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Put the metal to the petal: metal uptake and transport throughout plants

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Compared to other organisms, plants have expanded families of transporters that are involved in the uptake and efflux of metals. Fortunately, in many cases, the examination of double mutants has been sufficient to overcome the challenge of studying functionally redundant gene families. Plants that lack two heavy-metal-transporting P-type ATPase family members (HMA2 and HMA4) reveal a function for these transporters in Zn translocation from roots to shoots. Likewise, the phenotype of plants that lack two natural resistance associated macrophage protein (NRAMP) homologs (NRAMP3 and NRAMP4) implicate these metal uptake proteins in the mobilization of vacuolar Fe stores during seed germination. Most families of metal transporters are ubiquitous but the Yellow Stripe1-Like (YSL) family is plant specific and YSL family members have been implicated in the transport of metals that are complexed with a plant specific chelator called nicotianamine (NA).

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Introduction

As sessile organisms, plants have developed strategies to obtain essential metal micronutrients from soils of varying compositions [1]. Not surprisingly, they use a variety of mechanisms to assimilate metals while preventing toxicity, which involve the regulation of transport, chelation, and sequestration. As complex multicellular organisms, metal uptake and efflux at the cellular level must be coordinated with the needs of the whole plant to maintain metal-ion homeostasis. Plant genomes encode large families of metal transporters that vary in their substrate specificities, expression patterns, and cellular localization to govern metal translocation throughout the plant. Transporters that are involved in metal efflux from the cytoplasm, either by movement across the plasma membrane (PM) or into organelles, include the P_{1B}-ATPase family, which has eight *Arabidopsis thaliana* homologs [2], and the cation diffusion facilitator (CDF) family, which has 12 members in *A. thaliana* [3]. Proteins that are referred to as metal-uptake transporters either act at the PM to move metals into the cytoplasm or remobilize metals from intracellular compartments into the cytoplasm. The natural resistance associated macrophage protein (NRAMP) and zinc-regulated transporter, iron-regulated transporter-like protein (ZIP) families provide examples of such proteins. There are six NRAMP and 16 ZIP proteins in *A. thaliana* [4,5], whereas the plant-specific Yellow Stripe1-Like (YSL) family of metal uptake transporters includes eight *A. thaliana* homologs [6].

Beyond what has been reviewed previously [7,8], we highlight studies from the past two years that have improved our understanding of metal transport in plants. The movement of metals by PM-localized transporters in the *A. thaliana* root is depicted in Figure 1, whereas Figure 2 summarizes intracellular metal transport in *A. thaliana*. Tissue expression patterns, cellular localization, inducing conditions, and substrates for family members discussed here can be found in Table 1.

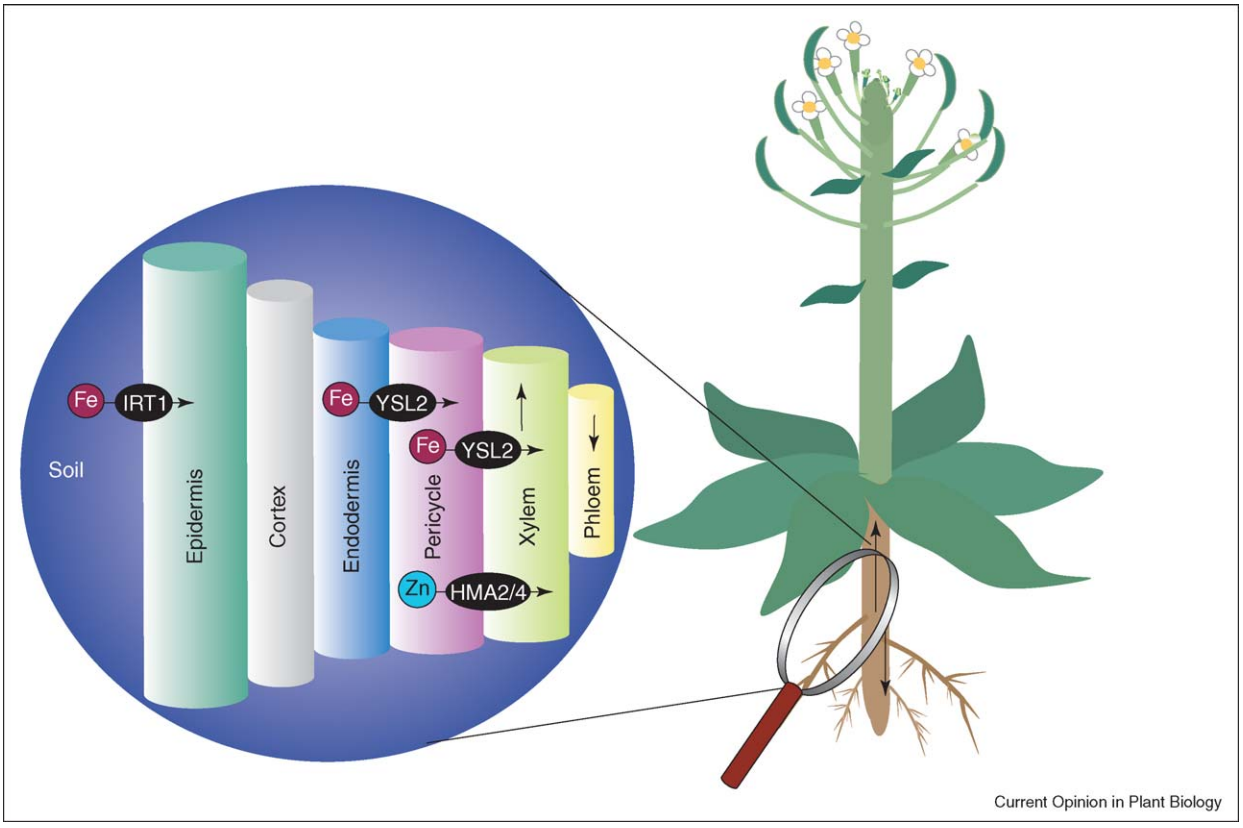
Metal efflux proteins

P_{1B}-ATPase family

The superfamily of P-type ATPases use energy from ATP hydrolysis to translocate cations across biological membranes and can be divided into several subfamilies, including the heavy-metal-transporting P_{1B}-ATPases [9,10]. Structural features of P_{1B}-ATPases, including eight predicted transmembrane domains, a CPx motif that is thought to play a role in translocation, and putative metal-binding domains in the amino or carboxyl terminus, distinguish them from other P-type ATPases. The eight P_{1B}-ATPases in *A. thaliana* are designated heavy-metal-transporting P-type ATPase (HMA)1 through HMA8 [2]. Four family members (HMA1–HMA4) group with the Zn/Cd/Pb/Co divalent cation transporter class of P_{1B}-ATPases, whereas *HMA5–HMA8* encode Cu/Ag monovalent cation transporters [10,11].

Metal transport studies of HMA2 and HMA4 in yeast provided evidence of their enzymatic functions and transport abilities. Measurements of ATPase activity in yeast expressing HMA2 show that HMA2 is indeed a Zn^{2+}-ATPase and, like classic P-type ATPases, HMA2 undergoes vanadate-sensitive phosphorylation in the presence of ATP and its metal substrate [12]. HMA2 is activated to a similar extent by the non-physiological heavy metal Cd^{2+} and to a lesser extent by other divalent cations [12], which is a common feature of Zn-ATPases. Kinetic determinations

Figure 1



Metal transport in the *A. thaliana* root. Metals including Fe and Zn are assimilated from the soil and must cross multiple cell membranes before being transferred to the xylem for delivery to growing tissues. PM localized transporters and their substrates are indicated, with arrows specifying the direction of transport. IRT1 acts at the epidermis to transport Fe^{2+} into the root. YSL2 is localized to the endodermis and pericycle where is it believed to transport Fe. Two P_{1B}-ATPases, HMA2 and HMA4, function redundantly to translocate Zn from the root to aerial tissues, probably by export into the xylem.

reveal that HMA2 is activated with high affinity by Zn ($K_{1/2}$ = 0.11 μM) and Cd ($K_{1/2}$ = 0.031 μM) [12].

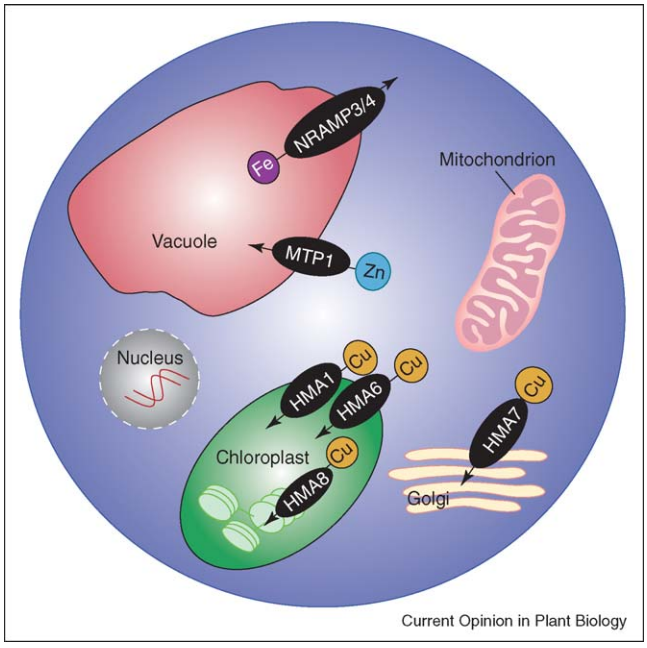
Heterologous expression of *HMA4* in wildtype and metal-sensitive yeast strains demonstrated that *HMA4* transports Zn, Cd, and Pb [13]. Radiolabeled ^{109}Cd- and ^{65}Zn-uptake assays show decreased accumulation of Cd and Zn in yeast transformed with *HMA4* compared to the empty vector control, consistent with a role for HMA4 in metal efflux [14]. Similar to results for HMA3 [15], mutation of the D phosphorylation site within a conserved DKTGT motif of HMA4 inhibits metal transport ability in yeast, indicating that functional complementation by HMA4 is due to metal transport rather than chelation [13].

Reverse genetic approaches have been successful in revealing the function of HMA family members *in planta*. No growth phenotypes were associated with soil-grown *hma2*, *hma3* or *hma4* single mutants [16••], but *hma4* plants show Zn and Cd sensitivity when grown in the presence of high Zn or Cd [14]. *hma2 hma4* double mutant plants are chlorotic, stunted, and fail to set seed — a phenotype that can be rescued by watering with high levels of Zn (1 or 3 mM), but not of Co or Cu [16••]. The severe phenotype of the double mutant (but not single mutants) indicates that these genes function redundantly and points to a specific defect in Zn homeostasis.

Zn levels of *hma2 hma4* plants were 2–4-fold lower than those of wildtype plants when grown under 10 μM Zn (+Zn conditions) and when Zn was omitted from the media (−Zn conditions) in all tissues examined with the important exception of −Zn roots, where the double mutant had twice the Zn concentration of wildtype plants [16••]. Consistent with the decrease in Zn and Cd concentrations in leaves of *hma4* loss-of-function mutants [16••,17•], *35S-HMA4* plants (in which *HMA4* is overexpressed) accumulate elevated levels of Zn and Cd in leaves [17•]. The *hma2 hma4* phenotype, along with the complementary HMA4 overexpressor data, supports a role for HMA2 and HMA4 in Zn translocation from roots to shoots.

A metal transport assay performed in yeast shows that HMA2 transports metal substrates out of the cytoplasm

Figure 2



Intracellular metal transport in *A. thaliana*. Metal delivery to and remobilization from intracellular compartments are important considerations in cellular ion homeostasis. The localization of intracellular metal transporters and their substrates are summarized in a generic cell, with arrows indicating the direction of transport. The related NRAMP proteins NRAMP3 and NRAMP4 function to access vacuolar Fe stores during seedling development. The CDF family member MTP1 is present in roots and leaves, where it pumps Zn into the vacuole. Four P_{1B}-ATPases that are implicated in Cu homeostasis have been localized. HMA6 (PAA1) and HMA1 are localized to the plastid envelope, HMA8 (PAA2) functions at the thylakoid membrane, and HMA7 (RAN1) resides at the Golgi for Cu loading [22,23].

[12]. This knowledge, in conjunction with the *in planta* localization of HMA2 [16••] and HMA4 [17•] to the PM, suggests that these proteins translocate heavy metals out of cells. The vascular expression of *HMA2* [12,16••] and *HMA4* [16••,17•] points to a possible role in xylem loading or unloading. A similar root expression pattern was previously observed for the boron transporter BOR1 [18••,19] and for the phosphate transporter PHO1 [20], which have both been implicated in xylem loading. The role of AtHMA2 and AtHMA4 in the translocation of heavy metals from root to shoot tissues makes these transporters potential targets for the generation of transgenic plants that are designed to clean up soils that have been contaminated with toxic metals, such as Cd and Pb. Engineering plants with an enhanced ability to accumulate heavy metals in aerial tissues is a crucial step in phytoremediation [21].

Members of the Cu/Ag-transporting class of P_{1B}-ATPases include HMA7 (RESPONSIVE TO ANTAGONIST1 [RAN1]), which is important for Cu delivery to hormone receptors at post-Golgi compartments [22,23]. More recently, *HMA5* was characterized as a root-enhanced, Cu-induced gene [24]. The amino-terminal MxCxxC Cu-binding motifs of HMA5 interact with *A. thaliana* ATX1-like Cu chaperones in yeast two-hybrid screens [24]. Considering the Cu hypersensitivity of *hma5* plants [24], it appears that this HMA family member is involved in Cu detoxification of roots in response to high Cu.

Three P_{1B}-ATPases are involved in Cu transport in the chloroplast. HMA1 [25] and HMA6 (P-type ATPase of *Arabidopsis*1 [PAA1]) [26,27] are localized to the plastid envelope where they deliver Cu to the stroma. Although HMA1 groups with the Zn/Cd/Pb/Co class of transporters, there is evidence of Cu and Zn transport by HMA1 in yeast and, in comparison with wildtype plants, *hma1* plants exhibit increased sensitivity to high light and reduced chloroplast Cu content [25]. *HMA1* and *HMA6* do not have completely redundant functions as each single loss-of-function mutant accumulates approximately half as much Cu in the chloroplasts as wildtype plants [25,26]. A complete loss of Cu accumulation in chloroplasts might be expected in *hma1 hma6* plants, although double mutants have not yet been analyzed. HMA8 (PAA2) resides at the thylakoid membrane and functions in Cu delivery to the thylakoid lumen [26,27]. *hma8* plants display greatly reduced Cu levels in thylakoids compared to whole chloroplasts, as determined by elemental analysis of chloroplast fractions. This is consistent with the localization of HMA8 and supports a role for this protein in Cu transport to the thylakoid lumen [26].

CDF family

The ubiquitous CDF family of metal transporters contain six transmembrane domains and encode proton antiporters that efflux heavy metals out of the cytoplasm [28]. The first CDF gene characterized in *A. thaliana* was the zinc transporter gene *ZAT1* [29], later renamed *METAL TOLERANCE PROTEIN1* (*MTP1*) [3]. Its overexpression confers Zn tolerance *in planta* [29]. The Zn-sensitive phenotype of insertion [30] and knockdown [31] *mtp1* mutants also supports a role for MTP1 in Zn homeostasis. Transient expression of AtMTP1::green fluorescent protein (GFP) in *A. thaliana* protoplasts [30,31], analysis of stable lines [31] and biochemical studies [30] indicate that AtMTP1 is localized to the vacuolar membranes of leaf and root cells, suggesting a role in Zn sequestration in the vacuole. Evidence that *mtp1* knockdown plants accumulate less Zn in various tissues indicates that the proposed defect in vacuolar Zn storage affects Zn uptake or distribution [31].

Understanding the mechanisms used by hyperaccumulating plant species has implications for phytoremediation. The upregulation of metal transporters from a variety of families is one way in which some hyperaccumulating species achieve increased metal translocation to aerial tissues, a hallmark feature of hyperaccumulating

Table 1

Summary of discussed genes.

	Tissue expression	Cellular localization	Inducing conditions	Proposed/known substrates	Reference(s)
Metal efflux proteins					
P_{1B}-ATPase (8)					
AtHMA2/HMA4	Vasculature of root and shoot, anther	PM		Zn, Cd	[12–14, 16••,17•]
AtHMA5	Root, flower		+Cu	Cu	[24]
AtHMA6(PAA1)	Root, shoot	Plastid envelope		Cu	[26,27]
AtHMA8(PAA2)	Shoot	Thylakoid membrane		Cu	[26]
AtHMA1	Root, shoot	Chloroplast envelope		Cu	[25]
CDF (12)					
AtMTP1	Root, shoot, flower	VM		Zn	[30,31]
AhMTP1	Root	VM	+Zn	Zn	[34]
TgMTP1		PM		Zn	[36]
Metal uptake proteins					
YSL (8)					
ZmYS1	Root, shoot		−Fe	Fe^{3+}–PS, Fe^{3+}-, Fe-, Ni-, Cu–NA	[6,37•,38•]
AtYSL1	Silique, leaf (xylem parenchyma), flower		+Fe	Fe–NA	[41]
AtYSL2	Root (endoderm pericycle), shoot	PM	+Fe, downregulated by −Zn		[39,40]
OsYSL2	Leaf (phloem), root, seed	PM	−Fe	Fe-, Mn–NA	[42]
NRAMP (6)					
AtNRAMP3/ NRAMP4	Root, shoot, seed	VM		Fe	[49••,50]
TjNRAMP4				Ni	[51]
ZIP (16)					
OsZIP4	Root, shoot (phloem meristem)	PM	−Zn	Zn	[59]
MtZIP1	Root, leaf		−Zn	Zn	[60]
MtZIP3	Root, leaf		Downregulated by −Mn, −Fe	Fe	[60]
MtZIP4	Root, leaf		−Zn	Mn	[60]
MtZIP5	Leaf		−Zn, −Mn	Zn, Fe	[60]
MtZIP6	Root, leaf			Zn, Fe	[60]
MtZIP7	Leaf			Mn	[60]
TjZNT1				Ni, Cd, Mn, Zn	[51]
TjZNT2				Ni, Cd, Mn	[51]
COPT (5)					
AtCOPT1	Root, pollen, embryo, stomata, trichome		Downregulated by Cu	Cu	[61,62]

Tissue expression, cellular localization, conditions known to increase steady-state transcript levels and proposed/known substrates are summarized for all reviewed genes, which are listed by gene family. The number of *A. thaliana* family members is shown in parentheses. Substrates are divalent unless otherwise indicated. VM = Vacuolar membrane.

plants. The Zn/Cd hyperaccumulator *A. halleri* safely accumulates 100-fold more Zn than non-accumulating species. Cross-species microarray analysis implicated increased expression of *MTP1* in shoots [32•] and a nicotianamine (NA) synthase gene in roots [33•] in Zn accumulation. *A. halleri* contains three independently segregating and differentially regulated *MTP1* genes, and in a backcross between the hyperaccumulator *A. halleri* and the non-accumulator *A. lyrata*, two *MTP1* loci co-segregate with Zn tolerance [34]. Evidence for Zn transport activity of *AhMTP1* and one of the *A. halleri* transporters, AhMTP1-3. is provided by functional complementation of the Zn-sensitive phenotype of *zrc1 cot1*, a Zn-hypersensitive strain that lacks the vacuolar CDF family members ZINC RESISTANCE CONFERRING1 (ZRC1) and COBALT TRANSPORTER1 (COT1) [34]. Like AtMTP1, AhMTP1-3 localizes to the vacuolar membrane, as shown by transient expression of GFP:AhMTP1-3 in *A. thaliana* protoplasts [34].

Thlaspi goesingense is a Ni/Zn hyperaccumulator that also relies on enhanced expression of CDF transporters for

metal tolerance [35]. When expressed in *zrc1 cot1*, TgMTP1b fused to an epitope of the influenza haemagglutinin protein (TgMTP1b-HA) localizes to vacuolar and plasma membranes, and the *zrc1 cot1* plants show a two-fold reduction in Zn levels [36]. By varying the copy number of *TgMTP1b* in the *zrc1 cot1* strain, the level of *TgMTP1b* expression was found to correlate positively with the rate of Zn efflux [36]. These results indicate that TgMTP1b does not confer resistance to high Zn by reversing the defect in vacuolar Zn compartmentalization of *zrc1 cot1*. Rather, long- and short-term uptake studies support a role for TgMTP1b in Zn efflux. This evidence, taken together with the PM localization of TgMTP1b::GFP in *A. thaliana* protoplasts [36], supports a role for MTP1 in Zn efflux at the PM in *T. goesingense*.

Metal uptake proteins

YSL family

Several transporter families translocate metals into the cytoplasm, either by uptake at the PM or remobilization from an intracellular organelle. YSL proteins are believed to mediate the uptake of metals that are complexed with plant-derived phytosiderophores (PS) or NA, a non-proteinogenic amino acid found throughout the plant kingdom that serves as a precursor for PS synthesis in grasses [6]. At the biochemical level, the best-studied member of this plant-specific family is YS1 from maize. ZmYS1 protein accumulates in the roots and leaves of Fe-starved plants and functions as a proton-coupled symporter to transport Fe–PS [37•,38•]. Yeast and oocyte transport studies indicate that ZmYS1 might have other substrates in addition to Fe, including Zn, Ni, and Cu [37•,38•].

On the basis of sequence similarity to ZmYS1, *A. thaliana* has eight predicted YSL proteins. Considering that non-grasses do not produce or use PS, AtYSLs most probably transport metal–NA complexes. Two family members, *AtYSL1* and *AtYSL2*, have recently been studied in some detail. A *YSL2*-promoter β-glucuronidase (GUS) fusion stains xylem-associated cells within the vasculature of expanded leaves and has a broad expression pattern within differentiated roots [39]. A more restricted root expression pattern in the pericycle and endodermis has also been reported [40]. *AtYSL2* transcript accumulation increases under conditions of Fe sufficiency or Fe resupply [39,40], and *AtYSL2* transcript levels also respond to Cu [39] and Zn [40]. The mRNA expression pattern of *AtYSL2* and its apparent protein localization in lateral membranes [39] suggest that AtYSL2 might function in the lateral transport of metals in veins. Although heterologous expression in yeast is often useful in determining substrates for metal transporters, conflicting results have been reported for AtYSL2. The expression of *AtYSL2* in metal-uptake-defective yeast strains using the pFL61 vector mediated the uptake of Fe–NA and Cu–NA [39]. Expression of the pFL61 vector alone, however, reduces that viability of yeast that lack the Fe-uptake proteins FET3/FET4, and the presence of AtYSL2 only restores growth to normal levels [40]. Complementation by AtYSL2 was clearly not observed using an alternate vector, pDR195 [40]. Therefore, AtYSL2 substrates have yet to be identified with certainty. The *ysl2* single mutant does not have an obvious phenotype; it did not show altered metal accumulation when grown on soil or under various metal conditions [39].

AtYSL1 is a shoot-specific gene whose transcript levels increase in response to high-Fe conditions [41]. Expression in young siliques and the chalaza [41], together with data from NA- and Fe-distribution studies, supports a role for *AtYSL1* in Fe loading of seeds. *ysl1* shoots contain elevated NA levels whereas *ysl1* seeds contain 2–4-fold less NA (and less Fe) than wildtype plants [41]. The germination of *ysl1* seeds under −Fe conditions is slower than that of wildtype plants, a defect that can be rescued by Fe supplementation during germination [41].

The rice genome contains 19 putative *OsYSL* genes. OsYSL2 has been shown to transport Fe^{2+}–NA and Mn^{2+}–NA but not Fe^{3+}–PS in *Xenopus* oocytes [42]. The accumulation of *OsYSL2* transcript is induced specifically in Fe-deficient leaves, and analysis of OsYSL2–GUS reporter plants supports a role for OsYSL2 in metal–NA transport in the phloem [42].

It is apparent that Fe and possibly Zn, Mn, and Cu homeostasis are dependent on YSLs. Further examination of the localization and substrate specificity of various YSLs, and genetic analysis of double (or higher order) mutant plants, will help assign functions to this recently identified plant-specific family of transporters.

NRAMP family

NRAMP proteins are a ubiquitous family of metal transporters that includes mammalian NRAMP2/divalent cation transporter 1 (DCT1)/divalent metal ion transporter 1(DMT1), which has a broad substrate range [43] and functions in intestinal Fe uptake [44]. Likewise, plant NRAMP family members have been implicated in the transport of several divalent cations, including Fe [45–48]. AtNRAMP1 can functionally complement an Fe uptake mutant of yeast, and increased resistance to toxic Fe levels is observed in plants that overexpress *AtNRAMP1* [46].

The closely related genes *AtNRAMP3* and *AtNRAMP4* share similar tissue-specific expression patterns, transcriptional regulation by Fe, and subcellular localization at the vacuolar membrane [49••,50]. Although neither single mutant has a dramatic phenotype, the *nramp3 nramp4* double mutant shows delayed root growth and cotyledon greening when grown on low-Fe media and cannot grow on Fe-limited soils [49••]. These effects can be reversed by growth in excess Fe, demonstrating a specific hypersensitivity to Fe deprivation. *nramp3*

nramp4 mutant seeds store Fe properly but the metal is retained in the vacuole globoids, whereas it is released by wildtype seeds as observed by microanalysis of Fe in seed storage vacuoles [49••]. These results indicate that AtNRAMP3 and AtNRAMP4 function redundantly in the mobilization of Fe from the vacuole during early seedling development.

An *AtNRAMP4* homolog, *TjNRAMP4*, was cloned from the Ni hyperaccumulator *Thlaspi japonicum* and might contribute to Ni tolerance [51]. Expression of *TjNRAMP4* increased the Ni^{2+} sensitivity of wildtype yeast and resulted in slightly elevated Ni accumulation, indicating that this protein might transport Ni into the cytoplasm [51]. TjNRAMP4 might have a specific function in Ni transport as it does not appear to transport Zn, Cd, or Mn [51]. As AtNRAMP4 and TjNRAMP4 share 86% amino-acid identity, it would be interesting to test the ability of TjNRAMP4 to transport Fe and also to compare *TjNRAMP4* expression levels in hyperaccumulating and nonaccumulating species of *Thlaspi*.

ZIP family

ZIP proteins generally contribute to metal-ion homeostasis through the transport of cations into the cytoplasm. They feature eight transmembrane domains and a histidine-rich variable loop between transmembrane domains III and IV. More than 100 ZIP proteins have been identified in bacteria, fungi, animals and plants [52,53]. Functional complementation in yeast has been useful in assigning metal transport abilities [54], and it is noteworthy that the involvement of *ZIP* genes in metal accumulation by hyperaccumulating species has previously been reported. High endogenous *TcZNT1* expression is associated with increased Zn uptake in the roots of the Zn/Cd hyperaccumlator *Thlaspi caerulescens* [55]. Compared to *A. thaliana*, the Zn hyperaccumulator *A. halleri* has elevated levels of *ZIP6* transcript in roots and shoots (23–24-fold), implying that the predicted Zn transporter contributes to buildup of this potentially toxic metal [32•]. Two *ZIP* genes, *TjZNT1* and *TjZNT2*, have been cloned from the Ni hyperaccumulator *T. japonicum*. Yeast expressing either *TjZNT1* or *TjZNT2* show increased resistance to Ni^{2+} [51], highlighting a potential role for these genes in Ni tolerance. Further studies are necessary to determine if/how these proteins function to hyperaccumulate Ni *in planta*.

A. thaliana IRON REGULATED TRANSPORTER1 (*AtIRT1*), the founding member of the ZIP family, encodes the major Fe transporter at the root surface in *A. thaliana* [56], whereas the closely related *OsIRT1* appears to play a similar role in Fe uptake under Fe-limiting conditions in rice [57]. Previous characterization of OsIRT1 [57] and more recent identification of OsIRT2 as Fe^{2+} transporters in the root reveals that grasses utilize two strategies for Fe uptake: the assimilation of Fe^{2+} and the uptake of Fe^{3+}–PS [58]. ZIP proteins also contribute to Zn homeostasis in rice, as had been previously shown for *A. thaliana*. OsZIP4, which shares greater than 50% identity with AtZIP1 and OsIRT1, was able to rescue the growth defect of the Zn-uptake-defective *zrt1 zrt2* yeast strain but no similar functional complementation was observed in the Fe-, Mn- or Cu-uptake mutants, indicating that OsZIP4 is selective for Zn transport [59]. *OsZIP4* mRNA accumulates under Zn deficiency and has been detected in the phloem and apical meristem of roots and shoots [59].

Six cDNAs encoding ZIP family members were recently identified in the model legume *Medicago truncatula* and tested for the ability to complement yeast metal-uptake mutants [60]. Each family member was able to rescue the growth of Zn-, Mn-, and/or Fe-uptake mutants, indicating a function in metal transport. In support of a role in metal homeostasis, expression analysis demonstrates that steady-state mRNA levels are metal responsive. For example, *MtZIP1* transcript was detected only in Zn-deficient roots and leaves, and like *AtZIP1* expression, expression of *MtZIP1* enabled the growth of *zrt1 zrt2* yeast on low Zn media [60]. MtZIP1 is 59.6% identical to AtZIP1 and appears to function as a Zn transporter.

COPT family

The Ctr family of high-affinity Cu uptake proteins are found throughout eukaryotes, including five members in *A. thaliana* named COPT1–COPT5 [61]. To date, only COPT1 has been characterized in detail. Plants that express antisense *COPT1* exhibit a decrease in Cu uptake and reduced Cu accumulation in leaves [62]. *COPT1* antisense plants display pollen development defects and increased root length, which can be reversed by Cu supplementation [62]. These observations are consistent with reports of *COPT1* expression in pollen and root tips and point to a role for COPT1 in growth and development [62].

Conclusions

Several families of metal transporters that have roles in essential metal uptake and distribution have been identified during the past decade. Primary sequence analyses have predicted potential metal-binding residues, and metal-regulated expression patterns have hinted at possible substrates. Further evidence of function has been provided by recent localization at the tissue and cellular levels, and by examination of single and higher-order mutant plants. Discovery of the function of transporters from multiple families has exciting biotechnological applications. For example, AtHMA2 and AtHMA4 probably serve to translocate potentially toxic metals from root to aerial tissues for sequestration and accumulation, and the study of hyperaccumulating species has revealed increased expression of *MTP1* as a mechanism to improve Zn tolerance and accumulation. Characterization of *ZIP*

family members in rice and a model legume are important steps towards improving the metal content of agriculturally significant plants.

Members of the recently identified YSL family of metal transporters are thought to transport metal–NA complexes, yet definitive substrate identification remains work-in-progress. Although specific transport functions have been assigned to members of other transporter families, in many cases it remains unclear which residues mediate metal binding and what motifs are required for metal transport. Studies eliminating the carboxy-terminus of AtHMA4 [14], overexpressing carboxy-terminal segments of TcHMA4 [63] and perturbing a double cysteine motif and an 11 histidine residue stretch within AtHMA4 [13] have begun to address these questions for the P_{1B}-ATPases. However, precise mapping of proteins from all families will be necessary to improve our current understanding of how various residues contribute to metal translocation.

Highlighting the importance of controlling mineral transport, transporters are regulated by their substrates at multiple levels, including transcription, metal-dependent protein trafficking, and protein turnover [28,64–66]. Recently, an essential transcription factor controlling Fe-uptake responses was identified in *A. thaliana* and tomato and was shown to be itself regulated by Fe [67–71], indicating that upstream regulatory components remain to be discovered. The B transporter BOR1, which resides at the PM, was shown to traffic to the vacuole for degradation in response to high B supply [18••]. IRT1 is also controlled post-translationally by metal-induced protein turnover, probably mediated by ubiquitination (EL Connolly, pers. comm.).

Further insight into the roles of many plant transporters awaits their subcellular localization and the characterization of loss-of-function mutants. Given the large number of family members involved in metal transport, it is likely that analysis of higher-order mutants might be required to assign functions *in planta*.

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- • of special interest
- •• of outstanding interest

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