Chapter 7
The Role of Membrane Transport in the Detoxification and Accumulation of Zinc in Plants

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7.1 Introduction

Plants incorporate inorganic elements and molecules, water, and organic compounds from the soil. The average content of Zn in the earth's crust is 80 mg/kg. Soils contain from 5 to 800 mg of Zn/kg of dry weight with an average content of 60 mg/kg. The most cultivated soils contain 70–110 mg of Zn/kg. Seawater has 50 mg/L and freshwater has less than 50 μg Zn/L (0.8 μM) (Greger 2004). The amount of Zn in soil, rivers, and lakes increases by human activities such as metal mining, refining, industry, and fertilization of crops. Automobile tires contain a significant amount of ZnO as a vulcanizing agent and the debris causes Zn contamination of soils on roadsides (Smolders and Degryse 2002).

Zn is a nutrient essential for the growth, differentiation, and survival of cells, and it affects the growth of all living organisms. The human body contains 2–4 g of Zn, and the recommended daily intake is 8–11 mg. The adequate concentration of Zn in plants is considered to be around 1.3 μg/g of fresh weight (20 μM). Zn-deficiency symptoms appear when a plant contains less than 20 μg Zn/g dry weight (Marschner 1995). Symptoms of Zn deficiency include reduction in growth of internodes and leaves, a puckered appearance along leaf margin, a decrease in stress resistance, and appearance of chlorotic spots in leaves (Broadley et al. 2007; Clemens 2010). Zn-deficient conditions tend to generate reactive oxygen species (ROS), which strongly

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inhibit the growth of plants (Cakmak 2000). Nearly 30% of the world's cultivated soil is deficient in Zn. The problem is serious in several countries, especially India, Turkey, China, and Australia. Application of Zn is an efficient way to improve crop yield in such Zn-deficient soils. Widespread Zn deficiency in humans is also notable in such areas.

Even though Zn is an essential cofactor for many enzymes and regulatory proteins, excessive amounts of Zn(II) (hereafter referred to as Zn) disturb the balance of metal elements in cells and is harmful to the plants. Excessive absorption of Zn suppresses absorption of Fe and Cu absorption by plant roots (Rogers et al. 2000; Kawachi et al. 2009). Excess Zn concentrations cause chlorosis and growth disorders in plants. Damage due to excess Zn appears when Zn content in plants is more than 0.4 mg/g of dry weight (Beckett and Davis 1977). In cells, Zn replaces Fe, Mg, or Mn in metal-containing proteins and enzymes and suppresses their functions. For example, an increase in Zn concentration reduces photosynthesis, because the metal substitution of Mg with Zn suppresses the affinity of Rubisco for CO_2 and the activity of chlorophyll (Monnet et al. 2005). Furthermore, ROS is generated in mitochondria not only when Zn is lacking, but also when Zn is present in excess. Excess Zn inhibits or interferes with the respiratory chain, which might trigger ROS production in mitochondria (Dineley et al. 2003).

There are many excellent articles on recent findings on Zn in plants (Alkorta et al. 2004; Broadley et al. 2007; Clemens 2010; Eide 2006; Krämer et al. 2007; Krämer 2010; Palmer and Guerinot 2009; Palmgren et al. 2008). These articles provide comprehensive, key information for experimental research and for improvement of crops, soils, and the environment. Membrane transport systems are generally crucial for cell growth through uptake, sequestration, and export of nutrients and signaling substances (Groeneveld et al. 2009). Here we discuss the importance of Zn in living cells including signaling; we focus on Zn transporters, which are involved in the homeostasis of Zn in plant cells.

7.2 Biological Importance of Zn as a Structural Element and a Signal

7.2.1 Zn as a Cofactor of Enzymes and Transcription Factors

Zn acts as a Lewis acid and is able to react with a Lewis base to form an additional product (adduct). In cells, Zn exists as a coordination compound with proteins or organic acids. Zn tends to form a tetrahedral complex. Histidine (His) and cysteine (Cys) residues in polypeptides have high affinity for Zn. The Zn finger and RING (really interesting new gene) finger are known as Zn-binding motifs in proteins. The Zn finger motif in transcription factors is formed with two His and two Cys residues (Cys_2/His_2 type) and is involved in the stabilization of the DNA-binding domains. RING finger motif is defined as $CX_2CX_{9-27}CX_{1-3}HX_{2-3}CX_2CX_{6-17}CX_2C$ ($Cys_3HisCys_4$ type), which binds two Zn cations (Gamsjaeger et al. 2007). Other

motifs containing Cys and His also bind Zn in cells. These Zn-binding proteins function in gene transcription, translation, mRNA trafficking, cytoskeleton organization, protein folding, chromatin remodeling, Zn transport, and Zn sensing in various organisms.

Plant cells contain many Zn-containing enzymes, for example, alcohol dehydrogenase, glutamate dehydrogenase, carbonic anhydrase, Cu,Zn-superoxide dismutase (Cu,Zn-SOD), ribonuclease, and DNA polymerase (Addepalli and Hunt 2008; Balasubrahmanyam and Hunt 2008; Clemens 2010; Gamsjaeger et al. 2007; Vallee and Auld 1990). Zn plays a key role in the assisting and structural stabilization of catalytic function in these enzymes. In carbonic anhydrase, the Zn that interacts with the water molecule attracts an electron in H_2O, depolarizes, and stabilizes the intermediate of the reaction, $H_2O \rightarrow H^+ + OH^-$; and finally completes the reaction, $CO_2 + H_2O \rightarrow HCO_3^- + H^+$(Lilsjas et al. 1994). Therefore, a deficiency in Zn causes dysfunctions in formation of functional enzymes and transcription factors and disrupts metabolism, cell proliferation (via DNA polymerase), synthesis of RNAs and ribosomes (RNA polymerase), and defense against ROS (Cu,Zn-SOD).

7.2.2 Zn as a Signal

Zn serves as a structural element for many proteins and plays a key role in the catalytic action of Zn-containing enzymes through attracting their substrates. Zn was recently highlighted as a dynamic signaling ion, especially in animal cells (Sensi et al. 2009). Zn acts as a neurotransmitter and mimics the actions of hormones and growth factors (Yamasaki et al. 2007). Zn binds to numerous proteins in cells, where it serves as a structural and functional element of proteins involved in various cell activities. For example, the Zn-sensitive protein tyrosine phosphatases, protein kinases, and transcription factors are thought to be transmitters of Zn signaling. Zn signaling may be involved in cell proliferation, cell differentiation, immune system, and senescence in animals (Sensi et al. 2009; Haase and Rink 2009).

In addition to cytoplasmic and nuclear proteins, Zn specifically binds to many membrane receptors, transporters, and channels, and modulates their activity (Fig. 7.1). A kind of voltage-gated K^+ channel requires Zn to form an active tetramer as an essential element (Strang et al. 2003). In human cells, intracellular Zn reversibly activates large-conductance Ca^{2+}-activated K^+ channels (Hou et al. 2010). These ion channels are possibly positive and direct effectors of the Zn signal in the cells. Zn has a negative effect on some types of voltage-dependent Ca^{2+} channels through binding to the extracellular metal-binding sites of the channels (Kang et al. 2010). In other cases, extracellular Zn activates a Na^+/H^+ exchanger mediated by a G protein-coupled, Zn-sensing receptor (Azriel-Tamir et al. 2004).

Zn has been found to act as a signal in plants. In wheat, Zn stimulates translation of several genes through enhancement of dimer formation of a translation initiation factor and a poly(A)-binding protein (Cheng et al. 2008). Proteins with the Zn-binding motif are present in numerous protein families in eukaryotes and the

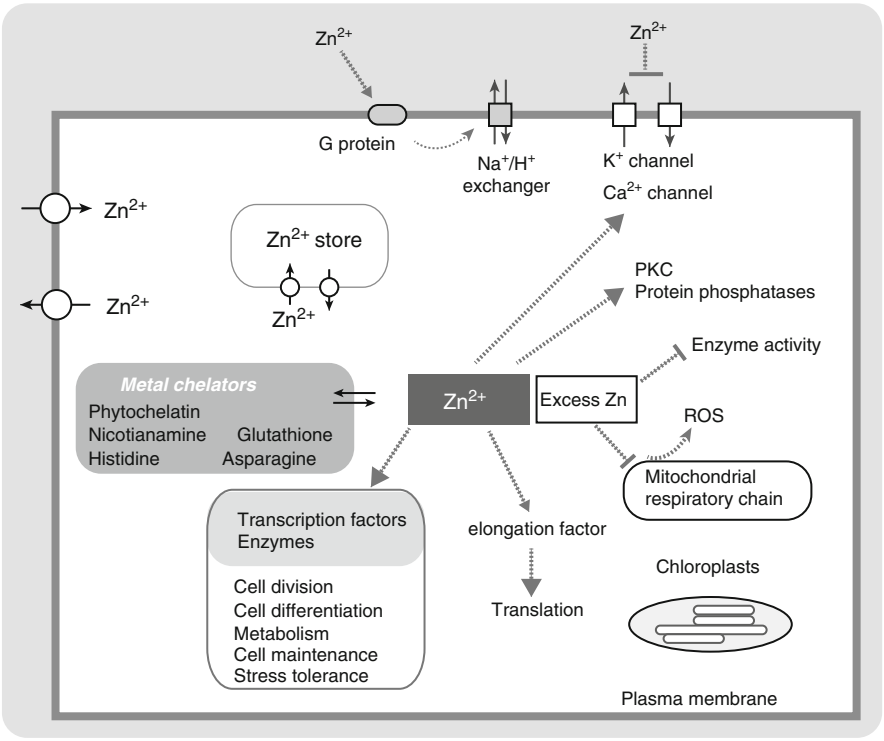

Fig. 7.1 *Zn-dependent cellular functions and homeostasis of Zn in plant cell.* Zn in the extracellular space (cell wall space) can regulate ion channels and ion transporters directly or indirectly. In the cell Zn is an essential element of many proteins, which are involved in various cell activities. Zn exists as a free ion, is stored in the organelles, or is chelated with organic compounds. Excess Zn damages many enzymes and stimulates the generation of reactive oxygen species (ROS). *G protein* guanine nucleotide binding protein, *PKC* protein kinase C

number of such proteins has been estimated to be more than 2,000 in plants (Gamsjaeger et al. 2007; Broadley et al. 2007). Several extracellular matrix metalloproteinases are included in this group (Flinn 2008). Some of these Zn-binding proteins and enzymes are targets of Zn signaling. Cell-imaging techniques reveal the homeostasis of Zn in the cytoplasm, vacuole, and nucleus, as shown for Ca in plant cells (Mazar et al. 2009). Zn signaling requires the maintenance of an adequate concentration of Zn in the cytoplasm, which is strictly regulated through the function of Zn transporters and Zn chelators. Loss-of-function mutants of Zn transporters in plants are key to understanding the function of Zn as a signal.

New experimental tools, including specific fluorescent probes (Zinpyr-1, etc.) coupled with transgenic plant lines that lack the Zn transporters, have revealed the dynamics of Zn in plant cells (Hanikenne et al. 2008; Kawachi et al. 2009). The function of many transcription factors and enzymes in the cytoplasm, nucleus, and other organelles is changed in response to the Zn level (Fig. 7.1). Membrane

transporters are involved in the regulation of Zn levels in the cytoplasm and cell organelles such as the vacuole (Krämer 2010; Martinoia et al. 2007).

7.3 Acquisition and Distribution of Zn

Zn exists in rocks, sands, and soils in several forms including ZnS, $ZnSO_4$, ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$, and Zn_2SiO_4 (Broadley et al. 2007). Fertilizers and agrochemicals provided to crop fields also contain Zn. Generally, a small part of the Zn in soils exists as free cations, the concentration of which increases at low soil pH and is available for plants. Concentrations of water-soluble Zn in the bulk soil solution have been estimated to be between 0.4 nM and 4 μM (Barber 1995). The Zn absorption properties of various plants suggest that the Michaelis–Menten parameter K_m for Zn has a submicromolar value (probably, <0.1 μM), close to the actual soil values (Broadley et al. 2007). Therefore, plant roots have a high potential to absorb Zn from the soil under normal conditions.

Zn absorbed by the epidermis including root hairs is transferred to cortical cells and then to the root xylem via the apoplast pathway, the transmembrane pathway, and the symplast pathway (xylem loading). The endodermis, which is lined between the cortex and the pericycle, has a Casparian strip that blocks the apoplast pathway. For this reason, the epidermal and endodermal cells might contain efficient Zn transporters in their plasma membranes. Zn is transported from the roots to shoots through the xylem and is redistributed to cells in stems and leaves by the apoplast and symplast pathways (xylem unloading).

Zn is compartmentalized in vacuoles, ER, Golgi apparatus, mitochondria, chloroplast, and the cell wall space (Fig. 7.2). There are numerous Zn-enzymes, Zn-proteins, and metal chelators with high and low affinity for Zn in the cytoplasm and cell organelles. Most Zn is required in the cytoplasm, nucleus, mitochondria, and chloroplasts, because there are many Zn-binding proteins in these compartments. Zn deficiency and toxicity occur at concentrations lower than 15–20 and higher than 100–300 μg/g of dry biomass, respectively (Krämer 2010). The adequate content of Zn in plants is 20–100 μg/g. However, the cytoplasmic Zn cation concentration ($[Zn]_{cyt}$) estimated for animal cells (<100 nM) (Sensi et al. 2009) and for *E. coli* (femtomolar level) (Outten and O'Halloran 2001) is very low. The $[Zn]_{cyt}$ might also be extremely low in plants (Broadley et al. 2007), although this value is not available because it is technically difficult to quantify. Most Zn is compartmentalized in cell organelles, especially vacuoles, and the cell wall space.

In mammalian and yeast cells, Zn-containing organelles called zincosomes have been identified. Zn-containing, vacuole-like small organelles have been shown to appear under conditions of excess Zn (Kawachi et al. 2009). It is still unknown what transporters of Zn exist in the organelles. Furthermore, ER and Golgi apparatus in plant cells may also function as a Zn store as shown in mammals (Ellis et al. 2005; Sensi et al. 2009).

Metal chelators with relatively high affinity to Zn include phytochelatin, glutathione, nicotianamine, histidine, and asparagine (Sharma and Dietz 2006). These

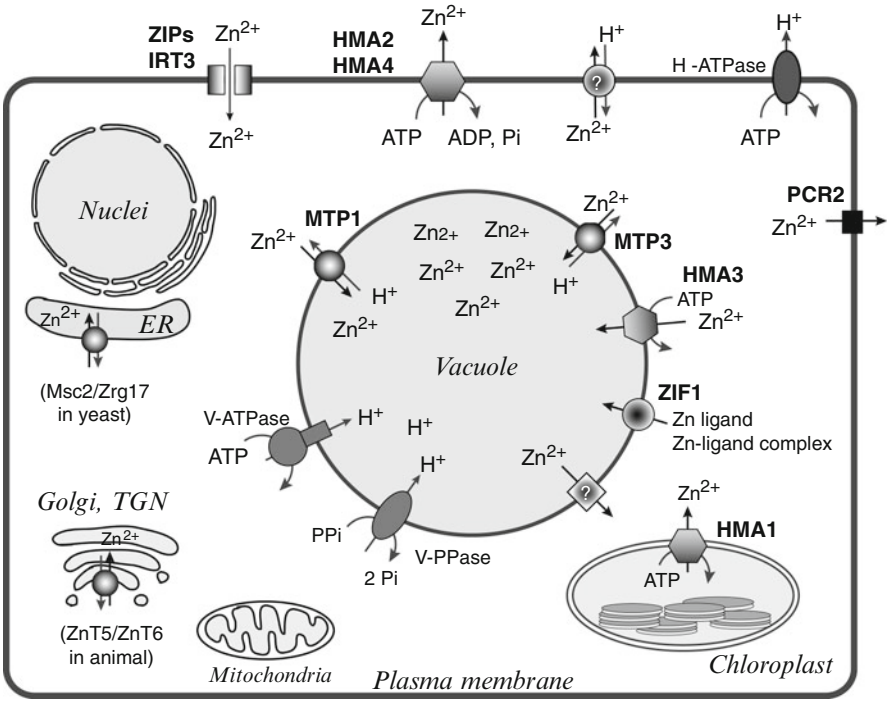

Fig. 7.2 *Membrane transport systems involved in homeostasis of Zn in the plant cell*. Various types of Zn transporters are involved in Zn homeostasis at the plasma membrane, vacuole, and chloroplast. *HMA* heavy metal ATPase, *IRT* iron-regulated transporter, *MTP* metal tolerance protein, *PCR* plant cadmium resistance, *TGN trans*-Golgi network, *V-ATPase* vacuolar H^+-ATPase, *V-PPase* vacuolar H^+-pyrophosphatase, *ZIF* zinc induced facilitator, *ZIP* ZRT1/IRT1-like protein, *ZnT* Zn transporter, *?* unknown proteins proposed to mediate transport of Zn

chelators immobilize Zn and play a role as a Zn pool in the cells. Zn is released from the chelators when $[Zn]_{cyt}$ decreases to the level of K_d of the chelators for Zn. Some phytochelatin is incorporated into vacuoles by an ATP-binding cassette (ABC) transporter and the vacuolar phytochelatin confers tolerance to Cd, but not Zn, in *Schizosaccharomyces pombe* (Mendoza-Cozatl et al. 2010). The plant homologue of the transporter remains to be identified.

7.4 Transport of Zn Across Membranes

7.4.1 Diversity of Zn Transporters

Plant cells contain several types of membrane transporters of Zn: metal tolerance protein (MTP), ZRT1/IRT1-like protein (ZIP) (also known as zinc–iron permease),

and heavy-metal ATPase (HMA) (P_{1B} subgroup of P-type ATPase) (Krämer et al. 2007). These are different in their structure and transport mechanism. ZIP is a passive transporter that facilitates the "downhill" flow of Zn until its electrical and/or concentration gradient is dissipated. MTP is a subfamily of the cation diffusion facilitator (CDF) and belongs to the active transporters. MTP utilizes a pH gradient across the membrane. HMA actively transports Zn using energy provided by ATP hydrolysis. Therefore, MTP and HMA pump Zn in a mode of thermodynamically "uphill" transport. It is unclear whether ABC transporters are involved in the transport of Zn or Zn-compound complexes in plants.

Recently, a zinc-induced facilitator 1 (ZIF1) has been reported to influence the detoxification and accumulation of Zn in *Arabidopsis thaliana* and to be different from the known Zn transporters, which transport free Zn cation (Haydon and Cobbett 2007). Carboxylic organic acids are effective ligands for chelation of Zn in plant cells. Therefore, ZIF1 is thought to transport low-molecular-mass Zn-ligands, such as organic acids, and/or Zn-ligand complexes into the vacuoles.

7.4.2 Zn Transporters in the Plasma Membrane

ZIP mediates electrodiffusion of Zn across the plasma membrane. ZIP members including ZIP1, ZIP2, ZIP3, ZIP9, and ZIP10 and IRT3 are thought to be responsible for Zn uptake into cells at the plasma membrane (Grotz et al. 1998; Talke et al. 2006). *ZIP4* gene expression is strongly induced in roots and shoots upon shortage of Zn supply and *ZIP4* transcription factors have been identified (Assunçao et al. 2010). ZIP members take up Zn using a pH gradient generated by plasma membrane H^+-ATPase (Palmgren et al. 2008). At present, it is unclear which member of the ZIP family is involved in Zn uptake from the soil at the epidermal cells and root hairs. The *IRT3* gene is also induced under Zn-deficient conditions in *A. thaliana* and is a candidate for a Zn uptake transporter (Krämer et al. 2007; Lin et al. 2009; van de Mortel et al. 2006). However, its ion selectivity remains to be investigated.

Active Zn pumps, HMA2 and HMA4, export excess Zn out of the cytosol. This process is essential to the release of Zn from the root symplasm into the apoplastic xylem vessels, which provide the primary pathway for the movement of Zn from the root to the shoot (Hanikenne et al. 2008; Palmgren et al. 2008). Indeed, HMA4 is highly expressed in the xylem parenchyma and the cambium of leaves in *A. thaliana* and *Arabidopsis halleri*, a metal hyperaccumulator. In other words, HMA4 is a key transporter in the process of loading Zn at roots and unloading Zn at leaves. AtHMA4 has eight predicted transmembrane domains with a long cytoplasmic loop and an extremely long C-terminal domain. This C-terminal tail, which is exposed to the cytoplasm, is involved in ion selectivity and transport activity (Mills et al. 2010).

Plant cadmium resistance 2 (PCR2), a new transporter, is also involved in Zn export at the plasma membrane in *A. thaliana* (Song et al. 2010). PCR2 might be essential for Zn distribution in plant organs and exclusion from the roots. The

functional mode of this transporter, which has only two transmembrane domains, is under investigation.

7.4.3 Zn Transporters in the Vacuolar Membrane

There is new information on the vacuolar Zn transporters that supports the vacuolar function as a Zn store in plant cells. MTPs function as Zn^{2+}/H^{+} exchanger and belong to the CDF family as do mammalian Zn transporters (ZnTs) and bacterial Zn transporters (YiiB and ZitB) (Lu et al. 2009; Ohana et al. 2009). MTP1 is localized in the vacuolar membrane and takes charge of active incorporation of cytoplasmic Zn into the vacuole using a proton gradient (Krämer 2010; Kawachi et al. 2008), which is generated by H^{+}-ATPase and H^{+}-pyrophosphatase (Maeshima 2001; Martinoia et al. 2007) (Fig. 7.2). A loss-of-function mutant of *AtMTP1* is sensitive to Zn as low as 0.4 mM (Kobae et al. 2004; Desbrosses-Fonrouge et al. 2005) and is defective in Zn accumulation in roots (Kawachi et al. 2009). A Zn hyperaccumulator *A. halleri* has been reported to have five gene copies of MTP1; its transcript levels of MTP1-A1, MTP1-A2, and MTP1-B are extremely high under normal and excess Zn conditions (Shahzad et al. 2010). Another Zn hyperaccumulator *Thlaspi goesingense*, which has three allelic variants of TgMTP1 (Kim et al. 2009), accumulates TgMTP1s at high levels in the vacuolar membranes in shoots (Gustin et al. 2009). Furthermore, overexpression of TgMTP1 leads to increased tolerance to Zn in transgenic *A. thaliana* (Gustin et al. 2009). Therefore, MTP1 plays a crucial role in the tolerance to Zn and accumulation of Zn.

The loss-of-function *atmtp1* plants of *A. thaliana* have a low Zn content. Roots of the wild-type and *atmtp1* seedlings grown under normal medium conditions contain Zn of approximately 207 and 116 μg/g dry weight, respectively (Kawachi et al. 2009). MTP1 contributes to a Zn accumulation of about 91 μg Zn/g dry weight. Dry weight is 8.4% of the fresh weight. The contribution of MTP1 on Zn uptake into vacuoles can be calculated to be 120 μM on the assumption that the volume of the vacuole accounts for 75% of root tissue.

MTP1 proteins of *A. thaliana*, *A. halleri,* and *T. goesingense* have a histidine (His)-rich loop between the fourth and fifth transmembrane domains. The His-rich region is absent in *E. coli* YiiP (Lu et al. 2009). Deletion of this region from AtMTP1 enhances the Zn transport velocity 11-fold. The His-rich loop might absorb Zn in the cytoplasm and transfer cations to the Zn transport pore of AtMTP1 (Kawachi et al. 2008). This Zn transfer process requires energy and lowers the transport rate. Structural and functional changes may occur during saturation of the loop with Zn. X-ray crystallography of YiiP, which lacks a His-rich loop (Lu et al. 2009), and further structural analyses of MTP1 proteins might shed light on the regulatory role and mechanism of the His-rich region.

MTP3 is also located in the vacuolar membrane and accumulates at a relatively high level in epidermal and cortex cell layers of the root hair zone when they are exposed to excess Zn (>30 μM) (Arrivault et al. 2006), although MTP1 is

expressed in the meristematic and elongating zones of the main and emerging lateral roots even under normal conditions. MTP3 might be involved in the compartmentalization and detoxification of Zn together with MTP1 under excess Zn conditions. Indeed, overexpression of MTP3 resulted in high accumulation of Zn in *A. thaliana* and conferred Zn tolerance compared with the wild-type plants (Arrivault et al. 2006). MTP3 has a short His-rich loop and an N-terminal region different from MTP1 (for sequence alignment, see Kawachi et al. 2008).

The vacuolar membrane contains HMA3, a P-type ATPase, which incorporates Zn, Cd, Co, and Pb ions into vacuoles (Morel et al. 2009). The gene is highly expressed in vascular tissues and the root apex. Overexpressing the gene enhances Zn tolerance in *A. thaliana*. AtHMA3 has been reported to be involved in the detoxification of biological (Zn) and other nonbiological heavy metals (Cd, Co, and Pb) by vacuolar sequestration. In rice, OsHMA3 of a *japonica* cultivar (Nipponbare), but not of an *indica* cultivar (Anjana Dhan), functions as a Cd transporter in the root vacuoles (Ueno et al. 2010). Cd is accumulated in the roots and the Cd content in the rice grains is kept low. The relationship between ion selectivity and specific amino acids is key to understanding the molecular evolution and physiological meaning of various metal transporters including MTP and HMA.

Zn exists in the form of free ions and complexes in vacuoles as well as the cytoplasm. In tobacco, almost 90% of Zn is sequestered by high levels of citrate in the vacuole while malate and oxalate hardly contribute to Zn accumulation in vacuoles (Wang et al. 1992). The stored Zn might be released from the vacuole and other compartments when the $[Zn]_{cyt}$ decreases. The transporter or channel for release of Zn from plant organelles remains to be identified.

7.4.4 Zn Transporters in Other Organelles

Recently, HMA1 has been identified in the chloroplast envelope of *A. thaliana* (Kim et al. 2009; Seigneurin-Berny et al. 2006). *AtHMA1* knockout plants are highly sensitive to high Zn levels and show increased accumulation of Zn in the shoot. In general, Zn can substitute easily for Mg and Fe because of similarity in ionic radius. Thus, many chloroplast proteins including plastoquinone, ferredoxin, and photosystems I and II are highly sensitive to excess Zn. AtHMA1 detoxifies Zn by transporting Zn from the chloroplast (plastid) stroma, which is rich in ATP, into the cytoplasm (Kim et al. 2009).

Mitochondria contain several Zn enzymes and require a minimum level of Zn in the matrix. Furthermore, mitochondria also have many Mg- and Fe-containing enzymes, such as aconitase and respiratory chain complexes, which might be sensitive to excess level of Zn. Therefore, Zn-uptake and Zn-export transporters may exist in mitochondria, although the transporters have not been identified.

In *Saccharomyces cerevisiae*, Zrg17p and Msc2p may form a heterodimer and transport Zn into the ER (Ellis et al. 2005). In mammals, ZnT5 and ZnT6, homologues of Msc2p and Zrg17, have been proposed to form a heterodimer and

transport Zn into the Golgi apparatus (Ellis et al. 2005). These four transporter proteins belong to the CDF family. Some members of the mammalian ZnT family are involved in Zn transport in the Golgi apparatus, lysosome, and synaptic vesicle (Sensi et al. 2009). Particular Zn transporters probably exist in the ER and Golgi apparatus membranes and regulate Zn homeostasis in these organelles in plants. The MTP members are plant homologues of mammalian ZnT and include candidates for proteins in the ER or Golgi apparatus. For example, proteins with accession numbers AC007231 and BT014889 have relatively high sequence similarity with human ZnT5.

7.4.5 Regulation of Zn-Related Genes

Quantitative regulation of Zn transporters is critical for plants to adapt to both deficiency and excess of Zn. A set of proteins responsive to Zn under Zn excess conditions was identified by proteomics techniques (Fukao et al. 2009). In yeast, Zn-responsive transcription factor Zap1 functions as a Zn sensor, activates its own transcription in Zn-limited conditions, and binds the Zn-responsive element of more than 100 genes. Under Zn-deficient conditions, Zap1 upregulates the plasma membrane Zn transporter gene *ZRT1* and the vacuolar membrane Zn transporter gene *ZRC1* and suppresses the genes for ADH1 and ADH3, which are abundant Zn-binding proteins in cells (Eide 2006). No plant homologues of Zap1 have been identified. In *A. thaliana*, two transcription factors, bZIP19 and bZIP23, have been identified (Assunçao et al. 2010). Both transcription factors have a leucine zipper motif and associate with the promoter regions of the genes encoding the proteins that constitute the primary response to zinc deficiency. The genes *ZIP1*, *ZIP3*, *ZIP4*, *ZIP5*, *ZIP9*, *ZIP12,* and *IRT3* are under regulation of bZIP19 and bZIP23. In wheat, Zn promotes the interaction between translation initiation factor (eIF4B) and poly (A)-binding protein (PABP) (Cheng et al. 2008). Formation of the complex is essential at the initiation of translation. Thus, Zn-related genes are regulated directly by Zn and indirectly through Zn-sensing transcription factors.

7.5 Conclusion

Zn functions as an essential metal for numerous enzymes and regulatory proteins. Recent studies highlight the role of Zn as a cell signal in various organisms through functional regulation of enzymes, membrane transport systems, and proteins involved in transcription and translation. Zn transporters, Zn-chelate compounds, and intracellular Zn-storage organelles take part in the homeostasis of Zn and in the function of Zn as a signal. In plants, excess Zn is removed from the cytoplasm and chloroplasts to the extracellular space or the intracellular stores, especially

vacuoles. Excessive Zn in the cytoplasm is incorporated in the vacuoles and Zn is supplied to the cytoplasm under Zn-deficient conditions. When the Zn concentration in cells increases beyond the capacity for Zn homeostasis, symptoms of excessive Zn appear. Zn accumulators resist excessive Zn by excluding Zn from the cytoplasm using Zn transporters or by detoxifying the high level of Zn by chelators.

Recent advances in genome database, metal quantification, and imaging techniques for Zn and Zn-related proteins have contributed knowledge on Zn homeostasis and on cross-homeostasis with other metals in plants. Studies on the structure–function relationship, functional regulation, and genetic regulation of Zn-related enzymes and transporters will enable us to fine-tune the homeostasis of Zn and grow plants adapted to the level of Zn in the environment. Zn signaling is an expanding area in plant science. The appearance of Zn-tolerant plants and high-Zn crops will improve the state of Zn nutrition in humans and livestock and enable phytoremediation of Zn-contaminated soil.

References

Addepalli B, Hunt AG (2008) Ribonuclease activity is a common property of *Arabidopsis* CCCH-containing zinc-finger proteins. FEBS Lett 582:2577–2582

Alkorta I, Hernandez-Allica J, Becerrisl JM, Amezaga I, Albizu I, Garbisu C (2004) Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Rev Environ Sci Biotechnol 3:71–90

Arrivault S, Senger T, Krämer U (2006) The *Arabidopsis* metal tolerance protein AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot under Fe deficiency and Zn oversupply. Plant J 46:861–879

Assunçao AGL, Herrero E, Lin YF et al (2010) *Arabidopsis thaliana* transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. Proc Natl Acad Sci USA 107: 10296–10301

Azriel-Tamir H, Sharir H, Schwartz B, Hershfinkel M (2004) Extracellular zinc triggers ERK-dependent activation of Na^+/H^+ exchange in colonocytes mediated by the zinc-sensing receptor. J Biol Chem 279:51804–51816

Balasubrahmanyam A, Hunt AG (2008) Ribonuclease activity is a common property of Arabidopsis CCCH-containing zinc-finger proteins. FEBS Lett 582:2577–2582

Barber SA (1995) Soil nutrient bioavailability, 2nd edn. Wiley, New York

Beckett PHT, Davis RD (1977) Upper critical levels of toxic elements in plants. New Phytol 79:95–106

Broadley MR, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. New Phytol 173:677–702

Cakmak I (2000) Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. New Phytol 146:185–205

Cheng S, Sultana S, Goss DJ, Gallie DR (2008) Translation initiation factor 4B homodimerization, RNA binding, and interaction with poly(A)-binding protein are enhanced by zinc. J Biol Chem 283:36140–36153

Clemens S (2010) Zn: a versatile player in plant cell biology. In: Hell R, Mendel RR (eds) Cell biology of metals and nutrients. Springer, Berlin

Desbrosses-Fonrouge AG, Voigt K, Schroeder A, Arrivault S, Thomine S, Krämer U (2005) *Arabidopsis thaliana* MTP1 is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation. FEBS Lett 579:4165–4174

Dineley KE, Votyakova TV, Reynolds IJ (2003) Zinc inhibition of cellular energy production: implications for mitochondria and neurodegeneration. J Neurochem 85:53–570

Eide DJ (2006) Zinc transporters and the cellular trafficking of zinc. Biochim Biophys Acta 1763:711–722

Ellis CD, MacDiarmid CW, Eide DJ (2005) Heteromeric protein complexes mediate zinc transport into the secretory pathway of eukaryotic cells. J Biol Chem 280:28811–28818

Flinn BS (2008) Plant extracellular matrix metalloproteinases. Funct Plant Biol 35:1183–1193

Fukao Y, Ferjani A, Fujiwara M, Nishimori Y, Ohtsu I (2009) Identification of zinc-responsive proteins in the roots of *Arabidopsis thaliana* using a highly improved method of two-dimensional electrophoresis. Plant Cell Physiol 50:2234–2239

Gamsjaeger R, Liew CK, Loughlin FE, Crossley M, Mackay JP (2007) Sticky fingers: zinc-fingers as protein-recognition motifs. Trends Biochem Sci 32:63–70

Greger M (2004) Metal availability, uptake, transport and accumulation in plants. In: Prasad MNV (ed) Heavy metal stress in plants: from biomolecules to ecosystems, 2nd edn. Springer, Berlin

Groeneveld P, Stouthamer AH, Westerhoff HV (2009) Super life: how and why "cell selection" leads to the fastest-growing eukaryote. FEBS J 276:254–270

Grotz N, Fox T, Connolly E, Park W, Guerinot ML, Eide D (1998) Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency. Proc Natl Acad Sci USA 95:7220–7224

Gustin JL, Loureiro ME, Kim D, Na G, Tikhonova M, Salt DE (2009) MTP1-dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn-hyperaccumulating plants. Plant J 57:1116–1127

Haase H, Rink L (2009) Functional significance of zinc-related signaling pathways in immune cells. Annu Rev Nutr 29:133–152

Hanikenne M, Talke IN, Haydon MJ et al (2008) Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of *HMA4*. Nature 453:391–396

Haydon MJ, Cobbett CS (2007) A novel major facilitator superfamily protein at the tonoplast influences zinc tolerance and accumulation in *Arabidopsis*. Plant Physiol 143: 1705–1719

Hou S, Vigeland LE, Zhang G et al (2010) Zn^{2+} activates large conductance Ca^{2+}-activated K^+ channels via an intracellular domain. J Biol Chem 285:6434–6442

Kang HW, Vitko I, Lee SS, Perez-Reyes E, Lee JH (2010) Structural determinants of the high affinity extracellular zinc binding site on $Ca_v3.2$ T-type calcium channels. J Biol Chem 285:3271–3281

Kawachi M, Kobae Y, Mimura T, Maeshima M (2008) Deletion of a histidine-rich loop of AtMTP1, a vacuolar Zn^{2+}/H^+ antiporter of *Arabidopsis thaliana*, stimulates the transport activity. J Biol Chem 283:8374–8383

Kawachi M, Kobae Y, Mori H, Tomioka R, Lee Y, Maeshima M (2009) A mutant strain *Arabidopsis thaliana* that lacks vacuolar membrane zinc transporter MTP1 revealed the latent tolerance to excessive zinc. Plant Cell Physiol 50:1156–1170

Kim Y-Y, Choi H, Segami S et al (2009) AtHMA1 contributes to detoxification of excess Zn(II) in *Arabidopsis*. Plant J 58:737–753

Kobae Y, Uemura T, Sato MH, Ohnishi M, Mimura T, Maeshima M (2004) Zinc transporter of *Arabidopsis thaliana* AtMTP1 is localized to vacuolar membranes and implicated in zinc homeostasis. Plant Cell Physiol 45:1749–1758

Krämer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61:517–534

Krämer U, Talke IN, Hanikenne M (2007) Transition metal transport. FEBS Lett 581: 2263–2272

Lilsjas A, Hikansson K, Jonsson BH, Xue Y (1994) Inhibition and catalysis of carbonic anhydrase-recent crystallographic analyses. Eur J Biochem 219:1–10

Lin YF, Liang HM, Yang SY et al (2009) Arabidopsis IRT3 is a zinc-regulated and plasma membrane localized zinc/iron transporter. New Phytol 182:392–404
Lu M, Chai J, Fu D (2009) Structural basis for autoregulation of the zinc transporter YiiP. Nat Struct Biol 16:1063–1067
Maeshima M (2001) Tonoplast transporters: organization and function. Annu Rev Plant Physiol Plant Mol Biol 52:469–497
Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London
Martinoia E, Maeshima M, Neuhaus E (2007) Vacuolar transporters and their essential role in plant metabolism. J Exp Bot 58:83–102
Mazar C, Bourque S, Mithofer A, Pugin A, Ranjeva R (2009) Calcium homeostasis in plant cell nuclei. New Phytol 181:261–274
Mendoza-Cozatl DG, Zhai Z, Jobe TO et al (2010) Tonoplast-localized Abc2 transporter mediates phytochelatin accumulation in vacuoles and confers cadmium tolerance. J Biol Chem 285: 40416–40426
Mills RF, Valdes B, Duke M et al (2010) Functional significance of AtHMA4 C-terminal domain *in planta*. PLoS One 5:e13388
Monnet F, Vaillant N, Hitmi A, Sallanon H (2005) Photosynthetic activity of *Lolium perenne* as a function of endophyte status and zinc nutrition. Funct Plant Biol 32:121–139
Morel M, Crouzet J, Gravot A et al (2009) AtHMA3, a P-1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in Arabidopsis. Plant Physiol 149:894–904
Ohana E, Hoch E, Keasar C, Kambe T et al (2009) Identification of the Zn^{2+} binding site and mode of operation of a mammalian Zn^{2+} transporter. J Biol Chem 284:17677–17686
Outten CE, O'Halloran TV (2001) Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. Science 292:2488–2492
Palmer CM, Guerinot ML (2009) Facing the challenges of Cu, Fe and Zn homeostasis in plants. Nat Chem Biol 5:333–339
Palmgren MG, Clemens S, Williams LE et al (2008) Zinc biofortification of cereals; problems and solutions. Trends Plant Sci 13:464–473
Rogers EE, Eide DJ, Guerinot ML (2000) Altered selectivity in an Arabidopsis metal transporter. Proc Natl Acad Sci USA 97:12356–12360
Seigneurin-Berny D, Bravot A, Auroy P et al (2006) HMA1, anew Cu-ATPase of the chloroplast envelope, is essential for growth under adverse light conditions. J Biol Chem 281: 2882–2892
Sensi SL, Paoletti P, Bush AI, Sekler I (2009) Zinc in the physiology and pathology of the CNS. Nat Rev Neurosci 10:780–791
Shahzad Z, Gostil F, Ferot H et al (2010) The five AhMTP1 zinc transporters undergo different evolutionary fates towards adaptive evolution to zinc tolerance in *Arabidopsis halleri*. PLoS Genet 6:e1000911
Sharma SS, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57:711–726
Smolders and Degryse (2002) Fate and effect of zinc from tire debris in soil. Environ Sci Technol 36:3706–3710
Song WY, Choi KS, Kim DY et al (2010) *Arabidopsis* PCR2 is a zinc exporter involved in both zinc extrusion and long-distance zinc transport. Plant Cell 22:2237–2252
Strang C, Kunjilwar K, DeRubeis D, Peterson D, Pfaffinger PJ (2003) The role of Zn^{2+} in Shal voltage-gated potassium channel formation. J Biol Chem 278:31361–31371
Talke IN, Hanikenne M, Krämer U (2006) Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*. Plant Physiol 142:148–167
Ueno D, Yamaji N, Kono I et al (2010) Gene limiting cadmium accumulation in rice. Proc Natl Acad Sci USA 107:16500–16505
Vallee BL, Auld DS (1990) Zinc coordination, function, and structure of zinc enzymes and other proteins. Biochemistry 29:5647–5659

van de Mortel JE, Villanueva LA, Schat H et al (2006) Large expression differences in genes for iron and zinc homeostasis stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 142:1127–1147

Wang J, Evangelou BP, Nielsen MT, Wagner GJ (1992) Computer, simulated evaluation of possible mechanisms for metal ion activity in plant vacuoles: II. Zinc. Plant Physiol 99: 621–626

Yamasaki S, Sakata-Sagawa K, Hasegawa A et al (2007) Zinc is a novel intracellular second messenger. J Cell Biol 177:637–645