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Lysosome-related Organelles as Mediators of Metal Homeostasis*

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Metal ion assimilation is essential for all forms of life. However, organisms must properly control the availability of these nutrients within the cell to avoid inactivating proteins by mis-metallation. To safeguard against an imbalance between supply and demand in eukaryotes, intracellular compartments contain metal transporters that load and unload metals. Although the vacuoles of *Saccharomyces cerevisiae* and *Arabidopsis thaliana* are well established locales for the storage of copper, zinc, iron, and manganese, related compartments are emerging as important mediators of metal homeostasis. Here we describe these compartments and review their metal transporter complement.

Metal ions are simple yet remarkably versatile protein cofactors. Because of this versatility, copper, zinc, iron, and manganese, in particular, occupy numerous and often essential positions within the chemical framework of the cell. They expand the repertoire of protein-catalyzed reactions and enable difficult chemistry, such as dinitrogen reduction and water oxidation. The latter reaction, which enabled bioenergetic diversification, was a monumental event in the evolution of modern metabolic capacity.

To utilize metal ions as catalysts, the cell has had to overcome the unique challenge of specificity and selectivity. With few exceptions, proteins have evolved to exploit the properties of a specific metal, and the interaction between a protein and a different metal is invariably unproductive. However, many metalloproteins bind Cu^{2+} with higher affinity than they do Zn^{2+} , followed in descending order by Fe^{2+} then Mn^{2+} (1). Thus, if these metals were presented in equimolar amounts, many apoproteins would consistently “pick” copper.

As a result, mechanisms evolved to ensure that each protein binds the correct metal to the exclusion of others. Apoprotein mismetallation in a non-native environment, either in a different organism (2–4) or in the wrong compartment within the cell (5, 6), demonstrates that when imbalance between metal

availability and apoprotein abundance occurs, mismetallation is inevitable.

A key determinant of cell-imposed metal specificity is the orchestrated uptake and distribution of individual metal ions. In addition to regulated transport of metals into/out of cells, protein-to-protein ligand-exchange pathways, as described for Cu^{1+} transport (7, 8), and involvement of metal-storage proteins, such as ferritin and metallothionein, serve to control the interaction between metals and proteins. Eukaryotes have the benefit of intracellular storage organelles, which provide an additional means to maintain metal homeostasis. In this mini-review, we present a synopsis of our current understanding on the role of intracellular transporters in modulating storage and mobilization of metal ions. Although plant and yeast vacuoles are well established organelles for sequestering and mobilizing metal ions, compartments, such as the acidocalcisome and other lysosome-related organelles, are also emerging as important mediators of metal homeostasis.

Metal Homeostasis and Organelles of the Endomembrane System

The endomembrane system is a complex network of membrane-bound compartments that includes the nuclear envelope, the endoplasmic reticulum, the Golgi apparatus, organism- or cell type-specific post-Golgi organelles, and the plasma membrane. A distinguishing feature of this system is the dynamic vesicle-mediated trafficking of lipids, proteins, metabolites, and ions between membranes. In addition to mediating endocytosis, secretion, and excretion, the coordinated exchange of membranes and cargo provides the means to establish a myriad of specialized compartments in response to the needs of the cell.

One such need is the balancing of metal concentrations. Several relatively well characterized compartments house metal transporters that are involved in both the metal-excess and the metal-deficient situations (Fig. 1). In microbes and plants, these compartments (specifically vacuoles) are generally constitutively present in the cell because they carry out functions in addition to metal homeostasis, but the abundance of metal transporters in their membranes changes in response to need (see below). There are other compartments whose presence may be transient and are typically either observed with metal-binding fluorescent probes or identified based on the localization of particular metal transporters.

The Vacuole in *Saccharomyces cerevisiae*

The vacuole of *S. cerevisiae* is frequently described as an organelle analogous to the lysosome in animals but is a jack-of-all-trades with respect to function. Because the vacuole is the largest organelle in the cell and is easily visualized by light microscopy, the localization of transporters to the vacuolar membrane is readily achieved by immunofluorescence (native or tagged protein) or by imaging a GFP (or other fluorescent protein) fusion.

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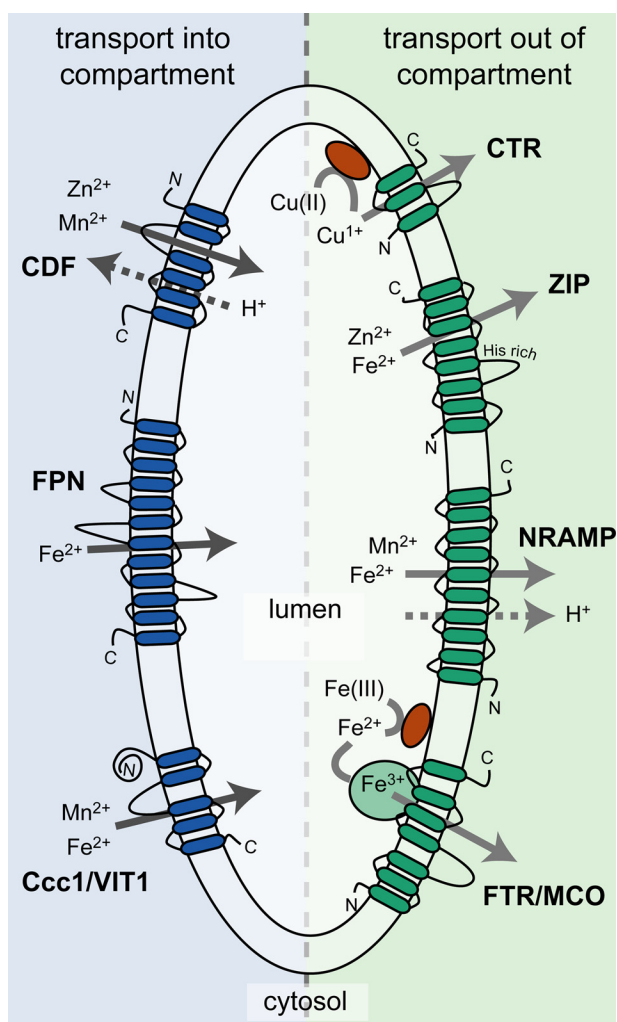


FIGURE 1. **Schematic of common transporter families found in the membranes of vacuoles and other lysosome-related organelles.** Predicted membrane topologies and common substrates for each transporter family are shown. Solid arrows represent the direction of metal ion transport. Metal reductases are represented as orange balls. Abbreviations: FPN, ferroportin; FTR, Fe transporter; MCO, multicopper oxidase.

The involvement of the vacuole in metal homeostasis is reliant in large part on the acidity of its lumen, which is maintained by vacuolar proton-translocating ATPases (V-type ATPases).² Defective vacuole acidification impacts a wide range of cellular activities including the homeostasis of transition metals (9, 10). At least two metal transporters in the vacuolar membrane are dependent on the proton gradient for activity (members of the NRAMP (metal/proton symporter) and CDF families (metal/proton antiporter)) (Fig. 1).

Although trafficking of metal transporters to the vacuole is uncharacterized, newly synthesized integral membrane proteins are expected to take one of two main routes from the Golgi to the vacuole. The carboxypeptidase Y (CPY) pathway takes cargo through the prevacuolar/late endosomal compartment, whereas vacuolar targeting by the alkaline phosphatase (ALP)

² The abbreviations used are: V-type ATPases, vacuolar proton-translocating ATPases; SAV, senescence-associated vacuole; CV, central vacuole; NRAMP, natural resistance-associated macrophage protein; CDF, cation diffusion facilitator; ZIP, ZRT, IRT-like protein; CTR, copper transporter.

pathway is dependent on the adaptor protein complex-3 (AP-3) and bypasses the prevacuolar compartment (11).

Plant Vacuoles

Depending on the developmental stage of the plant and the cell type, at least three different types of vacuoles are described in *Arabidopsis thaliana*: protein storage, senescent, and lytic. Protein storage vacuoles accumulate primarily in developing seeds and are less acidic than are the other two types but can contain membrane-bound compartments called globoids, which share characteristics with lytic vacuoles (12). The globoids are rich in phytic acid, which is likely responsible for chelating metals (13, 14). A second type of vacuole is the senescence-associated vacuole (SAV), which as the name implies, is involved in degradation of macromolecules during senescence (15). The liberation of metal ions during the degradation of metalloproteins would be expected to occur in SAVs, but the contribution of SAVs to metal homeostasis is unexplored. Lytic vacuoles (also referred to as central vacuoles (CVs)) are the main vacuoles found in vegetative tissue and are the major site of metal sequestration.

As with yeast, the plant CV is not just a site for macromolecule degradation. The contents of the CV reflect the specialized function of each tissue, with pigments in flower vacuoles and some defense metabolites concentrated in the vacuoles of epidermal cells. Both V-type ATPases and proton-translocating pyrophosphatases (V-type PPase) maintain the acidic pH of the lumen (16). The CV contains many different molecules that can bind metals. Glutathione and phytochelatins, also found in the yeast vacuole, have garnered much attention for their role in detoxifying metals such as cadmium and mercury (17, 18).

There are at least two separate routes for the trafficking of newly synthesized transporters to the vacuolar membrane: Ap-3-dependent and Ap-3-independent routes (19). Recently, a trafficking route that bypasses the Golgi has also been described in meristematic root cells (20).

Acidocalcisomes

First characterized in trypanosomatids, the acidocalcisome is a storage organelle defined by the presence of pyrophosphate and polyphosphate complexed with calcium and other divalent metal ions that appears as an electron-dense granule by transmission electron microscopy (21). Acidocalcisomes have been purified from parasitic protozoa, the green alga *Chlamydomonas reinhardtii*, the red alga *Cyanidioschyzon merolae*, and the amoeba *Dictyostelium discoideum* and are thought to be functionally equivalent to a ubiquitous group of polyphosphate-containing compartments formerly referred to as volutin granules or polyphosphate vacuoles. Analogous to the plant vacuole, acidocalcisomes contain both the V-type ATPases and the V-type pyrophosphatases that acidify this compartment.

These organelles contain zinc and iron, and transporters that are predicted to facilitate their uptake (CDF and Ccc1p/VIT1 families, respectively) have been detected by proteomic analysis (22, 23). The presence of copper in these organelles had not been documented until recently because researchers typically determine metal content with x-ray microanalysis of thin sections of biological material using copper grids.

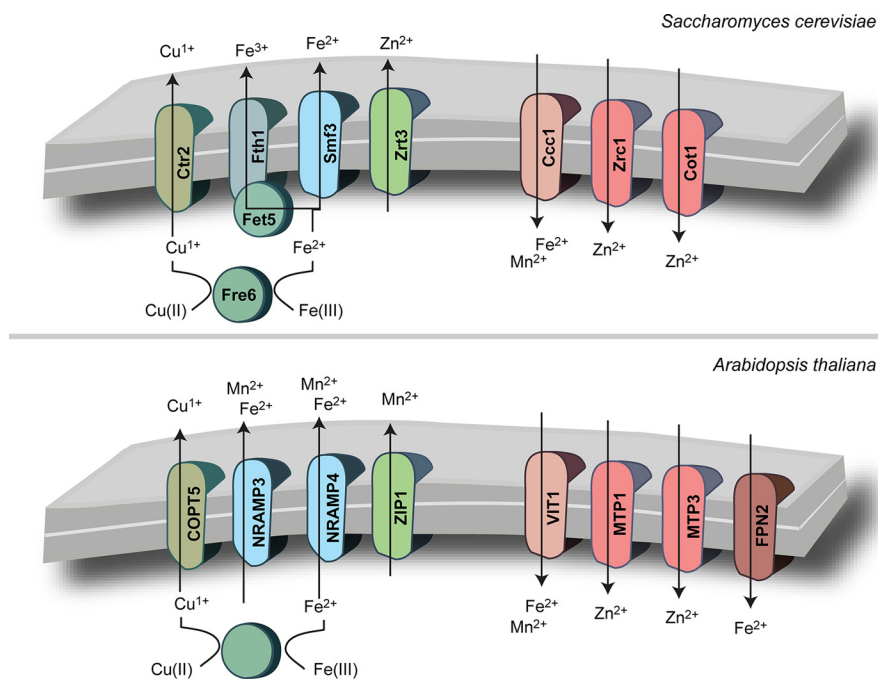


FIGURE 2. **Metal ion transporters in *A. thaliana* and *S. cerevisiae* that enable vacuole-mediated metal storage and mobilization.** Transporters from the same family are given the same color.

The trafficking of resident proteins to the acidocalcisome is poorly understood and likely varies between organisms. As with the yeast and plant vacuoles, a role for AP-3 has been established in both *Trypanosoma brucei* (24) and *Leishmania major* (25), but although AP-3 is essential for acidocalcisome biogenesis in *T. brucei*, this adaptor complex was important but not essential for *L. major* acidocalcisomes.

Zincosomes

In fungi and mammals, Zn^{2+} can be sequestered in small cytoplasmic compartments, which are typically visualized with the fluorescently labeled, zinc-binding probes Zinquin or FluoZin-3. In fungi, these zinc bodies are distinct from the vacuole (26–28) and correspond to an uncharacterized compartment. Because the predominant store of Zn^{2+} is in the vacuole (29), these compartments may be involved in a transient flux of cytosolic Zn^{2+} . In mammals, the term “zincosome” was coined to refer to similar cytoplasmic foci corresponding to accumulated Zn^{2+} (30). These foci have different functions depending on the cell type. Zn^{2+} -loaded “storage granules” function in Zn^{2+} loading of milk (31), neuron function (32), and insulin secretion (33). Zinc bodies also form in response to excess Zn^{2+} supplementation alone (30, 34) or in combination with either the overexpression or the silencing of intracellularly localized Zn^{2+} transporters (35, 36).

Gut Granules

Gut granules are acidic organelles that have lysosome-like characteristics and are found in the intestines of the nematode *Caenorhabditis elegans*. As shown for Zn^{2+} (37), these organelles may be able to mediate the uptake and distribution of metals from the diet, analogous to what the vacuoles do in plant roots. A high Zn^{2+} diet induces the formation of a specialized structure that sequesters Zn^{2+} ; the gut granules take on a

bilobed appearance, and one side of the granule is loaded with Zn^{2+} by the Zn^{2+} transporter CDF-2 (37). As shown for the yeast vacuole, whereas these Zn^{2+} compartments provide resistance to excess Zn^{2+} , the sequestered Zn^{2+} can be distributed to the rest of the organism when needed.

Metal Transporters

The ability of intracellular organelles to mediate metal availability is dependent on the transporters in their membranes (Fig. 2). In this review, we limit our discussion to those transporters that facilitate the transfer of uncomplexed metal ions across the vacuole membrane, but it should be pointed out that metal conjugates, such as complexes of phytochelatin (38), are also transported into the vacuole. Most of the transporter families were initially recognized for their involvement in the transport of metal ions across the plasma membrane (or assimilation). Subsequently, members of the family were localized to an intracellular compartment (for storage and distribution). In general, for a given transporter type, the direction of transport, *i.e.* into or out of the cytoplasm, is presumed to be conserved, regardless of the membrane in which the transporter resides.

Transport into the Cytoplasm

NRAMP

These permeases are typically divalent cation/proton symporters (as described for mammalian NRAMP2 and the yeast homolog Smf1p (39, 40)), whereas one NRAMP was shown to have a physiological role in Al^{3+} transport (41). Although NRAMPs can transport a broad range of divalent cations, as determined mainly with heterologous expression in *Xenopus laevis* oocytes, these transporters are most often implicated in the transport of Fe^{2+} and, to a lesser extent, Mn^{2+} (for a current review, we recommend Ref. 42).

The *S. cerevisiae* genome encodes three members of the NRAMP family, Smf1p, Smf2p, and Smf3p. Smf1p and Smf2p are involved in Mn²⁺ transport and are localized to the plasma membrane and small intracellular compartments of unknown function, respectively (43, 44). Smf3p has a steady-state localization to the vacuolar membrane (43). Based on regulation of gene expression (45) and higher iron content of vacuoles from a *smf3Δ* mutant (46), Smf3 is proposed to mobilize Fe²⁺ from this compartment in response to iron deficiency.

Of the six NRAMP homologs in *A. thaliana*, AtNRAMP3 and AtNRAMP4 localize to the vacuolar membrane, and as with *SMF3*, expression of both genes is increased by iron depletion (47, 48). In seeds, these transporters localize to the globoids, and *nramp3 nramp4* double mutant displays defects in mobilization of iron stored in globoids, which is critical during seed germination in iron-depleted soil (47). AtNRAMP3 and AtNRAMP4 are also located in mesophyll cells, the major site for photosynthesis, where they are involved in the mobilization of Mn²⁺ from vacuoles (49). This source of Mn²⁺ is required for photosystem II activity when plants face suboptimal manganese nutrition in the soil. Surprisingly, manganese nutrition affects neither the expression nor the abundances of these transporters.

The green alga *C. reinhardtii* provides a convenient single-celled reference system for studying metal metabolism that is comparable with the mesophyll cell in land plants. Of the four NRAMP members in *C. reinhardtii*, CrNRAMP4 is both orthologous to AtNRAMP4, and expression of the corresponding gene is increased during growth in iron-deficient medium (50), suggesting that CrNRAMP4 also resides in the membrane of an intracellular compartment and can transport Fe²⁺ into the cytosol.

ZIP

Members of the ZIP family are characterized as divalent cation transporters (for a current review, we recommend Ref. 51). Zn²⁺ transport is enhanced by the addition of bicarbonate to transfected mammalian cell lines, suggesting that these permeases are metal/bicarbonate symporters (52, 53). However, based on kinetics of a bacterial homolog in reconstituted proteoliposomes, ZIPs are proposed to mediate Zn²⁺ electrodiffusion (54).

Of the four ZIPs in *S. cerevisiae*, Zrt3p localizes to the vacuole. The corresponding gene is a direct target of the Zn²⁺-responsive regulator Zap1p, and expression is accordingly increased by zinc depletion (55). *ZRT3* is not associated with a growth defect in Zn²⁺-depleted medium unless the genes encoding the two major assimilatory transporters are also deleted (56). Even in the triple knock-out, an obvious growth phenotype is only observed within a narrow window of supplemental Zn²⁺. This phenotype is typical for vacuole-localized transporters because the transporter is only able to confer a fitness advantage if there is an adequate supply of metal in the vacuole to be mobilized. At low concentrations of Zn²⁺, there is not enough Zn²⁺ available to support growth and be stored, whereas at high concentrations of Zn²⁺, an internal supply is not necessary to support growth. If the vacuole is first loaded with Zn²⁺ by exposing cells to high levels of Zn²⁺ in the

medium, this store of Zn²⁺ can sustain growth in the absence of an external supply (29).

In contrast to yeast, the *A. thaliana* genome encodes 18 members of the ZIP family (57). Of these, only AtZIP1 has been localized to the vacuole so far (58). As with *ZRT3*, the abundance of AtZIP1 mRNA is higher in response to Zn²⁺ depletion (59, 60). Not surprising, given the possible redundancy of Zn²⁺ transport pathways, a growth phenotype linking AtZIP1 to Zn²⁺ homeostasis has yet to be found (58). However, AtZIP1 is associated with manganese homeostasis because disruption of AtZIP1 causes a growth defect on low manganese and defective root-to-shoot translocation of manganese (58).

CTR

Trimeric CTR is proposed to form a channel-like pore through the membrane; Cu¹⁺ is likely to transverse this pore by a succession of ligand-exchange reactions (61). A negative charge at the extracellular face attracts Cu¹⁺ ions, whereas a positive charge at the cytoplasmic face promotes the exit of Cu¹⁺ (62). As proposed for the ZIP transporters, transport is further facilitated by the concentration gradient across the plasma membrane that is maintained by Cu¹⁺ chelation within the cell. Unlike ZIP transporters, which can transport a broad spectrum of divalent metal cations, CTRs transport monovalent cations, lending substrate specificity to Cu¹⁺ (for a current review, we recommend Ref. 63). Accordingly, transport by CTR requires Cu²⁺ reduction either at the plasma membrane for assimilation or at the vacuole membrane for mobilization.

CTR_s at the vacuole membrane lack the extracellular methionine extensions (ectodomain) that are characteristic of CTR_s in the plasma membrane. Intriguingly, cleavage of the ectodomain in mammalian Ctr1 is required for Cu¹⁺ efflux from an endosomal compartment (64), suggesting that the acidic pH of the lumen likely alters the affinity of the ectodomain for Cu¹⁺ (65), hence inhibiting transport.

In *S. cerevisiae*, Ctr2p (66–68) and the reductase Fre6p are responsible for Cu¹⁺ transport from the vacuole. In the absence of the assimilatory transporters Ctr1p and Ctr3p, Ctr2p is required for Cu,Zn-Sod activity and Fe³⁺ uptake (because Cu¹⁺ is needed for Fet3p) (68). In *A. thaliana*, COPT5 is needed during severe copper limitation (69, 70), but unlike the situation in *S. cerevisiae* (61), neither *COPT5* mRNA abundance nor the protein amount is affected by copper (69). In *C. reinhardtii*, CrCOPT1 is predicted to localize to an intracellular compartment, but as with AtCOPT5, the mRNA abundance of CrCOPT1 is not affected by copper deficiency.

FTR (Fe Transporter)/MCO (Multicopper Oxidase) Complex

S. cerevisiae does not contain ferritin and relies on iron sequestration in the vacuole to balance iron levels in the cell (71). Paralogs of the iron-specific transporter Ftr1p/Fet3p are found in the vacuole membrane, and these function together to mobilize sequestered iron in a situation of iron deficiency (72). Although Smf3p also localizes to the vacuole and mediates Fe²⁺ transport into the cytosol, *SMF3* is negatively regulated by oxygen (73). Therefore, Smf3p is required for Fe²⁺ mobilization when Ftr1p/Fet3p is inactive due to the absence of oxygen, which is required for ferrous oxidation and transport by this

complex. The low affinity Fe^{2+} transporter in the plasma membrane Fet4p is co-regulated with Smf3p. Fet4p and Smf3p may represent an Fe^{2+} transport pathway that predates the evolution of Ftr1/Fet3 and Fth1/Fet5. The former evolved under anoxic conditions, whereas the latter was recently acquired.

Transport Out of the Cytoplasm

CDF

CDF transporters catalyze metal/proton antiport, and the acidic lumen of the vacuole therefore facilitates metal transport out of the cytosol. Based on sequence similarity, the CDF family can be divided into functional subgroups that correspond to characterized physiological substrates (57). Within the Zn^{2+} subgroup, the *S. cerevisiae* transporters Zrc1p and Cot1p are responsible for resistance to excess Zn^{2+} accumulation and are also induced by Zn^{2+} depletion in anticipation of Zn^{2+} shock, which is likely a more relevant pathway for excess Zn^{2+} to enter the cell rather than toxic levels in the environment (74). Indeed, this mechanism is probably conserved in *C. reinhardtii* (CrMTP1) (75) and *A. thaliana* (AtMTP2) (60), but these transporters have not yet been localized to an intracellular compartment. Two additional Zn^{2+} -type CDF transporters, AtMTP1 (76) and AtMTP3 (77), do localize to the tonoplast and can transport Zn^{2+} out of the cytosol, whereas a Mn^{2+} -type CDF, AtMTP11, localizes to a trans-Golgi compartment and mediates manganese tolerance (78).

Ccc1/VIT1

This transport family has not yet been characterized biochemically. Proteins containing a domain related to Ccc1p from *S. cerevisiae* and VIT1 from *A. thaliana* are found in all three kingdoms of life, but this domain is absent in animals (based on a PSI-BLAST search of GenBankTM; note that Vit1-like sequences found in *Lipotes vexillifer* and *Panholops hodgsonii* genomes are likely from a contaminating source of bacterial DNA given the high degree of similarity between these proteins and ones from Acinetobacter). Several bacterial proteins that contain the Vit1 domain (pfam01988) also contain a ferritin-like domain, which is predicted to extend into the cytosol (79). In bacteria, these homologs are involved in iron efflux from the cell (80, 81), whereas in eukaryotes they are involved in iron loading of the vacuole (82, 83). The Vit1 domain is also found in proteins that reside in endoplasmic reticulum bodies (84) and root nodules (85), but the function of these proteins remains to be determined.

In order for AtNRAMP3 and AtNRAMP4 to mobilize Fe^{2+} within the seed, Fe^{2+} must be preloaded into the compartment by AtVIT1. AtVIT1 is highly expressed in developing seeds, and AtVIT1 localizes to the vacuole membrane (86). The absence of this transporter does not affect the absolute concentration of iron in seeds but does lead to an aberrant distribution of iron within the seed (86), which is detrimental when seeds from the mutant are germinated on alkaline soil (because iron is less soluble in alkaline soil, plant iron assimilation is affected). Because of its constitutive role in seed maturation, AtVIT1 expression does not change in response to iron nutrition.

Expression of the two VIT1 homologs in *C. reinhardtii* is increased under iron deficiency (50) in contrast to the *S. cerevi-*

siae homolog, CCCI, whose expression is induced by iron excess (83). Whether these *C. reinhardtii* homologs also localize to the vacuole and mediate iron transport is unknown, but the differential expression between land plants, algae, and yeast suggests that members of this family have evolved to perform different physiological roles.

Ferroportin

Members of the ferroportin family are well characterized iron exporters in animals that mediate iron mobilization between tissues (87). In *A. thaliana*, FPN2/IREG2 transports Fe^{2+} (and other metal ions (88, 89)) into the vacuole. Unlike AtVIT1, FPN2 is expressed in the two outermost layers of the root in response to iron deficiency (88). FPN2 may either buffer the influx of Fe^{2+} from the soil or serve to protect the plant from iron shock. The protein and its response to iron deficiency is conserved in *C. reinhardtii* (50).

A Shifting Perspective on Metal Homeostasis

Our picture of metal economy (the uptake and distribution of metal ions) has largely focused on one metal at a time. When faced with metal deficiency, the goal is to increase supply relative to demand (90, 91), while prioritizing the distribution of the limiting cofactor to essential metalloproteins.

However, metal homeostasis can be viewed another way. Deficiency of one metal causes a relative excess of the other metal ions in the cell. Consequently, those proteins lacking their cofactor due to the insufficiency are more susceptible to mismetallation. In several organisms, iron deficiency can lead to the misincorporation of Zn^{2+} into protoporphyrin IX (92), whereas in yeast, manganese deficiency (generated by deletion of a Mn^{2+} transporter) can cause misincorporation of Fe^{2+} into Sod2p (93). Because these "errors" are analogous to those that occur in the metal overload situation resulting from toxic levels in the environment (94–96), the compartmentalization of conditionally excess metal ions is a relatively unexplored mechanism of metal economy.

Metal Sequestration during Deficiency

Poor nutrition of a single metal often affects the cell quota of one or more other metals. The molecular basis for this apparent cross-talk is not completely understood. In several cases, unexpected perturbations to the cellular metal content are attributed to the promiscuity of transport pathways. As an example, the vacuoles of iron-deficient roots of *A. thaliana* over-accumulate manganese, zinc, and cobalt. IRT1, the major Fe^{2+} transporter in roots during iron deficiency, can also transport these metals based on 1) heterologous expression in yeast, and 2) the dependency of metal hyper-accumulation on IRT1 expression in *A. thaliana* (97, 98).

C. reinhardtii hyper-accumulates metal ions specifically during Zn^{2+} limitation. The metal quota for iron, manganese, and copper is increased to differing degrees (75). Unexpectedly, Zn^{2+} -limited cells are phenotypically copper-deficient as indicated by expression of the copper deficiency regulon and absence of plastocyanin (the major copper-dependent protein in the cell), but not iron-deficient based on reduced abundance of known iron-regulated transcripts (75). Therefore, the copper

within the cell is relatively inaccessible to both plastocyanin and the copper regulator CRR1.

An explanation for these observations was attained by analyzing the copper distribution within the cell. Hong-Hermesdorf *et al.*³ observed the accumulation of copper in punctate cellular bodies in live cells using a fluorescently labeled copper probe, Coppersensor-3, and in fixed cells with nano-scale secondary ion mass spectrometry (NanoSIMS). As with acidocalcisomes, these compartments are electron-dense when viewed by transmission electron microscopy and contain polyphosphate and calcium.

Copper sequestration may be a mechanism to balance the ratio of copper and zinc, thus preventing mismetallation of Zn²⁺-dependent apoproteins. Plastocyanin, an essential protein for electron transfer in photosynthesis, is dispensable in *C. reinhardtii* because of the presence of cytochrome *c*₆, a functionally equivalent heme-dependent protein. Therefore, to balance the Cu-Zn ratio, the cell can sequester the plastocyanin-accessible copper pool with little consequence. Cytochrome *c* oxidase in the mitochondrion, which does not have a copper-independent backup, does not appear to be affected by copper sequestration during zinc limitation. Copper hyper-accumulation (in contrast to copper sequestration) is likely a consequence of compartmentalization and the activation of the CRR1 regulon, which includes the assimilatory Cu¹⁺ transporters CTR1 and CTR2. Because plastocyanin places a relatively large intracellular demand on *C. reinhardtii* for Cu¹⁺ acquisition (estimated to be 8 × 10⁶ atoms/cell (99)), copper hyper-accumulation provides a ready supply of copper when zinc homeostasis is restored. Indeed, upon Zn²⁺ resupply, the copper within these bodies is mobilized and used to metallate plastocyanin.

Looking Forward

Metal homeostasis serves to ensure that the accessibility of metal ions is controlled, and apo-proteins only interact with the right metal. One such mechanism is the fine-tuning of metal balance afforded by the ability to partition metal ions through compartmentalization. Presently accessible imaging techniques such as x-ray fluorescence are being successfully employed for the simultaneous analysis of multiple metal ions in tissues with resolution at the single cell level (100). With increased resolution, and with the development of more sophisticated fluorescence-based metal sensors, we expect that many novel aspects of intracellular metal sequestration and distribution will be illuminated.

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