View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by RERO DOC Digital Library

Journal of Plant Biology, July 2008, 51(4) : 240-247

REVIEW ARTICLE

Long-distance Transporters of Inorganic Nutrients in Plants

Jiyoung Park[1], Yu-Young Kim[1], Enrico Martinoia[1,2], and Youngsook Lee[1,*]
[1]POSTECH-UZH Global Research Laboratory, Division of Molecular Life Sciences, POSTECH, Pohang, 790-784, Korea
[2]Institut für Pflanzenbiologie, Universität Zürich, Zollikerstrasse 107, 8008 Zürich, Switzerland

In plants, long-distance transport of inorganic nutrients is important for mineral nutrition, ion homeostasis, nutrient recycling, and the detoxification of toxic or excess inorganic ions. Here, we review information on the transporters involved in the loading/unloading of inorganic nutrients to and from the vascular bundle. We also describe the methods used to obtain such information.

Keywords: inorganic nutrients, long-distance transport, mineral nutrition, plant transporters, root-to-shoot translocation, vascular bundle

Plants deliver minerals absorbed from the root to the shoot, and also re-mobilize them from senescing leaves to young developing tissues using long-distance transport systems. Efficient translocation is important for normal plant development, especially when the supply of mineral nutrients from the environment is scarce. Study of their translocation is necessary to understand how plants efficiently use inorganic nutrients, which are a limited resource. Such information about transporters enables researchers to produce nutritionally improved crops (Grusak et al., 2002; Jean et al., 2005; Miwa et al., 2006). In addition, knowledge of mineral nutrient translocation can be useful when plants are genetically engineered for the phytoremediation of toxic heavy metals, for crops containing reduced heavy metal contaminations, and for crops that are resistant to harsh conditions such as excess salt (Shi et al., 2003).

To fully understand how different plant organs are connected in terms of mineral transport, one must first identify the transporters. Transporters of different inorganic ions are likely to vary in their modes of action, depending on the chemical nature of the substrate. For example, some ions require chelators in order to be transported over a long distance, while others move as free ions. However, through comprehensive reviews of the many genes involved in such transport, some common features have emerged, which will help us learn how metal ions are generally transported throughout the whole plant and how we can find new transporters with similar functions. Here we review transporters that are involved in long-distance transport of inorganic nutrients. We also summarize the experimental methods used to obtain results that have led to such informative conclusions.

METHODS USED FOR TRANSLOCATION STUDIES

Experiments on transporters use single-cell systems and whole plants. Although both have advantages and disadvantages, they can complement each other. The former includes plant protoplasts, plant suspension-culture cells, and heterologous systems, e.g., budding yeast, *E. coli*, or *Xenopus* oocytes. Heterologous expression systems are useful for the identification of substrates and biochemical properties of those transporters. Mutant yeasts and *E. coli* strains provide valuable expression systems into which a plant gene can be introduced for complementation tests (Figure 1F). The most commonly used mutant strains are *zrc1/cot1* yeast to test Zn(II), Co(II) transport; *crt1* yeast to test Cu(II) transport; *ycf1* yeast to test Cd(II), Pb(II), and As(V) transport; and *zntA E. coli* to test Zn(II), Pb(II), and Cd(II) transport (Kamizono et al., 1989; Conklin et al., 1992; Dancis et al., 1994; Szczypka et al., 1994; Rensing et al., 1997; Song et al., 2003). *Xenopus* oocytes express only a limited number of transporter proteins compared with plant cells, which express many different transporters constitutively. Thus, those oocytes maintain a quiet background above which small currents or a small flux of isotope ions, through a heterologously expressed transporter protein, can be detected (Gaymard et al., 1998).

Using single-cell systems such as protoplasts or suspension-cultured cells, scientists often show the localization of a transporter at the plasma membrane as supporting evidence for its involvement in translocation (Fig. 1B). Plasma membrane localization is important because it is a starting point from which the transporter moves substrates among cells and, eventually, from one tissue to others. Fluorescent proteins, such as Green Fluorescence Protein (GFP), or other tag proteins, are fused to the transporter. These are then expressed transiently in plant cells by such methods as PEG-mediated transformation or particle bombardment. Localization of the fusion protein can be either observed directly or visualized via antibodies tagged with a fluorescent dye. This procedure is simple and often provides clear results. However, there are caveats: GFP can interfere with the transport function, mask the signal peptides, or be cleaved off in the cell. To show that the GFP-fused protein is targeted to the functional site and that GFP does not interfere with the function of the transporter, scientists perform functional complementation with the GFP-fused transporter in a heterologous system or in a knockout mutant plant. To show that the fluorescence observed through the fluorescence microscope is from the whole fused protein and not from a cleaved fragment containing GFP, Western blot analysis with GFP antibodies is performed. The molecular weight of the protein band that crossreacts with GFP antibody should correspond with that

*Corresponding author; fax +82-54-279-2199
e-mail ylee@postech.ac.kr

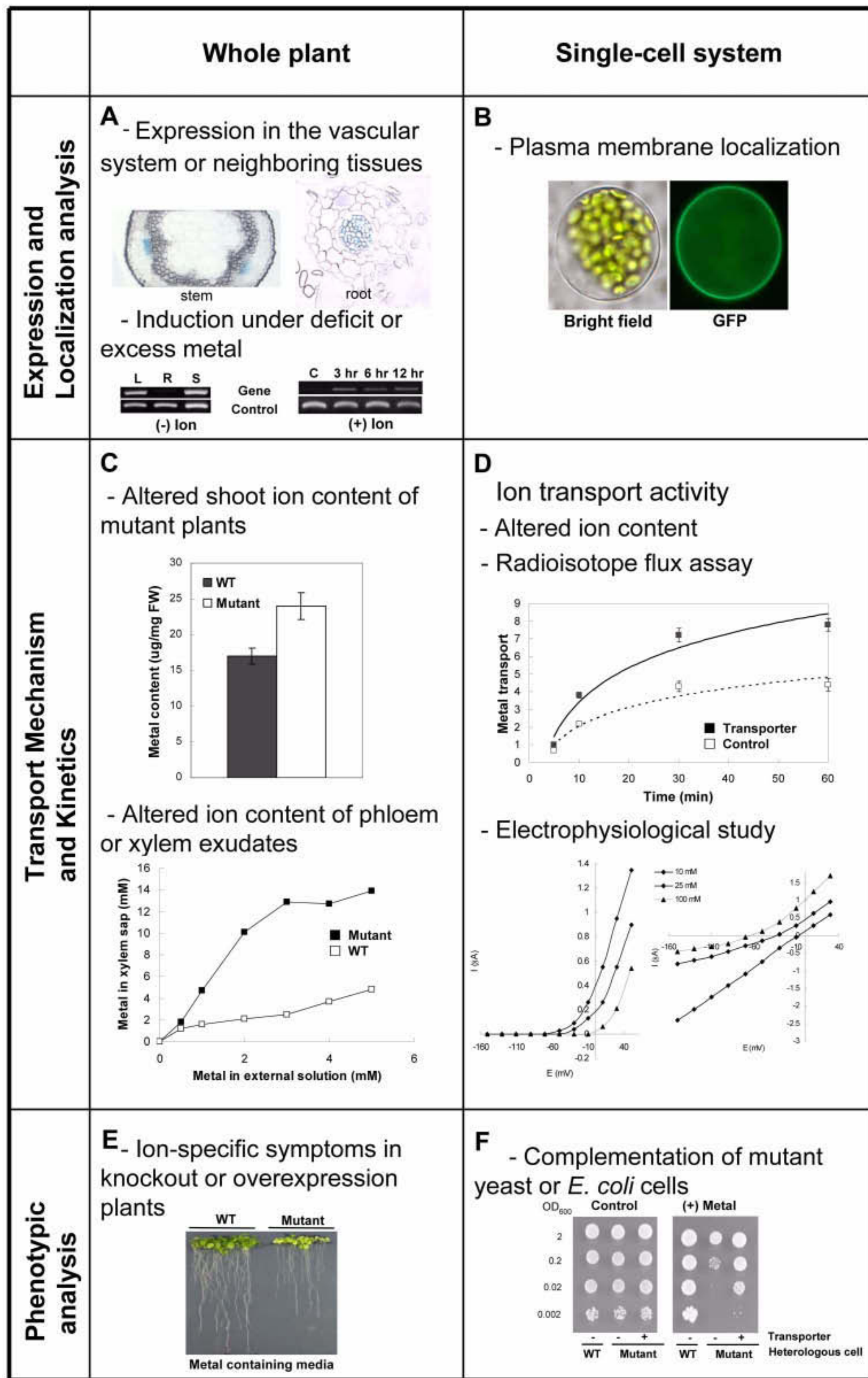

Figure 1. Criteria used to define a role of a transporter in long-distance transport of mineral nutrients.

predicted for the GFP-fused transporter protein.

The single-cell system is especially useful for flux assays (Fig. 1D). It allows one to control the composition of the external media, e.g., pH, ion concentrations, and the concentration of substrates of interest. One can also analyze the effect of inhibitors or activators in order to characterize the transport mechanisms. Radioisotope-labeled substrates can be used to identify substrates and transport kinetics. When the transport results in charge redistribution across the cell membrane, electrophysiological analyses can be used to

identify the substrate specificity and kinetics of the transporter. Among the many electrophysiological methods, the patch-clamp technique is the most rigorous for identifying a transport mechanism because it allows one to control not only the external medium but also the internal medium composition and membrane potential.

Although single-cell systems can reveal much information, they cannot provide evidence for a role of the transporter in long-distance transport. Plants mutated in expression of transporters are necessary if one is to demonstrate root-to-shoot translocation. Transgenic plants expressing the GUS protein under control of innate promoter are used to detect tissue-specific expression. Localization of the transporter in the vascular system or neighboring tissues can support its functioning in ion translocation (Fig. 1A). Knock-out or over-expressing transgenic plants are utilized for detecting altered phenotypes and ion contents, for which individual organs can be separately collected and analyzed. Even when the total ion content of transgenic plants does not differ from that of the wild type, root-to-shoot ratios can differ. Such alterations in transgenic plants provide strong evidence for a role of the protein in long-distance translocation (Fig. 1C). Analyses of the ionic contents of the xylem or phloem sap of the mutant and wild-type plants can also help identify transporters involved in long-distance transport. Advanced techniques that rely upon high-energy X-ray sources now enable researchers to detect ion levels with high resolution and/or *in vivo* (Takahashi et al., 2003; Kim et al., 2006). In many studies, mutants show distinct phenotypes (Fig. 1E) that indicate changes in shoot mineral content. For example, zinc-deficient symptoms are found from *hma2 hma4* double knockout plants that exhibit uneven chlorosis in leaves of reduced size, shorter internodes, and infertility (Hussian et al., 2004). This phenotypic analysis demonstrates the importance of long-distance transport of inorganic nutrients in homeostasis of the ion within the whole plant. Thus, the use of mutants has allowed us much progress in our understanding of long-distance transport of mineral nutrients. As more mutant pools with improved features become available, many more gene products will be found to be involved in this important aspect of transport in plants.

TRANSPORTERS INVOLVED IN LONG-DISTANCE TRANSPORT

Metal transporters involved in the long-distance delivery

Table I. Characteristics of transporters implicated in the long-distance transport of ions in plants.

Transporter	Substrate	Single-cell study		Transgenic plant study	
		Yeast and/or *E. coli*	*Xenopus* oocytes (Electrophysiological assay)	Loss-of-function	Gain-of-function
Xylem-loading transporter					
AtSKOR	K^+		K^+-selective outward current	$\downarrow[K^+]$ in shoot	
AtHMA4	Zn(II),Cd(II)	Zn(II) and Cd(II) efflux activity		$\downarrow[Zn^{2+}]$ and $[Cd^{2+}]$ in shoot, Zn(II)-deficient symptoms	$\uparrow[Zn^{2+}]$ and $[Cd^{2+}]$ in shoot
AtBOR1	B	$\downarrow$[B]		$\downarrow$[B] in xylem exudate, reduced growth and sterility	$\uparrow$[B] in shoot, Increased growth under B-deficiency
AtPHO1	Pi(V), As(V)			$\downarrow$[Pi] in shoot, reduced growth	
AtSOS1	Na^+	$\downarrow[Na^+]$, Na^+ efflux activity		$\downarrow[Na^+]$ in shoot (moderate salt) $\uparrow[Na^+]$ in xylem sap and plant (high salt)	
OsLsi1	Si		Si uptake activity	$\downarrow$[Si] in shoot and xylem sap, decreased growth and seed production	
OsLsi2	Si		Si efflux activity	$\downarrow$[Si] in plant, decreased growth and seed production	
X-QUAC	NO_3^-, Cl^-, and anions		NO_3^-, Cl^-, and anion-dependent current		
Phloem-loading transporter					
AtHKT1	Na^+		Na^+-selective inward current	$\uparrow[Na^+]$ in shoot, $\downarrow[Na^+]$ in root and phloem (need to be confirmed)	
OsYSL2	Fe(II)-NA, Mn(II)-NA		Fe(II)-NA, Mn(II)-NA-selective inward current		
AtALS3	Al(III)			Sensitive to Al(III)	
Transporter involved in unloading					
VfVFK1			K^+-selective current		

$\downarrow$[Ion] = decreased ion content compared with the corresponding control; $\uparrow$[Ion] = increased ion content compared with the corresponding control.

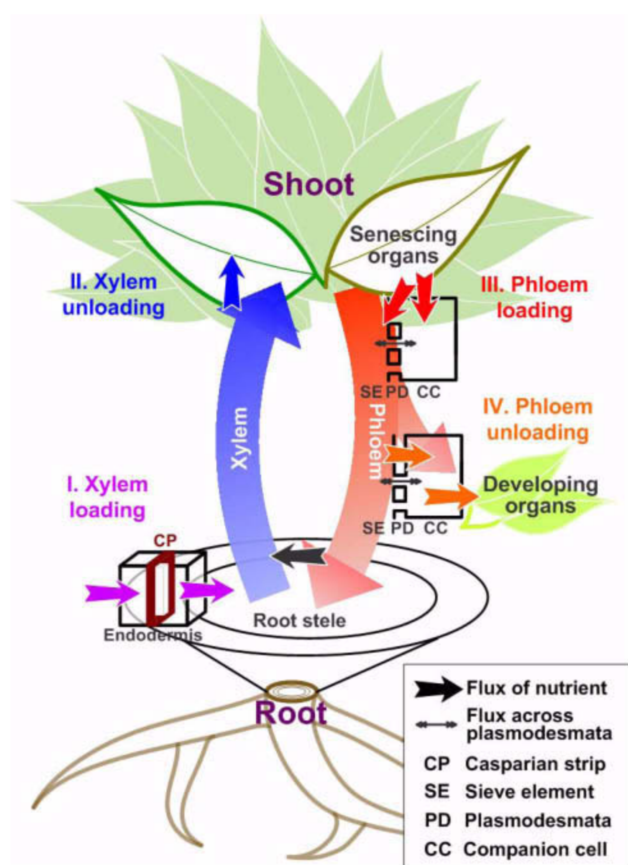

Figure 2. A diagrammatic representation of long-distance transport in the plant. Mineral ions absorbed by the root is translocated to the shoot via xylem (blue flow, I and II). In this pathway, transporters can be involved in two steps: xylem loading (purple arrow) and xylem unloading (blue arrow). Transporters localized in the root endodermis increase apoplastic ion levels near the vascular bundle and enhance xylem loading. Long-distance transport via phloem re-mobilizes minerals from organs that undergo senescence to developing organs (red flow, III and IV). The identities and characteristics of transporters involved in phloem loading (red arrow) or unloading (orange arrow) of mineral ions remain unclear.

of minerals belong to diverse families, including channels and pumps. Those associated with xylem-loading commonly increase apoplastic ion levels around xylem vessels. The mechanism of phloem loading of mineral ions remains unclear, although it seems reasonable to assume that both symplastic and apoplastic loading should be possible, as in the case of sucrose-loading into the phloem. Here we summarize information on transporters involved in long-distance transport of inorganic nutrients in plants (Illustrated in Figure 2; reviewed in Table I).

XYLEM-LOADING TRANSPORTERS

AtSKOR

SKOR, stelar K^+ outward rectifier, in the shaker super family, was the first-identified outward K^+ rectifier in *Arabidopsis*. It is expressed only in the root pericycle and stellar parenchyma cells. Using two-electrode voltage clamp, it was found that SKOR is an outward K^+-selective rectifier. *skor* loss-of-function mutant plants show a reduced potassium ion content in the shoot but not the root. This strongly suggests that SKOR is involved in the release of K^+ into the root stellar apoplasm, thereby contributing to its loading into the xylem sap. On the whole, this work is a good model for studying long-distance transport of ions because it appropriately combines transgenic techniques and electrophysiology (Gaymard et al., 1998).

AtHMA4

AtHMA4 is a P_{1B}-ATPase, predicted to be a heavy metal transporter, based on its homology to bacterial heavy metal transporters. Recent experimental results support its involvement in Zn(II) translocation (Hussian et al., 2004; Verret et al., 2004; Mills et al., 2005). Plants of the *hma4* knockout mutant have diminished shoot Zn and Cd contents when grown under mild Zn(II) or Cd(II) conditions, suggesting that HMA4 is important for supplying metals to the shoot. In line with data from null mutants, HMA4-overexpressing lines accumulate more Zn and Cd in their shoots than do wild-type plants (Verret et al., 2004). HMA4 is closely related to another P_{1B}-ATPase, AtHMA2. As expected, *hma4 hma2* double knockout plants show more severe Zn(II)-deficient symptoms, such as small, chlorotic leaves and short internodes, which can be rescued by Zn(II) supplementation (Hussian et al., 2004). This confirms their roles in supplying zinc. In contrast to these data from mutant plants, which seem to indicate that HMA4 is involved in metal acquisition, HMA4-expressing yeast cells efflux radio-labeled Zn(II) and Cd(II) into the medium (Mills et a., 2005), suggesting that AtHMA4 is an efflux pump. P-type ATPases are generally exporters; therefore efflux of the metals is more likely function of this transporter. These seemingly contradictory data are reconciled by information on tissue-specific expression of the transporter: AtHMA4 is mainly expressed in the root stellar cells surrounding the root vascular vessels and localized at the plasma membrane (Hussian et al., 2004; Verret et al., 2004). In this location, the efflux of ions from the live cells helps loading of the ions to the xylem of vascular bundle, thereby facilitates supply of the ions to the shoot. Thus the authors propose that AtHMA4 pumps Zn(II) and Cd(II) to the apoplasm surrounding the xylem, enhances the loading of metals into the xylem, thereby helps deliver them to the shoot. Taken together, these experimental data strongly support the idea that HMA4 takes part in long-distance transport, especially in root-to-shoot translocation, of Zn(II) and possibly Cd(II).

AtBOR1

AtBOR1 was the first boron transporter found in any organism, and has been suggested to load boron into the xylem of *Arabidopsis thaliana*. The *bor1* knockout mutant was discovered due to its reduced growth and fertility under boron-deficient conditions (Noguchi et al., 1997). BOR1 is expressed in the root pericycle and located at the plasma membrane. Tracer assay experiments using radioisotopic boron reveal that *bor1* has a lower soluble-B concentration in xylem exudates than does the corresponding wild type, although this mutant does not differ from the wild type in its

boron concentration in the root cell sap. When BOR1 is expressed in a yeast strain lacking YNL275w, its yeast homolog, it decreases the accumulation of boron, suggesting that BOR1 has boron efflux activity. Therefore, BOR1 is most likely involved in transporting boron out of the pericycle cells and loading it into the xylem, thus contributing to the supply of B to shoots (Takano et al., 2002). The transport mechanism for BOR1 remains unclear, although its sequence similarity with bicarbonate transporters in mammals implies a $Cl^-/B(OH)_4^-$ exchanging mechanism. However, no evidence has excluded the possibility that BOR1 is a boron uniporter or a H^+/boric acid antiporter. More studies are necessary to clarify its transport mechanism.

AtPHO1

AtPHO1 is a putative phosphate (Pi) transporter involved in xylem-loading. Shoots of *pho1* knockout plants have strongly reduced inorganic Pi contents compared with the wild type under low-phosphate conditions. Mutants show Pi-deficient symptoms, e.g., reduced growth, thinner stalks, small leaves, and delayed flowering, which imply an impaired Pi status. However, uptake levels in the roots of *pho1* plants do not differ from the wild type, nor is the level of Pi transport through the xylem of the hypocotyl to leaves. A defect in xylem-loading of Pi is proposed as the main reason for low Pi contents in mutant shoots (Poirier et al., 1991). GUS protein under the control of the *PHO1* promoter is expressed in the stellar cells of the roots and lower hypocotyls. Low ion contents in mutant leaves as well as stellar cell-specific expression are similar to those of SKOR, the potassium channel that loads K^+ into the xylem. This supports the possibility that PHO1 has a role in Pi-loading into the xylem. In addition, the expression of *PHO1* mRNA is elevated under Pi-deficient conditions, which supports its role in phosphate acquisition. However, it is not clear whether PHO1 delivers Pi directly because PHO1 is not similar in structure to any known Pi transporters, and because Pi transport activity by PHO1 has not yet been demonstrated in any heterologous system (Hamburger et al., 2002). Therefore, it is possible that PHO1 is a regulator of Pi transport activity and not, *per se*, a Pi transporter.

AtSOS1

AtSOS1, a plasma membrane Na^+/H^+ antiporter, mediates Na^+ transport across the plasma membrane. The *SOS1* knockout mutant *sos1,* together with *sos2* and *sos3* mutants, was first identified by its phenotype of sensitivity to high salt concentrations. SOS1 regulates salt levels in *Arabidopsis* in collaboration with the activity of the kinase SOS2 and the Ca^{2+}-binding protein SOS3 (Wu et al., 1996; Zhu et al., 1998; Qiu et al., 2002; Quintero et al., 2002). Recent experimental results support a role of SOS1 in the long-distance transport of Na^+ as well. When expressed in a salt-sensitive yeast mutant, SOS1 improves salt resistance and decreases Na^+ contents under moderate salt stress condition (Quintero et al. 2002; Shi et al., 2002). In *Arabidopsis*, SOS1 seems to load Na^+ into the xylem sap under normal conditions; this is demonstrated by *sos1* mutants that have low sodium contents in shoots when grown under mild salt stress. This hypothesis is supported by the preferential expression of SOS1 in parenchyma cells around the xylem. However, under severe salt stress such as >100 mM NaCl, *sos1* mutants have higher sodium contents in the xylem sap and in whole plants compared with the wild type. To explain such a phenomenon, Shi et al. (2002) have proposed that SOS1 unloads sodium from the xylem of roots grown in excess salt, in contrast to plants grown under control conditions where it loads Na^+ into the xylem sap. Thus, the direction of Na^+/H^+ exchange mediated by SOS1 seems to change depending on the ratio of extracellular and intracellular substrate levels. Reversibility in the direction of transport has been shown experimentally with SOD2 protein in fission yeast (Hahnenberger et al., 1996), but not yet with SOS1. Tests on bi-directional transport activity by the latter will be necessary to clearly understand the role of this transporter in transport through the xylem.

OsLsi1 and OsLsi2

The rice mutant *lsi1* has low silicon (Si) concentrations in its shoots and xylem sap, but does not differ from the wild type in root Si level. When Si is increased gradually in the external solution, the very low silicon concentration in the mutant xylem sap rises linearly, while that of the wild type is saturated at a much higher level, thus demonstrating that *lsi1* is defective in active xylem-loading. Short-term experiments have revealed that Si uptake in the mutants is much lower than in wild-type plants. Si uptake of the wild type, but not that of *lsi1* is inhibited by metabolic inhibitors (Ma et al., 2002). NMR has shown that the soluble form of Si in the symplasm of plant cells is monosilicic acid ($Si(OH)_4$), the content of which is lower in the xylem sap of the mutant, although no differences are detected in the leaf or root cell sap of either genotype (Ma et al., 2004). Therefore, the *lsi1* mutant seems to be defective in Si-loading into the xylem via an active transporter. Lsi1 is a member of the aquaporin family; when expressed in *Xenopus* oocytes, it shows Si uptake activity. In rice, Lsi1 is very specifically expressed on the distal side of the root exodermis and endodermis. Taken together, these data suggest that Lsi1 loads silicon into the xylem by transporting silicon into cells coated with the Casparian strip (Ma et al., 2006).

The rice mutant *lsi2* is also defective in Si uptake. Interestingly, Lsi2 is expressed in the same exodermis and endodermis cells where Lsi1 is found, albeit on the opposite, or proximal side. Lsi2 is a putative anion transporter that manifests silicon efflux activity when expressed in *Xenopus* oocytes. It has been suggested as a Si transporter that exports those ions from root cells of the endodermis to increase levels near the vascular bundle, resulting in the loading of Si into the xylem (Ma et al., 2007). These two transporters show complementing characteristics in their expression patterns, and their concerted actions enable efficient loading of Si into xylem.

X-QUAC

X-QUAC, quickly-activating anion conductance, has been identified as the main anion conductance in xylem parenchyma cells from barley roots. This conductance is gener-

ated by the movements of nitrate, chloride, and other anions, and can be important in loading these anions into the xylem sap. When xylem-parenchyma protoplasts are supplied with nitrate from the extracellular side, the voltage dependency of X-QUAC activity is changed to the direction that increases nitrate efflux from the cell. If this holds true under physiological conditions of plants, a high concentration of NO_3 in the xylem sap should enhance nitrate-loading activity, implying that X-QUAC represents an efficient xylem-loading transporter. As most studies on X-QUAC have been conducted with electrophysiological tools, further research on its molecular biological aspects is necessary to identify and characterize the transporter protein and the gene that encodes the protein carrying X-QUAC conductance (Köhler et al., 2002; Gilliham and Tester, 2005).

PHLOEM-LOADING TRANSPORTERS

AtHKT1

AtHKT1 belongs to the plant HKT family, which has sequence homology with yeast Trk1, Trk2, and *E. coli* TrkH, transporters involved in K^+ acquisition. Electrophysiological analyses using *Xenopus* oocytes have indicated Na^+ transport activity by AtHKT1. For example, Na^+-selective conductance is detected in oocytes injected with *AtHKT1* cRNA. A reduced level of the current is detected when oocytes were injected with *sas2-1* cRNA, a point mutated form of *AtHKT1* (Berthomieu et al., 2003). In *Arabidopsis*, both point and insertional mutations of *AtHKT1* elevate Na^+ accumulations in shoots, while contents of K^+ and other ions are not altered. Interestingly, both mutant types exhibit diminished Na^+ levels in their roots (Mäser et al., 2002; Berthomieu et al., 2003), which results in an increase in the shoot to root ratio of Na^+. This altered ratio suggests a role of AtHKT1 in long-distance transport of Na^+. Based on several lines of evidence, the proposed direction is from shoot to root via the phloem. First, Na^+ levels are lower in the phloem sap of the *sas2-1* mutant than in the wild type, even though shoot Na^+ contents are similar (Mäser et al., 2002). Second, AtHKT1 promoter-GUS transgenic plants exhibit GUS activity only in phloem tissues. Third, Na^+ levels in the xylem sap and Na^+ uptake efficiency in the roots of *sas2-1* plants are not altered (Mäser et al., 2002). These results suggest that AtHKT1 loads Na^+ into the phloem, thereby enhancing the recirculation of sodium from shoot to root, and improving resistance to salt stress.

However, recent studies have presented different roles of AtHKT1. Sunarpi et al. (2005) have suggested that it functions in xylem-unloading, based on a high Na^+ content in the xylem sap of the AtHKT1 insertional mutant and expression of the protein in the xylem parenchyma, as detected with a specific antibody (Sunarpi et al., 2005). This hypothesis is further supported by short-term transport experiments using radioisotopic Na^+. In *athkt1* mutants, the shoots accumulate more Na^+ within 1 h, suggesting that xylem-unloading may be defective. However, unloading from pre-loaded shoots, which represents the recirculation of sodium via phloem transport, remains similar to that of the wild type (Davenport et al., 2007). These results imply that AtHKT1 has a role in xylem-unloading of Na^+ rather than phloem-loading.

OsYSL2

OsYSL2 is a rice homolog of ZmYS1, which transports a phytosiderophore-metal complex at the root surface (Wirén et al., 1994; Curie et al., 2001). *OsYSL2* expression in the shoot is highly up-regulated under iron-deficient conditions, supporting its function in Fe nutrition. However, unlike ZmYS1, which transports metals bound to deoxymugeneic acid and other phytosiderophores (chelators used by graminaceous plants to take up Fe from the soil), OsYSL2 binds nicotianamine (NA). NA is important in the long-distance transport of Fe(II) and Mn(II) (Takahashi et al., 2003) as well as other NA-bound metals. When expressed in *Xenopus* oocytes, OsYSL2 transports NA complexes of Fe(II) or Mn(II), but not phytosiderophore-bound Fe(II) or Mn(II) (Koike et al., 2004). Furthermore, GUS under control of the *OsYSL2* promoter is expressed in phloem and companion cells. Therefore, OsYSL2 is likely to function as a phloem transporter of NA-Fe(II) or -Mn(II) complexes (Takahashi et al., 2003).

AtALS3

AtALS3 is an ABC transporter-like protein whose loss-of-function causes hyper-sensitivity to $AlCl_3$, suggesting that it may be involved in the Al(III) tolerance mechanism of *Arabidopsis thaliana*. Because ALS3 is localized at the plasma membrane and expressed in the phloem of most tissues and leaf hydathodes, it may redistribute Al(III) to less-sensitive tissues and/or to the hydathodes for excretion. Consistent with this hypothesis, in the *als3* mutant, Al resides in the region proximal to the root tip, in contrast to the generally diffuse distribution of Al(III) in wild-type roots (Larsen et al., 2005).

TRANSPORTERS FOR UNLOADING

Only a few reports have described the involvement of transporters in unloading inorganic nutrients from the vascular bundle. Even with sucrose, the compound at the highest concentration in phloem sap, the unloading transporter has been identified only recently (Carpaneto et al., 2005). This may be because unloading mechanisms are complex, use both plasmodesmata and a symplastic pathway, are regulated by many factors, and are promoted by multiple driving forces, including a concentration gradient. Apoplastic unloading of sucrose and other mineral nutrients (Jeschke and Pate, 1991; Marschner et al., 1996) has been suggested based on results from physiological experiments, which necessitates transporters at the plasma membrane of phloem sieve element/companion cells.

VFK1

VFK1 is a member of K^+ import channels found in *Vicia faba* that shows high sequence homology with AtAKT3, a phloem-localized K^+ channel. VFK1 is dominantly expressed in the flower, stem, and leaf. *VFK1* mRNA is detected in the

phloem of stems and petioles, similarly as *AtAKT3*. When expressed in *Xenopus* oocytes, VFK1 mediates potassium-selective currents. Therefore, it may function as a phloem-localized potassium transporter. Interestingly, cotyledon expression of VFK1 is observed mainly during the early stage of seed development, at the beginning of the storage phase. Moreover, its expression is higher in sink leaves than in source leaves at the same stage. This sink-specific expression is consistent with the possibility that it is involved in K^+ retrieval at the sink. However, more evidence at the molecular level is necessary to establish the physiological role of VFK1 as a K^+ retriever from the phloem.

CONCLUSION AND FUTURE DIRECTIONS

Most plant transporters involved in long-distance transport have homologs in yeast or *E. coli*. Therefore, plants apparently were not forced to invent new classes of transporter proteins for long-distance transport of mineral nutrients but instead use similar proteins, or at least domains that resemble those found in other organisms. Thus, their role in translocation seems to be conferred by tissue-specific expression and not by any unique characteristics of those transporters.

Very interesting new developments are the discoveries of endodermis-specific transporters that exhibit polar distributions (Ma et al., 2006, 2007), and of the multiple mechanisms for regulating transporter proteins depending on the available substrate levels: both transcriptional and post-transcriptional as well as via endocytosis and degradation (Takano et al., 2005). No doubt, future research will uncover similar interesting features of transporters for many essential ions. Relatively unknown aspects of long-distance transport are the identity of signals for the deficiency of essential metals, including those sent from the shoot to the root, and the identity and mode of action of transporters involved in unloading minerals at each sink organ. Because of the great interest in this field and the availability of powerful tools in genetics, biochemistry, and cellular biology, we are confident that an overall picture of long-distance transport will soon emerge.

ACKNOWLEDGEMENT

This work was supported by a grant awarded to Y.L. and E.M. from the Global Research Laboratory program of the Ministry of Science and Technology of Korea.

Received May 9, 2008; accepted May 11 , 2008.

LITERATURE CITED

Ache P, Becker D, Deeken R, Dreyer I, Weber H, Fromm J, Hedrich R (2001) VFK1, a *Vicia faba* K^+ channel involved in phloem unloading. Plant J 27(6): 571-580

Berthomieu P, Conéjéro G, Nublat A, Brackenbury WJ, Lambert C, Savio C, Uozumi N, Oiki S, Yamada K, Cellier F, Gosti F, Simonneau T, Essah PA, Tester M, Véry A-A, Sentenac H, Casse F (2003) Functional analysis of AtHKT1 in *Arabidopsis* shows that Na^+ recirculation by the phloem is crucial for salt tolerance. EMBO J 22(9): 2004-2014

Carpaneto A, Geiger D, Bamberg E, Sauer N, Fromm J, Hedrich R (2005) Phloem-localized, proton-coupled sucrose carrier ZmSUT1 mediates sucrose efflux under the control of the sucrose gradient and the proton motive force. J Biol Chem 280(22): 21437-21443

Conklin DS, McMaster JA, Culbertson MR, Kung C (1992) COT1, a gene involved in cobalt accumulation in. Mol Cell Biol 12(9): 3678-3688

Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat J-F, Walker EL (2001) Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. Nature 409: 346-349

Dancis A, Yuan DS, Haile D, Askwith D, Eide D, Moehle C, Kaplan J, Klausner RD (1994) Molecular characterization of a copper transport protein in.: An unexpected role for copper in iron transport. Cell 76: 393-402

Davenport RJ, Muòoz-Mayor A, Jha D, Essah PA, Rus A, Tester M (2007) The Na^+ transporter AtHKT1;1 controls retrieval of Na^+ from the xylem in *Arabidopsis*. Plant Cell Environ 30: 497-507

Gaymard F, Pilot G, Lacombe B, Bouchez D, Bruneau D, Boucherez J, Michaux-Ferrière N, Thibaud J-B, Sentenac H (1998) Identification and disruption of a plant shaker-like outward channel involved in K^+ release into the xylem sap. Cell 94: 647-655

Gilliham M, Tester M (2005) The regulation of anion loading to the maize root xylem. Plant Physiol 137: 819-828

Grusak MA (2002) Enhancing mineral content in plant food products. J Amer Col Nut 21: 178S-183S

Hahnenberger KM, Jia Z, Young PG (1996) Functional expression of the *Schizosaccharomyces pombe* Na^+/H^+ antiporter gene, sod1, in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 93: 5031-5036

Hamburger D, Rezzonico E, Petétot JM-C, Somerville C, Poirier Y (2002) Identification and characterization of the arabidopsis PHO1 gene involved in phosphate loading to the xylem. Plant Cell 14: 889-902

Hussian D, Haydon MJ, Wang Y, Wong E, Sherson SM, Young J, Camakaris J, Harper JF, Cobett CS (2004) P-type ATPase Heavy metal transporter with roles in essential zinc homeostasis in *Arabidopsis*. Plant Cell 16: 1327-1339

Jean ML, Schikora A, Mari S, Briat J-F, Curie C (2005) A loss-of-function mutation in AtYSL1 reveals its role in iron and nicotianamine seed loading. Plant J 44: 769-782

Jeschke D, Pate JS (1991) Cation and chloride partitioning through xylem and phloem within the whole plant of *Ricinus communis* L. under conditions of salt stress. J Exp Bot 42: 1105-1116

Kamizono A, Nishizawa M, Teranishi Y, Murata K, Kimura A (1989) Identification of a gene conferring resistance to zinc and cadmium ions in the yeast *Saccharomyces cerevisiae*. Mol Gen Genet 219(1-2): 161-167

Kim SA, Punshon T, Lanzirotti A, Li L, Alonso JM, Ecker JR, Kaplan J, Guerinot ML (2006) Localization of iron in *Arabidopsis* seed requires the vacuolar membrane transporter VIT1. Science 314: 1295-1298

Köhler B, Wegner LH, Osipov V, Raschke K (2002) Loading of nitrate into the xylem: Apoplastic nitrate controls the voltage dependence of X-QUAC, the main anion conductance in xylem-parenchyma cells of barley roots. Plant J 30(2): 133-142

Koike S, Inoue H, Mizuno D, Takahashi M, Nakanishi H, Mori S, Nishizawa NK (2004) OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. Plant J 39: 415-424

Larsen PB, Geisler MJ, Jones CA, Williams KM, Cancel JD (2005) ALS3 encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in *Arabidopsis*. Plant J 41: 353-363

Ma JF, Tamai K, Ichii M, Wu GF (2002) A rice mutant defective in Si uptake. Plant Physiol 130: 2111-2117

Ma JF, Mitani N, Nagao S, Konishi S, Tamai K, Iwashita T, Yano M (2004) Characterization of the silicon uptake system and molecular mapping of the silicon transporter gene in rice. Plant Physiol 136: 3284-3289

Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Ishiguro M, Murata Y, Yano M (2006) A silicon transporter in rice. Nature 440: 668-691

Ma JF, Yamaji N, Mitani N, Tamai K, Konishi S, Fujiwara T, Katsuhara M, Yano M (2007) An efflux transporter of silicon in rice. Nature 448: 209-213

Marschner H, Kirkby E, Cakmak I (1996) Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. J Exp Bot 47: 1255-1263

Mäser P, Eckelman B, Vaidyanathan R, Horie T, Fairbairn DJ, Kubo M, Yamagami M, Yamaguchi K, Nishimura M, Uozumi N, Robertson W, Sussman MR, Schroeder JI (2002) Altered shoot/ root/ Na^+ distribution and bifurcating salt sensitivity in *Arabidopsis* by genetic disruption of the Na^+ transporter *AtHKT1*. FEBS Lett 531: 157-161

Mills RF, Francini A, Rocha PF, Baccarini PJ, Aylett M, Krijger GC, Williams LE (2005) The plant P_{1B}-type ATPase AtHMA4 transports Zn and Cd and plays a role in detoxification of transition metals supplied at elevated levels. FEBS Lett 579: 783-791

Miwa K, Takano J, Fujiwara F (2006) Improvement of seed yields under boron-limiting conditions through overexpression of BOR1, a boron transporter for xylem loading, in *Arabidopsis thaliana*. Plant J 46: 1084-1091

Noguchi K, Yasumori M, Imai T, Naito S, Matsunaga T, Oda H, Hayashi H, Chino M, Fujiwara T (1997) bor1-1, an *Arabidopsis thaliana* mutant that requires a high level of boron. Plant Physiol 115: 901-906

Poirier Y, Thoma S, Somerville C, Schiefelbein J (1991) A mutant of arabidopsis deficient in xylem loading of phosphate. Plant Physiol 97: 1087-1093

Qiu Q-S, Guo Y, Dietrich MA, Schumaker KS, Zhu J-K (2002) Regulation of SOS1, a plasma membrane Na^+-H^+ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. Proc Natl Acad Sci USA 99(12): 8436-8441

Quintero FJ, Ohta M, Shi H, Zhu J-K, Pardo JM (2002) Reconstitution in yeast of the *Arabidopsis* SOS signaling pathway for Na^+ homeostasis, Proc Natl Acad Sci USA 99(13): 9061-9066

Rensing C, Mitra B, Rosen BP (1997) The *zntA* gene of *Escherichia coli* encodes a Zn(II)-translocating P-type ATPase. Proc Natl Acad Sci USA 94: 14326-14331

Shi H, Quintero FJ, Pardo JM, Zhu J-K (2002) The putative plasma membrane Na^+/H^+ antiporter SOS1 controls long distance Na^+ transport in plants. Plant Cell 14: 465-477

Shi H, Lee B-H, Wu S-J, Zhu J-K (2003) Overexpression of a plasma membrane Na^+/H^+ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. Nat Biotech 21: 81-85

Song W-Y, Sohn EJ, Martinoia E, Lee YJ, Yang, Y-Y, Jasinski M, Forestier C, Hwang I, Lee Y (2003) Engineering tolerance and accumulation of lead and cadmium in transgenic plants. Nat Biotech 21(8): 914-919

Sunarpi, Horie T, Motoda J, Kubo M, Yang H, Yoda K, Horie R, Chan W-Y, Leung H-Y, Hattori K, Konomi M, Osumi M, Yamagami M, Schroeder JI, Uozumi N (2005) Enhanced salt tolerance mediated by AtHKT1 transporter induced Na^+ unloading from xylem vessels to xylem parenchyma cells. Plant J 44: 928-938

Szczypka MS, Wemmie JA, Moye-Rowley WS, Thiele DJ (1994) A yeast metal resistance protein similar to human Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) and multidrug resistance-associated protein. J Biol Chem 9: 22853-22857

Takahashi M, Terada Y, Nakai I, Nakanishi H, Yoshimura E, Mori S, Nishizawa NK (2003) Role of nicotianamine in the intracellular delivery of metals and plant reproductive development. Plant Cell 15: 1263-1280

Takano J, Noguchi K, Yasumori M, Kobayashi M, Gajdos Z, Miwa K, Hayashi H, Yoneyama T, Fujiwara T (2002) *Arabidopsis* boron transporter for xylem loading. Nature 420: 337-340

Takano J, Miwa K, Yuan L, Wirén N, Fujiwara T (2005) Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana*, regulated by boron availability. Proc Natl Acad Sci USA 102(34): 12276-12281

Verret F, Gravot A, Auroy P, Leonhardt N, David P, Nussaume L, Vavasseur A, Richaud P (2004) Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. FEBS Lett 576: 306-312

Wirén N, Mori S, Marschner H, Romheld V (1994) Iron inefficiency in maize mutant ysl (*Zea mays* L. cv Yellow-Stripe) is caused by a defect in uptake of iron phytosiderophores. Plant Physiol 106: 71-77

Wu S-J, Ding L, Zhu J-K (1996) SOS1, a genetic locus essential for salt tolerance and potassium acquisition. Plant Cell 8: 617-627

Zhu J-K, Liu J, Xiong L (1998) Genetic analysis of salt tolerance in *Arabidopsis*: Evidence for a critical role of potassium nutrition. Plant Cell 10: 1181-1191