulphate transporter mRNA levels upon altering the nutritional status of the plants indicate that mRNA turns over rapidly in roots [5]. This is mirrored by measurements of sulphate uptake rates indicating that the functional sulphate transporter proteins also turn over rapidly. Turnover of the barley sulphate transporter in approximately 2.5 h has been suggested [33] whilst a shorter half-life of less than 2 h has been suggested for the mRNA [5]. Western blot analyses indicate that phosphate transporters also turn over rapidly [34]. This rapid turnover would enable fast modulation of transport rates by direct transcriptional control.

Since sulphate transport is the first step in the acquisition and assimilation of sulphur the flux of sulphur through the assimilatory pathway is likely to be linked to regulation of sulphate transporters. Transcriptional regulation of a number of the genes encoding enzymes of the sulphur assimilatory pathway in response to the plant sulphur status has been observed [9]. A model has been proposed [35] linking regulation of the assimilatory pathway and sulphate transport. This model embraces the negative transcriptional control of sulphate transporters outlined above. It also includes a positive transcriptional control loop. This loop was demonstrated in experiments in which barley plants, well supplied with external sulphate, were fed *O*-acetylserine, a precursor in the synthesis of cysteine [5,31]. De-repression of the sulphate transporter occurred and there was a rapid increase in *HVST*1 mRNA, sulphate uptake rates and cysteine and glutathione levels in the tissues. Transcription activated by *O*-acetylserine partially over-rides the negative feedback associated with an adequate external sulphate supply and increases flux of sulphur through the assimilatory pathway. Transcriptional regulation of the genes encoding high-affinity sulphate transporters is thus linked to the availability of sulphate, the demand for reduced sulphur compounds and the supply of a C/N skeleton precursor needed in the assimilatory pathway. In this way the number of available transporters in the membrane is regulated. Further fine tuning of the transport process may be exercised by post-transcriptional modifications of the transporter proteins and by allosteric regulation of the activity of transporters.

A different pattern of regulation was observed for the lower-affinity SHST3 transporter from *Stylosanthes* [3] and the AST68 homologue from *Arabidopsis* [9]. These transporters are expressed in both roots and shoots. In situ hybridisation studies with *AST*68 indicate that this gene is expressed in root tips and in cells of the central vascular cylinder in both roots and leaves. No expression was detected in the xylem, endodermis, cortex or epidermal cells. Expression in roots is transcriptionally regulated by the sulphur status of the plant in a similar manner to the genes encoding the high-affinity SHST1, SHST2 and

HVST1 transporters, although there appear to be fewer SHST3 transcripts than for the high-affinity transporters [3]. Up to 9-fold increases in abundance of *AST*68 mRNA were noted in *Arabidopsis* roots following sulphate starvation [9]. Expression of these genes in shoots, however, does not appear to be up-regulated by sulphate deprivation. In fact, studies with the *SHST*3 gene suggest, at least in the initial stages of sulphate deprivation, its expression may be down-regulated in leaf tissues [3]. Expression of *AST*56, an isoform of *AST*68 that is also likely to be a low-affinity sulphate transporter from *Arabidopsis*, is reported to be enhanced 1.5- to 2-fold in roots by sulphur deprivation, but not in leaves [9].

Similar transcriptional regulation of phosphate transporters by negative feedback has been reported. Genes encoding the APT1 and APT2 phosphate transporters of *Arabidopsis* are only expressed in roots and their expression is considerably enhanced by phosphate deprivation [12]. Root phosphate transporters isolated from potato [13], tomato [14,15], *Medicago* [17], and barley [18] are similarly regulated. These data have been summarised in a recent review by Mimura [29]. Localisation experiments with the tomato *LePT*1 phosphate transporter [14] revealed that, although mRNA for this transporter could be detected in all vegetative organs of tomato seedlings, it was primarily expressed in the peripheral cell layers of the root including the root cap, root hairs, epidermis, and outer layers of the cortex. When plants were deprived of phosphate, *LePT*1 was also expressed in some cells in the stelar region. Using a specific antibody to LePT1 it has been shown that LePT1 protein levels also increase in response to phosphate deprivation [34]. Further, enriched levels of LePT1 protein were associated with plasma membrane preparations from phosphate starved roots in these studies. These data indicate that the enhanced levels of mRNA that result from phosphate deprivation are translated into LePT1 protein and this protein is targeted to the plasma membrane. Thus there is an increase in the total number of phosphate transporters in the plasma membranes of root cells in close proximity to the soil solution. This gives rise to the increased capacity of phosphate starved plants to take up phosphate [36–39].

Post-translational modifications of plant phosphate transporters present another potential means of regulation. Interestingly, most of these transporters possess highly conserved sites that could be phosphorylated or glycosylated [11–13]. Biochemical studies targeting these sites are under way in some laboratories. Interaction with other proteins presents another avenue for regulating the activity of plant phosphate transporters. It is now clear that interactions between a number of proteins are involved in regulating the transport of phosphate into yeast cells [40–43]. Plant homologues of the yeast genes encoding these interacting proteins have not yet been identified. It has been suggested that poor complementation of the yeast PHO84 phosphate transporter mutant by plant phosphate transporters may be due to these interacting proteins [44].

Physiological data suggest that feedback regulation of sulphate and phosphate uptake by plant roots is a systemic effect rather than a localised effect. Split root studies indicate that uptake rates by a phosphate or sulphate deprived root respond to the overall phosphate or sulphate status of the plant [45,46]. This is in contrast to regulation of the *ANR*1 gene that encodes a transcription factor involved in root development [47]. High nitrate levels adjacent to a section of the root result in localised expression of this gene in that section of the root system. Molecular studies indicating that transcriptional regulation of genes encoding sulphate and phosphate transporters primarily respond to the sulphate or phosphate status of the whole plant [15,32] are now confirming the physiological evidence. This is important since it implies that signals responsible for transcriptional regulation are likely to be delivered via some inter-organ transport system. Experiments on the role of phloem-translocated glutathione in the regulation of the AST68 root sulphate transporter and ATP sulphurylase, an enzyme of the sulphur assimilatory pathway, provide evidence for such signalling [32]. A further interesting observation is the failure of root specific sulphate and phosphate transporters to be up-regulated during some dual nutrient deficiencies [18]. It seems likely that the transport capacity of the roots may remain down-regulated if growth is limited by some second nutrient deficiency.

6. Roles of different phosphate and sulphate transporters

Sufficient data on functional characteristics such as affinity type and localisation of expression are becoming available to permit some of the root phosphate and sulphate transporters to be delegated probable roles. Using sequence comparisons to construct a phylogenetic tree, together with the available information on the sites of expression of plant sulphate transporters, Hawkesford and colleagues [33] have classified these proteins into their sub-classes. This classification is particularly valuable for assigning tentative roles to some of these transporters. A group represented by SHST1, SHST2, HVST1, a transporter TTST1 isolated from wheat (Prosser and Hawkesford, unpublished) and AST101 from *Arabidopsis* contains the high-affinity sulphate transporters that are only expressed in roots. Localisation studies with the *SHST*1 and *HVST*1 clones suggest that these transporters are expressed in the root tip and in the epidermal and outer cortical cells of the root (Rae et al., unpublished). Similar data have been obtained for the high-affinity LePT1 phosphate transporter from tomato [14]. In situ hybridisation studies indicate that this transporter is also mainly expressed in root hairs, epidermal cells, outer layers of the cortex and the root cap. These plant tissues are in contact with the soil solution and are known to be active in ion uptake. Further, the low concentrations of phosphate and sulphate in most soil solutions requires involvement of high-affinity transporters for uptake of these ions. It is therefore very likely that high-affinity phosphate transporters of the LePT1 and PHT1 [26] types and high-affinity sulphate transporters in the group represented by the SHST1 and HVST1 transporters mediate the initial uptake of these ions into roots from the soil solution. Once inside the symplast, these nutrients can move radially towards the stelar region of the root through the suberised endodermis without encountering further membrane barriers [51].

A second group of sulphate transporters is represented by AST68 [9] and SHST3 [3]. These are lower-affinity transporters (SHST3 has K_m for sulphate of 100 μM) expressed in both roots and shoots. In situ hybridisation studies with *AST*68 indicate that this gene is expressed in vascular tissues of both the root and shoot [9]. Expression in roots occurs in the tips and in most of the cell types within the central stelar region, with the exception of the xylem. No expression has been observed in the endodermis, the cortex or the epidermis. To date, no low-affinity sulphate transporters of this group have been identified in the monocot cereal species in spite of specific searches for them in barley, rice and wheat. However, in situ hybridisation studies using the high-affinity *HVST*1 sulphate transporter to probe sections of barley roots have suggested that this gene is also expressed within the stelar region (Rae, unpublished data). Positive signals have been obtained in cells of the endodermis, the pericycle and xylem parenchyma surrounding the xylem vessels. This pattern of expression of the high-affinity *HVST*1 sulphate transporter in the stele of barley roots is similar to that of the lower-affinity *AST*68 transporter in the stele of radish roots and suggests they may be fulfilling a similar role. Another piece of evidence for expression of a high-affinity transporter in the stelar region can be found in the in situ hybridisation micrographs showing expression of the gene encoding the LePT1 phosphate transporter in tomato roots [14]. Here it was noted that, when induced by phosphate deprivation, *LePT*1 was expressed in the young stelar tissues. These findings raise the question of the role of these transporters within the stele. Unloading of the millimolar concentrations of nutrients within cells into the xylem only requires a low-affinity transport system and could involve these transporters. However unloading is an efflux process and presumably only involves the xylem parenchyma cells adjacent to the xylem vessels. Takahashi et al. [9] have suggested that AST68 may play a role in the symplastic movement of sulphate from the endodermis towards the xylem. An alternative explanation is that these transporters that are expressed throughout the stelar region provide a scavenging mechanism for recovering nutrients leaked from the vascular tissues. Such a process would involve influx and, at the concentrations that occur in vascular tissues, could be achieved by either high-affinity or low-affinity transporters. Further, it might be expected that such a mechanism may be particularly important in low nutrient status plants and thus be up-regulated under conditions of low external nutrient supply.

Regrettably, there are insufficient details available

on the localisation of expression of specific shoot phosphate and sulphate transporters to enable roles to be delineated. We might expect these to be low-affinity types involved in such processes as the loading of leaf cells, phloem loading and transfer of nutrients to meristems and storage tissues. Similarly, transporters involved in the efflux of sulphate or phosphate from plant cells have not yet been identified. It is possible that some of the same transporter molecules used for influx may be used for efflux, but it is more likely that efflux occurs through low-affinity channels or transporters. Other transporters are likely to play a role in the transfer of nutrients to organelles such as chloroplasts. Several phosphate transporters belonging to a different family to the H^+/phosphate cotransporters and containing six MSDs have been cloned from plastids [48–50]. It is also likely that additional sulphate and phosphate transporters similar to the Na^+/sulphate and Na^+/phosphate cotransporters found in some fungi, yeasts and vertebrates may play a role in at least some plant species. Another very important process for which appropriate transporters or channels have not yet been identified is the movement of sulphate and phosphate into and out of vacuoles through the tonoplast membrane.

7. Phosphate transport and mycorrhiza–plant interactions

Vesicular–arbuscular mycorrhizal fungi form symbiotic associations with the root systems of many plants and play a very important role in the transfer of phosphate to the root systems. These fungi colonise cortical cells where they form arbuscules and extend a network of hyphae from these out into the surrounding soil. The hyphae can gather nutrients from the soil solution and transfer them back to the host plant thereby expanding the effective volume of soil that the plant can exploit. Acquisition of phosphate through mycorrhizal associations involves transport across membranes during uptake of phosphate by the fungal hyphae, unloading that phosphate from the fungal arbuscules at the arbuscule–cortical cell interface and uptake of that phosphate by the cortical cells. A high-affinity phosphate transporter expressed in the hyphae has been isolated from the mycorrhizal fungus *Glomus versiforme* [52]. This transporter is structurally very similar to the yeast PHO84 transporter and to the plant phosphate transporters. It complements the gene encoding the yeast PHO84 transporter and has similar characteristics to it. It is most likely to be a high-affinity phosphate transporter involved in the initial uptake of phosphate from the soil solution into the hyphae. The other fungal transporter involved in unloading phosphate from the fungus at the arbuscule–cortical cell interface has not yet been identified. Similarly, plant transporters responsible for uptake from this interface into the cortical cells have not been specifically identified. Depending upon the concentration of phosphate at the interface, these could be low-affinity types. They could also be among the root phosphate transporters already isolated, but regulated in such a way as to enable the plant to exploit the mycorrhizal association.

Two genes encoding phosphate transporters have been isolated from the roots of *Medicago truncatula* [17]. The expression of these genes is up-regulated during phosphate deprivation in a similar manner to the other root specific phosphate transporters discussed earlier. However, following mycorrhizal infection of *M. truncatula*, the expression of these phosphate transporters and other phosphate starvation inducible genes is down-regulated. Data suggest that this down-regulation is in response to growth of the fungus and occurs prior to significant transport of phosphate [52,53]. This implies that down-regulation of the plant phosphate transporters may be in response to another signalling pathway initiated specifically by mycorrhizal infection. If this does prove to be a general phenomenon, it raises important questions about the role of plant phosphate transporters in the roots of mycorrhizal plants and their contribution to the overall phosphate nutrition of the plant.

8. Conclusion

Application of molecular techniques to the nutritional physiology of plants over the past 5 years has resulted in rapid advances in our understanding of the mechanisms of phosphate and sulphate transport in plants and their regulation. However, many ques-

tions remain to be adequately addressed. Much of the work to date has concentrated on transport in plant roots. Whilst this is obviously of great importance, throughout this review we have drawn attention to many of the other transport systems in plants about which relatively little is known at present. Many of these are likely to be involved in the internal cycling of phosphate and sulphate throughout the plant and may therefore be important not only to improving the efficiency of nutrient use, but also to the quality of plant products. They are therefore worthy of study both academically and for their potential importance to agriculture and the environment.

Attempts at manipulating the expression of sulphate and phosphate transporters are being made in various centres. By putting the expression of phosphate and sulphate transporters under control of different promoters, the feedback regulation that attenuates expression of some of these genes could be overridden. This type of intervention may offer new approaches to improving the use of nutrients by commercial crop plants, reducing nutrient loads in the environment and improving the quality of plant products. However, a great deal of caution is required. For example, overexpression of a high-affinity phosphate transporter in plant roots could lead to the accumulation of toxic levels of phosphate under many circumstances. Excess sulphate is less likely to be a problem but it should be kept in mind that plants have evolved regulatory controls to maintain the nutrient content of their tissues within biologically defined limits. Attempts to exceed those limits by increasing the expression of genes encoding phosphate and sulphate transporters are unlikely to be successful. It will be interesting to determine whether this technology can define how flexible these limits may be and what the effects on levels of other nutrients in plant tissues will be. The likely implications of these types of molecular interventions require better understanding of the coordinate regulation of nutrient transport processes and their linkages to plant growth and development.

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