Chapter 13
Transport System of Mineral Elements in Rice

Namiki Mitani-Ueno, Naoki Yamaji, and Jian Feng Ma

Abstract Plant requires 14 mineral elements for their growth and development. These elements in the soil are taken up by the roots, translocated from the roots to the shoots, and distributed to different organs depending on their demands (Marschner P, Mineral nutrition of higher plants, 3rd edn. Academic, London, 2012). In addition to these essential elements, toxic elements such as Cd and As are also transported from the soils to aboveground parts. All these processes require various transporters (membrane proteins). During the last decades, a number of transporters for uptake, translocation, and distribution of mineral elements have been identified, especially in model plants such as *Arabidopsis* and rice; however, most transporters remain to be identified. In this chapter, transporters identified so far in rice are described, and the regulation mechanisms of transporters in response to environmental changes are also discussed.

Keywords Transporter · Mineral elements · Uptake · Distribution

13.1 Brief Introduction of the Root and Node Structure in Rice for Mineral Transport

Rice grown in paddy field shows a distinct transport system for uptake and distribution due to the structural features of its roots and nodes. Rice roots are characterized by two Casparian strips at both the exodermis and endodermis (Enstone et al. 2002). Furthermore, mature roots have a highly developed aerenchyma in which almost all of the cortex cells between the exodermis and endodermis are destroyed. Therefore, movement from the soil solution to the stele requires both influx and efflux transporters in both the exodermis and endodermis of the roots (Sasaki et al. 2016, Fig. 13.1).

N. Mitani-Ueno · N. Yamaji · J. F. Ma (✉)
Institute of Plant Science and Resources, Okayama University, Kurashiki, Japan
e-mail: maj@rib.okayama-u.ac.jp

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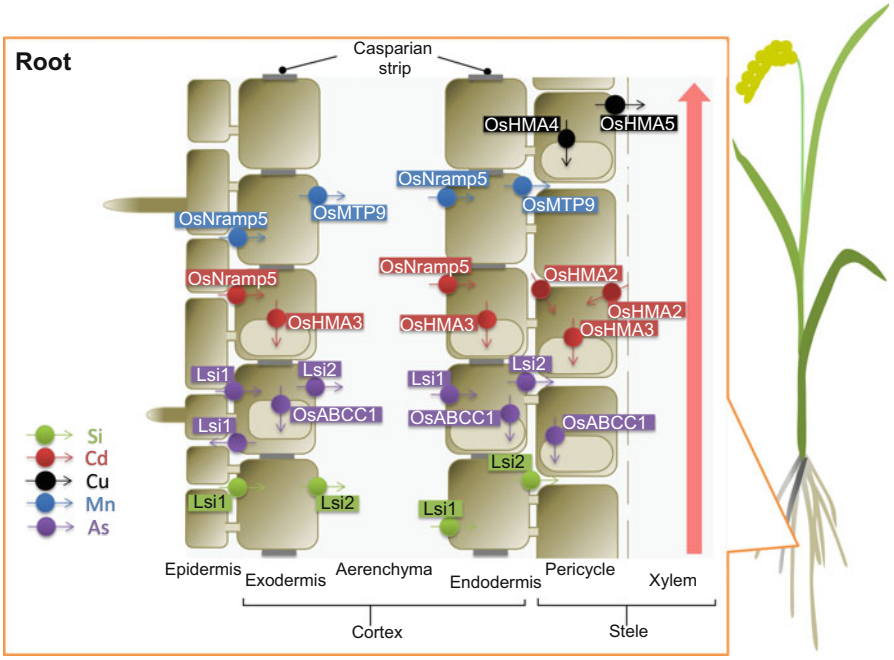

Fig. 13.1 Schematic diagram of transporters identified so far for uptake and sequestration of essential and toxic elements in rice roots. Pink arrow indicates xylem flow; green Si channel Lsi1 and efflux transporter Lsi2; red Cd influx transporter OsNramp5 and OsHMA2 and tonoplast-localized transporter OsHMA3 for Cd sequestration; black Cu efflux transporter OsHMA5 and tonoplast-localized transporter OsHMA4 for Cu sequestration; blue Mn influx transporter OsNramp5 and Mn efflux transporter OsMTP9; purple As channel Lsi1, As efflux transporter Lsi2, and tonoplast-localized transporter OsABCC1 for As sequestration

In terms of distribution of mineral elements, rice nodes have been demonstrated to play an important role in the distribution. Rice nodes have highly developed vascular systems, mainly consisting of enlarged vascular bundles (EVBs) and diffuse vascular bundles (DVBs). EVBs come from the two lower nodes and are connected to the leaf attached to the node, while DVBs start at the node and are connected to the upper two nodes or panicle. Therefore, an intervascular transfer of mineral elements from EVBs to DVBs is required for their preferential distribution to developing tissues and reproductive organs. Various transporters for different mineral elements are also required for this intervascular transfer (Fig. 13.2).

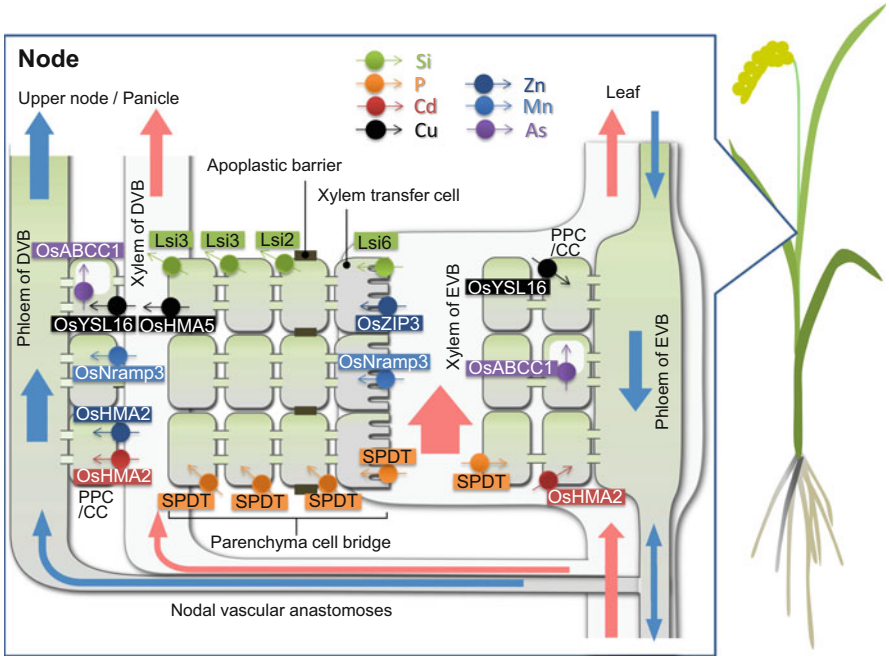

Fig. 13.2 Schematic diagram of transporters identified so far for distribution of essential and toxic elements in rice nodes. Pink and light blue arrows indicate xylem and phloem flow, respectively; green Si channel Lsi6 and Si efflux transporters Lsi2 and Lsi3; orange P influx transporter SPDT; red Cd transporter OsHMA2; black Cu efflux transporter OsHMA5 and Cu-nicotianamine transporter YSL16; dark blue Zn transporters OsZIP3 and OsHMA2; blue Mn influx transporter OsNramp3; purple tonoplast-localized transporter OsABCC1 for As sequestration

13.2 Transporters Involved in Uptake and Distribution of Essential Elements

13.2.1 Transporters for Macronutrients

Plants need six macronutrients (N, P, K, Mg, S, Ca) for their healthy growth. Nitrogen (N) is one of the most important nutrients for rice productivity and can be available in several different forms, including ammonium ions (NH_4^+) and nitrate ions (NO_3^-) and organic molecules, such as amino acids. Rice is usually cultivated under flooded condition, where NH_4^+ is the dominant inorganic N form. For the uptake of NH_4^+, rice has two uptake systems: a high-affinity transport system (HATS) and a low-affinity transport system (LATS) (Wang et al. 1993). Two types of ammonium transporters (AMTs) have been implicated in the ammonium uptake. The first ammonium transporter (AMT1) involved in HATS was identified in *Arabidopsis* (Ninnemann et al. 1994). In rice genome, there are 12 potential AMT members, but only few of them have been characterized.

OsAMT1;2 may play a role in NH_4^+ uptake and xylem loading (Sonoda et al. 2003). *OsAMT1;2* expression is induced by ammonium supply, and its mRNA is detected in the exodermis, sclerenchyma, endodermis, and pericycle cells of primary root tips by in situ hybridization (Sonoda et al. 2003). Two other members of AMT1 such as OsAMT1;1 and OsAMT1;3 were also reported as transporters of NH_4^+ uptake (Hoque et al. 2006; Ferreira et al. 2015); however their exact roles remain to be investigated.

Rice is also able to take up NO_3^-, which is oxidized from ammonium in the rhizosphere of rice through oxygen release from the root aerenchyma (Kirk and Kronzucker 2005). The *indica* cultivars usually have greater nitrate uptake ability than *japonica* cultivars, probably because *indica* cultivars are more frequently cultivated in upland soil where nitrate is the dominant N form. Plant nitrate transporters (NPF) belong to the nitrate transporter 1/peptide transporter (NRT1/PTR) family. There are at least 93 NPF members in the rice genome (Li et al. 2015), but only a few have been characterized. Recently, one gene (NRT1.1B/NPF6.5), responsible for the genotypic difference in nitrate uptake between *japonica* and *indica* cultivars, was identified (Hu et al. 2015; Chen and Ma 2015). It encodes a plasma membrane-localized transporter for nitrate and is mainly expressed in the epidermis, root hairs, and vascular tissues. Furthermore, its expression is induced by nitrate. In addition to NRT1.1B/NPF6.5, a plasma membrane-localized OsNPF2.2 has been implicated in the root-to-shoot translocation of nitrate (Li et al. 2015). It is mainly expressed in the parenchyma cells around the xylem. Disruption of OsNPF2.2 resulted in increased nitrate concentration in the shoot xylem exudate (Li et al. 2015). OsNPF2.2 might be involved in the xylem unloading of NO_3^- and effects on root-shoot NO_3^- translocation. OsNRT2.3a expressed in parenchyma cells around the root xylem is also responsible for long-distance transport of NO_3^- from root to shoot (Tang et al. 2012). OsNRT2.3b expressed in the leaf phloem plays a key role in NO_3^- remobilization and phloem pH balance (Fan et al. 2016).

Phosphorus (P) is also an important nutrient for plant growth and often becomes a limiting factor for crop productivity due to its low availability and strong fixation to soil particles. There are two forms of inorganic P (Pi), $H_2PO_4^-$ and HPO_4^{2-}, depending on the soil pH. The uptake of Pi is mediated by plasma membrane-localized Pi transporters belonging to the phosphate transporter (PHT1/PT) family. Thirteen PHT1 genes have been identified in rice (Raghothama 1999; Goff et al. 2002), and some of them have been implicated in root Pi uptake. OsPht1;4 (PT4) and OsPht1;8 (PT8) are expressed in the roots and show a transport activity of Pi in yeast or oocytes (Jia et al. 2011; Zhang et al. 2015). Phenotypic analysis showed that root and shoot Pi concentration was significantly lower in the knockout/knockdown lines of OsPht1;4 or OsPht1;8 than in that of the wild-type rice (Jia et al. 2011, Zhang et al. 2015), indicating their role in Pi uptake. Two other members, OsPht1;2 (OsPT2) and OsPht1;6 (OsPT6), are expressed in both the roots and shoots (Ai et al. 2009). OsPT2 is localized exclusively to the stele of primary and lateral roots, whereas OsPT6 is expressed in both epidermal and cortical cells of the younger primary and lateral roots. Knockdown of either

OsPT2 or *OsPT6* expression significantly decreased both the uptake and the long-distance transport of Pi from the roots to the shoots, suggesting that they are also involved in Pi uptake and translocation, but their relative contribution to the entire Pi uptake is unknown.

PHO1, belonging to a unique membrane protein family that is distinct from PHTs, has been implicated in the root-to-shoot translocation of Pi. In the rice genome, there are three PHO1 homologues. Interestingly, all of them have *cis*-natural antisense transcripts (*cis*-NAT). *OsPHO1;2* showed the most abundant expression in the roots (Secco et al. 2010). Knockout of OsPHO1;2 resulted in reduced Pi transfer from the roots to the shoots (Secco et al. 2010). A more recent study revealed that the *cis*-natural antisense transcript of PHO1;2 (*cis*-$NAT_{PHO1;2}$) has a function for promoting the translation of PHO1;2 and affecting phosphate homeostasis and finally plant fitness (Jabnoune et al. 2013).

Following xylem loading by PHO1, P is preferentially distributed by SPDT (SULTR-like phosphorus distribution transporter) at the nodes (Fig. 13.2). SPDT belongs to the sulfate transporter family but transports Pi rather than sulfate (Yamaji et al. 2017). It is highly expressed in the nodes, particularly in the uppermost node I at the reproductive stage. SPDT is localized at the xylem transfer cells and parenchyma cell bridge (PCB) and responsible for preferential distribution of P to developing new tissues and grains (Yamaji et al. 2017).

Potassium (K) plays an important role especially in regulating osmotic pressure. Transporters for K uptake have been identified in *Arabidopsis*. Two transporters, HAK5 (potassium transporter KUP/HAK/KT family) and AKT1 (ARABIDOPSIS K^+ TRANSPORTER1; shaker family potassium channel), have been reported to be involved in K^+ uptake (Hirsch et al. 1998; Gierth et al. 2005). However, little is known about transporters involved in K^+ uptake in rice. A homologue of *Arabidopsis* AKT1, OsAKT1, functions as an inward-rectifying channel and contributes to K^+ uptake (Li et al. 2014). Knockout of OsAKT1 resulted in decreased biomass and K^+ concentration of the roots and shoots at both vegetative and reproductive growth stages in the presence of low or normal K^+ (Li et al. 2014).

There are high- and low-affinity uptake systems for magnesium (Mg) (Tanoi et al. 2014). Recently, OsMGT1, a CorA-like gene encoding a member of the mitochondrial RNA splicing 2/magnesium transporter (MRS2/MGT) family in rice, was found to be involved in Mg uptake (Chen et al. 2012). OsMGT1 is localized at the plasma membrane. Knockout of this gene resulted in decreased Mg uptake (Chen et al. 2012). However, since Mg uptake in the *osmgt1* mutant is still remaining, it seems that other unidentified transporters are also responsible for Mg uptake from the soil solution to the root cells. In addition, OsMGT1 contributes to the alleviation of Al toxicity by increasing Mg concentration in the cell (Chen et al. 2012).

Sulfate (SO_4^{2-}) is the major form of sulfur (S) that plants can use for synthesizing sulfur-containing compounds, such as cysteine and methionine, proteins, lipids, coenzymes, and various secondary metabolites (Leustek et al. 2000; Saito 2004). Sulfate is absorbed by the SULTR transporter family members in plants. Extensive work has been done in *Arabidopsis* on SULTR transporters. There are

12 SULTR members in *Arabidopsis* (Takahashi et al. 2012a, b). In rice, there are 14 SULTR members showing different expression patterns (Kumar et al. 2011; Takahashi et al. 2012a). Among them, OsSULTR1;1, OsSULTR1;2, OsSULTR2;1, and OsSULTR5;2 are highly expressed in the seedling roots based on microarray analysis (Kumar et al. 2011). Moreover, most OsSULTR genes are up- or downregulated by several biotic and abiotic stresses (Kumar et al. 2011). Based on tissue expression, OsSULTR1;1 has been implicated in S uptake in rice roots (Godwin et al. 2003). However, the functions and physiological roles of most S transporter genes in rice have not yet been characterized. Following uptake of sulfate from the soil, it is loaded into xylem for subsequent translocation to aerial parts. However transporters involved in these steps are still unclear.

Transporters for calcium (Ca) are poorly understood. Some transporters belonging to the cation/H^+ exchanger family protein showed Ca^{2+} transport activity when they were expressed in yeast (Kamiya et al. 2005). However, the contribution of these transporters to Ca transport in rice remains to be determined because phenotypic analysis using knockout/knockdown of OsCAXs has not yet been investigated.

13.2.2 Transporters for Micronutrients

Manganese (Mn) is involved in many biological processes, such as water splitting activity in photosystem II, elimination of superoxide, and various secondary metabolisms. Plants require 50–100 mg kg^{-1} of Mn for their growth (Vlamis and Williams 1964). However, rice is able to accumulate Mn up to 5000 mg kg^{-1} DW without showing toxicity symptoms. It is now clear that this high Mn uptake is cooperatively mediated by an influx transporter (OsNramp5) and an efflux transporter (OsMTP9). *OsNramp5*, a member of the NRAMP (natural resistance-associated macrophage proteins) family, is mainly expressed in the root through all its growth stages (Sasaki et al. 2012). Furthermore, *OsNramp5* is highly expressed in the mature zone of the root, and its expression is not affected by Mn availability. It is a plasma membrane-localized influx transporter for Mn. OsNramp5 protein is polarly localized at the distal side of both the exodermis and endodermis (Sasaki et al. 2012, Fig. 13.1). In the knockout mutant of OsNramp5, the Mn uptake ability is almost lost (Sasaki et al. 2012), indicating that OsNramp5 is a major transporter for Mn uptake in rice (Ishikawa et al. 2012; Ishimaru et al. 2012; Sasaki et al. 2012), which is responsible for transporting Mn from the soil solution into the root cells. On the other hand, OsMTP9 (metal tolerance protein 9) belongs to the cation diffusion facilitator (CDF) family. It is a plasma membrane-localized efflux transporter for Mn (Ueno et al. 2015). Similar to *OsNramp5*, *OsMTP9* is also mainly expressed in the mature root zones. OsMTP9 protein is also localized to exodermis and endodermis cells, but, in contrast to OsNramp5, it is polarly localized at the proximal side of both cell layers (Ueno et al. 2015, Fig. 13.1). Knockout of OsMTP9 significantly decreased Mn uptake and root-

to-shoot translocation. These results indicated that the efficient Mn uptake in rice requires both OsNramp5 and OsMTP9.

A transporter belonging to the NRAMP family, OsNramp3, plays an important part in distributing Mn to different tissues in response to environmental Mn changes (Yamaji et al. 2013a). OsNramp3 is a plasma membrane-localized transporter of Mn localized in both xylem transfer cells of EVBs and phloem region of DVBs at both basal nodes at vegetative growth stage and upper nodes at reproductive growth stage (Fig. 13.2). Under Mn-limited condition, OsNramp3 at the transfer cells mediates unloading of Mn from the xylem of EVBs, followed by reloading Mn to the phloem of DVBs in nodes, delivering limited Mn preferentially to the developing new leaves and panicles. However, under excess Mn conditions, OsNramp3 protein was rapidly degraded, and the distribution of Mn follows the transpiration rate (Yamaji and Ma 2014).

Zinc (Zn) plays a key role in plants as an enzyme cofactor and a structural component of protein mediating binding to other proteins, DNA, RNA, or small molecules. Two members belonging to the ZIP (zinc-regulated transporters, iron-regulated transporter-like protein) family, OsZIP1 and OsZIP3, were suggested to be responsible for Zn uptake and/or homeostasis in rice (Ramesh et al. 2003; Bashir et al. 2012). However, recent work showed that OsZIP3 localized to the node is involved in the distribution of Zn to the developing tissues, rather than uptake (Sasaki et al. 2015). OsZIP1 is expressed in the epidermis and vascular tissues of roots and leaves (Ramesh et al. 2003, Bashir et al. 2012). Therefore, OsZIP1 might be involved in the uptake of Zn, but more physiological evidence is required for supporting this hypothesis.

Most of the Zn taken up by the roots is preferentially distributed to the nodes and developing organs but is not allocated to developed leaves (Obata and Kitagishi 1980a, b; Obata et al. 1980; Suzuki et al. 2008). The nodes accumulate Zn at very high level that is more than ten times higher than that of other tissues (Kitagishi and Obata 1986; Yamaguchi et al. 2012; Moore et al. 2014). For efficient distribution of Zn, two transporters localized at the node were identified. One is OsZIP3 described above, and the other is OsHMA2 (Sasaki et al. 2015; Yamaji et al. 2013b). OsZIP3 is localized at the xylem transfer cells in EVB and is involved in unloading of Zn from the xylem of EVB (Sasaki et al. 2015, Fig. 13.2). On the other, OsHMA2 is localized at the phloem region of both EVBs and DVBs and responsible for loading Zn to the phloem of DVBs and EVBs (Yamaji et al. 2013b, Fig. 13.2).

Iron (Fe) is essential for many cellular functions in plants, including chlorophyll biosynthesis, photosynthesis, and respiration. There are two strategies for iron (Fe) acquisition from soil depending on the plant species. Dicots and non-gramineous monocots adopt Strategy I, which is characterized by the reduction of ferric Fe (Fe^{3+}) to ferrous Fe (Fe^{2+}) by ferric chelate reductase (FRO) on the plasma membrane and subsequent uptake by the IRT1 transporter (Eide et al. 1996; Robinson et al. 1999). By contrast, gramineous plants adopt Strategy II, which is characterized by the secretion of mugineic acid (MA) and subsequent uptake of the mugineic acid–ferric complex (Ma and Nomoto 1996; Murata et al. 2006). However, rice seems to harbor both Strategy I and II. Although rice does not have

effective ferric chelate reductases in the roots (Ishimaru et al. 2006), ferrous Fe is rich in paddy fields due to reductive status in flooded paddy soil. Ferrous Fe was suggested to be taken up by OsIRT1 and OsIRT2, two members of the ZIP transporter family (Bughio et al. 2002; Ishimaru et al. 2006). Both OsIRT1 and OsIRT2 show a transport activity for ferrous Fe (Ishimaru et al. 2006). *OsIRT1* is expressed in the root epidermis and stele, and its expression is upregulated by Fe deficiency (Ishimaru et al. 2006). However, the contribution of these transporters to Fe uptake remains to be determined because phenotypic analysis using knockout/knockdown of OsIRT1 and OsIRT2 has not yet been performed.

On the other hand, rice is also able to take up the MA–Fe complex, which is mediated by OsYSL15, a member belonging to the yellow stripe-like family (Inoue et al. 2009; Lee et al. 2009). OsYSL15 is localized to the plasma membrane and shows transport activity for the MA–Fe complex (Inoue et al. 2009; Lee et al. 2009). *OsYSL15* in roots is strongly induced by Fe deficiency, but not by Zn, Mn, or Cu deficiency (Inoue et al. 2009). *OsYSL15* is expressed in the epidermis, endodermis, cortex, and vascular bundles of the roots as well as in the leaves under Fe deficiency condition (Inoue et al. 2009; Lee et al. 2009). Knockout of *OsYSL15* decreased the Fe concentration in both the shoots and roots (Lee et al. 2009). However, the contribution of this transporter in paddy soil would be small because secreted MA will be diffused out of the rhizosphere.

After Fe uptake, a citrate transporter, OsFRDL1, is involved in the translocation of Fe from the roots to the shoots (Yokosho et al. 2009). OsFRDL1 is localized at the plasma membrane of root pericycle cells. Knockout of this gene resulted in increased Fe accumulation in the roots, but decreased Fe in the shoots. On the other hand, OsYSL2 is implicated in long-distance transport of NA-chelated Fe into sink tissues (Ishimaru et al. 2010).

Boron (B) is essential for the formation of cross-links between polysaccharides in the cell walls. Two transporters (AtNIP5;1 and AtBOR1) for B uptake have previously been identified in *Arabidopsis* (Takano et al. 2002, 2006). A homologue of *Arabidopsis* B uptake transporter AtBOR1 in rice, OsBOR1, was also reported to be involved in B uptake as well as in xylem loading (Nakagawa et al. 2007). OsBOR1 is expressed in the root exodermis, endodermis cells, and the stele (Nakagawa et al. 2007). Knockout of this gene resulted in decreased B levels in the shoot and xylem sap. OsBOR1 is an efflux transporter for B; therefore, an influx transporter-like AtNIP5;1 is required for transporting B from the soil solution to the root cells in rice. OsNIP3;1, the closest homologue of AtNIP5;1, is also localized at the plasma membrane and is permeable to boric acid. It is expressed in both roots and shoots (Hanaoka et al. 2014), but knockdown lines of *OsNIP3;1* did not significantly affect B uptake (Hanaoka et al. 2014). Recently, however, *OsNIP3;1* was identified as a gene responsible for the dwarf phenotype observed in rice mutant *dte1* (*Dwarf and tiller-enhancing 1*) and was implicated in B uptake (Liu et al. 2015). *OsNIP3;1*/*DTE1* is expressed abundantly in the exodermal cells in the roots as well as in the nodal region of adult leaves. Furthermore, the expression was induced by B deficiency (Liu et al. 2015). Although the shoot B concentration in the *dte1* mutant was not changed from the WT, growth and total B content were

significantly decreased in the mutant (Liu et al. 2015). Therefore, OsNIP3;1/DTE1 and OsBOR1 may constitute a cooperative uptake system like Lsi1–Lsi2 for silicon (Si) uptake described below, although further investigation is required particularly in terms of protein localization in the roots.

Copper (Cu), as a cofactor in proteins, is involved in a wide variety of physiological processes. Cu uptake in the roots is likely mediated by a member of copper transporter (COPT), a homologue of the yeast Cu transporter CTR1 (Sancenón et al. 2003; Yuan et al. 2011). There are seven members of COPT1-like transporters in rice; some of them are expressed in the roots, and their expression is induced by Cu deficiency (Yuan et al. 2011). These COPT1-like transporters can transport Cu in Cu^{+} form. However it is still unclear which of these COPT-like transporters are involved in Cu uptake in rice roots. On the other hand, OsHMA5, a member of heavy metal-transporting P-type ATPase (HMA), mediates the xylem loading of Cu (Deng et al. 2013). OsHMA5 is mainly localized at the pericycle, and knockout of this gene resulted in decreased root-to-shoot translocation of Cu (Deng et al. 2013, Fig. 13.1). OsHMA5 is also localized at the xylem region of DVBs in node I (Deng et al. 2013, Fig. 13.2). It is likely that OsHMA5 also plays an important role in releasing Cu from these tissues into the xylem connected to the grains (Deng et al. 2013). Its homologue, OsHMA4, has been involved in Cu accumulation in grain (Huang et al. 2016). OsHMA4 is also a Cu transporter but localized to the tonoplast. It is highly expressed in the vascular tissues of the stele, mainly in pericycle cells in the roots (Fig. 13.1). The mRNA expression of *OsHMA4* is induced by high Cu treatment. Knockout of OsHMA4 resulted in lower Cu accumulation in roots but higher accumulation in the shoots, indicating that OsHMA4 functions to sequester Cu into root vacuoles, limiting Cu accumulation in the grain at high Cu condition (Huang et al. 2016).

Another transporter involved in the Cu transport is OsYSL16, which is a transporter belonging to the yellow stripe-like family (Zheng et al. 2012). OsYSL16 is expressed in phloem regions of EVBs and DVBs in the nodes (Fig. 13.2). In addition, OsYSL16 is highly expressed in vascular tissues of the leaves (Zheng et al. 2012). Knockout of OsYSL16 resulted in decreased remobilization of Cu-nicotianamine complex from old leaves to new leaves and from the flag leaf to the panicle (Zheng et al. 2012). So OsYSL16 is involved in the delivery of Cu-nicotianamine complex to young tissues and grains by phloem transport.

13.3 Transporters for Beneficial Elements

Silicon (Si) is a beneficial element for plant growth, protecting plants from various stresses (Ma and Yamaji 2006, 2015). Plants take up Si from the soil in the form of silicic acid, a non-charged form below pH 9.0. Two transporters (Lsi1 and Lsi2) for Si uptake have been identified in rice (Ma et al. 2006, 2007). Lsi1 belongs to the Nod26-like major intrinsic protein (NIP) subfamily of aquaporins (Ma et al. 2006)

and is a Si-permeable channel. On the other hand, Lsi2, belonging to a putative anion transporter protein family, is an efflux transporter of Si (Ma et al. 2007). Both of them are localized at the root exodermis and endodermis with different polarity; Lsi1 is at the distal side and Lsi2 is at the proximal side (Fig. 13.1). Knockout of either Lsi1 or Lsi2 in rice results in a defect in Si uptake. Therefore, a cooperative transport by both Lsi1 and Lsi2 is required for the efficient uptake of Si in rice. Mathematical modeling revealed that the polar localization of Lsi1 and Lsi2 and the presence of Casparian strips are essential for efficient Si uptake in rice (Sakurai et al. 2015).

Following uptake by the roots through Lsi1 and Lsi2, Si is translocated to the shoot by transpirational mass flow in the xylem. In rice, more than 90% of Si taken up by the roots is translocated to the shoots (Ma and Takahashi 2002). Si in xylem of EVBs is unloaded by another Si transporter Lsi6 (Fig. 13.2), moved to adjacent cells through plasmodesmata, and then effluxed to the apoplast and reloaded to xylem of DVBs by Lsi2 and Lsi3, a homologue of Lsi2 (Yamaji and Ma 2009; Yamaji et al. 2008, 2015, Fig. 13.2). These three transporters are responsible for the preferential distribution of Si to the grain, which is required for high Si accumulation in the husk.

13.4 Transporters for Toxic Elements

Uptake of toxic elements such as cadmium (Cd) and arsenic (As) will affect our health through food chain. Rice is a major dietary source of Cd and As. Several transporters involved in Cd accumulation have been identified in rice. OsNramp5 described above as a Mn uptake transporter also transports Cd (Sasaki et al. 2012). A *Nramp5* knockout mutant and RNAi lines grown in soil show a strongly reduced Cd concentration of the straw and the grain. Other transporters such as OsIRT1, OsIRT2, and OsNramp1 (these are Fe^{2+} transporters induced under Fe deficiency) are potentially implicated in Cd uptake. However because OsNramp5 was shown to account for most of the rice Cd uptake under different Fe supply conditions, OsIRTs and OsNramp1 appear to play only a minor role in Cd uptake from the soil (Sasaki et al. 2012).

Part of Cd taken up is sequestered into vacuoles in the roots. This vacuolar sequestration process is facilitated by P_{1B}-type ATPase, OsHMA3 (heavy metal ATPase 3) (Ueno et al. 2010; Miyadate et al. 2011). OsHMA3 is localized at the tonoplast of all root cells (Fig. 13.1) and has an efflux transport activity of Cd. When OsHMA3 was overexpressed in rice, the transgenic plants greatly increased the Cd accumulation in the roots and strongly reduced Cd accumulation in the shoots (Sasaki et al. 2014). Another HMA family protein, OsHMA2, is responsible for the root-to-shoot translocation of Cd in rice (Satoh-Nagasawa et al. 2012; Takahashi et al. 2012b; Yamaji et al. 2013b). OsHMA2 is a transporter of Zn in rice and is localized in the plasma membrane of root pericycle cells at the vegetative growth stage (Fig. 13.1). Knocking out this transporter significantly

decreased the Zn concentration in the root tips and shoot elongating zone, suggesting that OsHMA2 transports Zn and Cd from the apoplast to the symplast to facilitate translocation via the phloem.

Low-affinity cation transporter 1 (OsLCT1) and OsHMA2 are involved in intervascular transfer of Cd in the nodes (Uraguchi et al. 2011; Yamaji et al. 2013b). The transcripts of *OsLCT1* were detected in cells surrounding the EVBs and DVBs and the phloem parenchyma cells of EVBs of node I. Knockdown of *OsLCT1* resulted in the reduction of grain Cd after grown in Cd-contaminated soil. The Cd concentration is reduced in the phloem sap but not in the xylem sap of the mutant. It seems that OsLCT1 plays a role in xylem-to-phloem transfer in node I.

OsHMA2 is also expressed in the upper nodes during the reproductive stage (Yamaji et al. 2013b). OsHMA2 was detected in the plasma membrane of phloem companion cells in EVBs and DVBs (Fig. 13.2). Mutation of OsHMA2 resulted in lower Cd accumulation in the tissues above node I, suggesting that OsHMA2 plays a role in the preferential delivery of Cd to these tissues.

On the other hand, As is a highly toxic metalloid for animals including human, and exposure to excessive As causes numerous diseases, such as cancers, diabetes, cardiovascular diseases, and developmental disorders (Abdul et al. 2015). A number of As species may be present in soil depending on soil conditions and the history of As contamination. These include arsenate [As(V)], arsenite [As(III)], and methylated As species such as monomethylarsonic acid [MMA(V)] and dimethylarsinic acid [DMA(V)]. Paddy rice shows about ten times higher accumulation of As compared with other cereal crops (Williams et al. 2007). High accumulation of As in paddy rice is caused by the mobilization of As in flooded paddy soil (Xu et al. 2008) and the highly efficient uptake of As by the silicon (Si) pathway in rice (Ma et al. 2008). In the anaerobic paddy field, As is mainly present in the form of arsenite (As(III)), which is taken up by two Si transporters, namely, Lsi1 and Lsi2 (Ma et al. 2008), which have high expression in the roots (see above; Fig. 13.1). Not only Lsi1 and Lsi2 but also other transporters might be involved in the transport of As(III) (Li et al. 2016).

After As(III) is taken up by the root cells, a part of this is released from the cytosol to the rhizosphere by the bidirectional transport of Lsi1 (Zhao et al. 2010a, Fig. 13.1). However, the remaining As are sequestrated into the root vacuoles or translocated to the shoots (Zhao et al. 2010b). For the detoxification of As by vacuolar sequestration, the complexation of As(III) with phytochelatins (PCs) is important. Recently, a tonoplast-localized ABC transporter, OsABCC1, was found to be involved in the As(III)–PC sequestration into the vacuoles (Song et al. 2014). *OsABCC1* was expressed in many organs, including the roots, leaves, nodes, peduncle, and rachis (Figs. 13.1 and 13.2). Knockout of OsABCC1 in rice resulted in decreased tolerance to As. At the reproductive stage, the As concentration was higher in the nodes and in other tissues of wild-type rice than in those of knockout mutants but was significantly lower in the grain. Therefore, OsABCC1 restricts the translocation of As into grains by sequestering As into the vacuoles in the nodes.

13.5 Control Mechanisms for Mineral Transport: Perception, Signaling, and Responses

Availability of mineral elements for plants in natural environment varies greatly depending on the weather, soil, growth conditions, and so on. But plants have many strategies to overcome mineral unbalances. Transporters involved in the uptake and translocation respond to these environmental changes at transcriptional and/or translational levels. For example, the gene expressions of OsPT6 and OsPT8 were upregulated by P deficiency condition (Ai et al. 2009; Jia et al. 2011). Fe^{2+} transporters *OsIRT1* and *IRT2* were also highly upregulated in response to iron deficiency condition. Several transcription factors including IDE1 and IDE2 and OsIRO2 and OsIRO3, which control Fe deficiency-induced genes, have been identified in rice (Kobayashi and Nishizawa 2012). Recently, two iron- and zinc-binding RING ubiquitin ligases, OsHRZ1 and OsHRZ2, were suggested to be Fe-binding sensors (Kobayashi et al. 2013). Knockdown of these genes resulted in increased tolerance to low iron availability but inferior growth under extreme iron excess conditions. The knockdown lines showed derepressed expression of various genes involved in iron uptake and transport, mainly under enough or excess iron availability, indicating that HRZ ubiquitin ligases negatively regulate the expression of the genes involved in iron uptake, transport, and accumulation, inhibiting excess iron accumulation.

Mn in soil solution fluctuates greatly from 0.5 to 200 μM depending on soil conditions. The transporters involved in Mn uptake (OsNrmap5 and OsMTP9) did not respond to external Mn concentration, whereas OsNramp3 localized at the nodes responded to high Mn at the protein level. Under Mn-limited condition, OsNramp3 mediates preferential distribution of Mn to the developing tissues. However, under high Mn condition, this protein was degraded within few hours although the transcript level of *OsNramp3* is unchanged.

More detailed mechanisms for the regulation of mineral acquisition have been investigated for some transporters. For example, in rice, Pi transporters are regulated at the transcriptional and posttranslational levels (Bayle et al. 2011; Wu et al. 2013). Recently, a CK2 kinase was found to regulate the trafficking of Pi transporters in response to phosphate availability (Chen et al. 2015). In P-sufficient conditions, Pi transporters (OsPHT1;2/OsPT2 and OsPHT1;8/OsPT8) are phosphorylated by the CK2α3/β3 holoenzyme, resulting in an inhibition of the interaction of PT8 with PHF1(phosphate transporter traffic facilitator1), a key cofactor regulating the exit of PTs from the endoplasmic reticulum (ER) to the plasma membrane. By contrast, low-Pi stress leads to a reduction of the phosphorylation and abundance of CK2β3 which limits CK2α3 access to PTs for phosphorylation. Therefore, the non-phosphorylated PTs can interact with PHF1 for efficient trafficking from the ER to the PM (Chen et al. 2015). In *Arabidopsis*, it was found that the PHO2 ubiquitin-conjugating E2 enzyme modulates the degradation of PHO1 in the endomembranes to maintain Pi homeostasis (Liu et al. 2012), but it is not known whether similar mechanisms are present in rice.

Regulatory mechanism of nitrate uptake has been extensively investigated in *Arabidopsis*. First, nitrate is sensed by nitrate transporter 1.1 (NRT1.1), which is switched from low to high affinity due to the phosphorylation of threonine-101 (T101) (Ho et al. 2009). Under low-nitrate conditions, CBL-interacting protein kinase 23 (CIPK23) can phosphorylate T101, making NRT1.1 function as a high-affinity nitrate carrier and then leading to a weak induction of gene expression of *NRT2.1* (Ho et al. 2009). *NRT2.1* is a high-affinity nitrate transporter gene, which shows a rapid induction of gene expression in response to nitrate treatments independent of protein synthesis. But it is not known whether similar mechanisms are present in rice.

13.6 Perspective

During the last decade, several transporters involved in the uptake and distribution of mineral elements have been identified as described above (Figs. 13.1 and 13.2); however, most transporters remain to be identified in the future. Plants use different transporters for different mineral elements. For the same mineral element, different transporters are required for uptake, root-to-shoot translocation, and distribution/redistribution. Furthermore, due to the distinct root anatomy of rice, a pair of influx and efflux transporters is required for the radial transport of mineral elements from the soil to the stele in order to cross the two Casparian strips at both exodermis and endodermis. However, the exact roles of most transporters are unknown, especially in terms of tissue and cellular localization. The regulation of these transporters in response to changing environments (deficiency or excess of mineral elements) also remains to be examined. Further investigation of these transporters will not only help to better understand mineral transport system in rice but also provide new tools for breeding rice with high nutrient use efficiency and low accumulation of toxic mineral elements. On the other hand, large genotypic differences in mineral accumulation and use efficiency have been reported. Identification of new alleles by using these genotypic differences will also provide new tools for breeding.

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