Plant Nutrition 3: Micronutrients and Metals

INTRODUCTION TO MICRONUTRIENTS

Micronutrients are nutrients that are required for normal plant growth. By definition, micronutrients are required in smaller amounts than macronutrients. Iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), molybdenum (Mo), and nickel (Ni) are metal micronutrients that extend the range of a cell's chemical reactions or contribute to protein structure. Boron (B) and silicon (Si) are metalloid elements that have properties between those of metals and non-metals, are mainly used in the apoplast, and contribute to the plant's structure. The major role of the non-metal chorine (Cl) is to serve as an abundant and nontoxic anion that contributes to electrical and osmotic balance; its function is similar and complementary to that of potassium.

Although essential, micronutrients are required in very small concentrations, ranging from 0.1 mg/kg dry mass (Mo and Ni) to 100 mg/kg (Cl and Fe). By contrast, the macronutrient composition of plants ranges from 1000 to 15,000 mg/kg dry mass. The optimal concentration range of most micronutrients is quite narrow in the plant body, so plants must maintain them in sufficient but not excessive amounts; homeotic regulatory strategies are employed at both the low end and high end of the optimal nutrient range. In the soil, the optimal amount of each micronutrient varies by type of crop, soil pH, nutrient form, extent of organic material in the soil, microbial activities, and relative amounts of other nutrients, all of which can affect the mineral forms or species of micronutrients and their efficiency of uptake. As an example, Fe is orders of magnitude more soluble at pH 6.0 than pH 7, and its solubility decreases even further as soil is alkalinized to pH 8, whereas Mo becomes more soluble with soil alkalinization over the same pH range.

Plants respond dynamically to supply and demand to maintain nutrient levels in the optimal range. For example, at the low end or deficiency end of the optimal range, plants increase the activity of selected nutrient transporters to increase uptake and can excrete protons or small molecules into the soil to enhance the nutrient's bioavailability. Root system architecture also can change to enhance uptake, for example, by increasing the production of root hairs or lateral roots. When demand greatly exceeds supply, a condition known as limitation, the plant's growth rate slows but the plant can respond by switching to alternative metabolic pathways that rely less on the limiting micronutrient, for example, by repressing the synthesis of non-essential metalloproteins in favor of those that are essential. This strategy is referred to as nutrient sparing. Nutrient limitation can also lead to reallocation or recycling of nutrients so that they can be used optimally to support the plant's metabolic, growth, and defense needs. At the high end or luxury end of the optimal range, excess nutrients can be stored safely for later use. When a nutrient is present in very high levels, the plant's growth rate can be restricted by nutrient toxicity. However, far from being a hapless victim, the plant responds by increasing the appropriate nutrient efflux and detoxification pathways. Some plants have evolved mechanisms by which they can tolerate quite high levels of various micronutrients and even hyperaccumulate them for defensive purposes.

When soil levels are low, micronutrient fertilizers can be applied to soil or sprayed onto leaves. Analytical testing of plant tissues or the appearance of characteristic deficiency symptoms can indicate whether a plant will benefit from micronutrient supplementation. Deficiency symptoms reflect the nutrients' biological roles and properties. For example, Fe and Mn are poorly mobile in plants and are required for chlorophyll production, so deficiency of either leads to interveinal chlorosis in which photosynthetic tissues turn yellow due to a lack of chlorophyll, and tissues farther away from vascular tissue supplies are first affected.

Plant nutrition has a big impact on human health. Here, efforts to apply our understanding of plant physiology to improve human nutrition, especially for the critical elements iron, zinc, and selenium (Se), are described, as are efforts to limit the harm of a few of the nonessential toxic elements that can enter plants and animals through the plant's nutrient uptake machinery.

METAL MICRONUTRIENTS: WHAT'S SO SPECIAL ABOUT METALS?

Elements are defined by their properties and positions in the periodic table as metals, non-metals, or metalloids. Most elements are metals, but the most abundant biological elements are non-metals (e.g., H, C, N, O, P, and S). Fe, Cu, Zn, Mn, Mo, and Ni are *d* block metals, sometimes called transition metals. Heavy metal is an ambiguous term that sometimes refers to an element's density but also can be used to imply toxicity. Running diagonally through the periodic table are the metalloids, of which we discuss B, Si, and arsenic (As).

If you include the loose association with Mg, between one-third and one-half of cellular proteins associate with a metal ion in some way. About one-quarter of cellular proteins are metalloproteins that association with *d*-block metals. The polypeptide portion of a metalloprotein is called the apoprotein, which has to be united with a metal or metal cofactor for proper function. Metals greatly expand the range of chemical reactions that can take place in the cellular environment. As examples, the electron transport proteins in plastids and mitochondria associate with Fe and Cu for their oxidation and reduction capabilities. In some cases, particularly for Zn, the metal is required to maintain a protein's structure.

Metals require special handling to maintain solubility and prevent toxicity. Unconjugated Fe or Cu, and to a lesser extent

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Ni, can generate dangerous free radicals via the Fenton reaction, as shown below for Fe:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\cdot + OH^-$$

To protect against oxidative damage and to maintain their solubility, metals are rarely found in free form in cells. Rather, they are almost always bound by proteins, peptides, or small molecules.

MICRONUTRIENT TRANSPORTERS AND TRANSPORT

Membrane-localized nutrient transporters are crucial for micronutrient homeostasis. Plasma membrane transporters in root cells are required for nutrient import and passage beyond the impermeable endodermis and/or exodermis. Transporters are needed for nutrients to enter and exit the vacuole for storage or other organelles for functional purposes. Translocation through the xylem requires nutrient efflux from the symplast into the apoplast through transporters and subsequent influx through transporters back into the symplast of distal tissues. Finally, some nutrients are mobilized and transported for recycling during senescence or in response to nutrient limitation, a process that usually involves nutrient transporters.

An element that was translocated solely and nonselectively through the xylem stream would preferentially accumulate in the most highly transpiring tissues, such as the leaves. However, many micronutrients are preferentially translocated into young, developing tissues, which they reach by way of the phloem. Thus, transport into and out of the xylem and phloem are important control points for nutrient transport and homeostasis, and studies in grasses have highlighted that leaf nodes are sites for nutrient redistribution between xylem and phloem.

Membrane Transporters for Micronutrients

Several families of membrane transporters contribute to micronutrient uptake, storage, long-distance transport, and remobilization. Micronutrient transporters are often not very selective and many are able to transport more than one type of micronutrient. Here, some of the major transporter families are introduced, particularly those that are involved in the transport of multiple micronutrients. This is not an exhaustive list; additional transporters are discussed separately where appropriate.

Heavy metal-transporting P-type ATPases (HMAs) are P_{1B}-type ATPases related to plasma membrane H^+-ATPases that are also found in prokaryotes, animals, and fungi. In plants, these transporters reside in diverse membranes and contribute to metal efflux from the cytosol into the xylem, the plastid, or the endomembrane compartments. HMA1-HMA4 transport divalent cations (Zn^{2+}, Cd^{2+}, Pb^{2+}, and Co^{2+}), whereas HMA5-HMA8 transport monovalent cations (Cu^+ and Ag^+). ECA (ER-type calcium ATPase) transporters are related P_{2A}-type ATPases.

Metal tolerance proteins (MTPs) are cation efflux transporters from plants that are part of the cation diffusion facilitator family found in all organisms. There are at least seven groups of MTPs that transport ions, including $Zn^{2+}$and Mg^{2+}. Various members have been identified in the tonoplast, Golgi, or plasma membranes, and their role is to remove metals from the cytosol. As their name suggests, functional studies indicate that MTPs have a role in metal tolerance, and in some metal-hyperaccumulating plants, elevated MTP activity increases the sequestration of metals into the vacuole.

Arabidopsis thaliana has 15 genes encoding zinc-regulated transporter, iron-regulated transporter-like proteins (ZIPs), which are metal influx transporters. Many are plasma membrane-localized metal importers with different specificities that collectively transport Zn, Fe, Mn, Cu, and Cd. Some have been identified through studies in yeast and others through genetic studies in plants. For example, IRT1 (IRON-REGULATED TRANSPORTER1; a ZIP family member) was identified by its ability to functionally complement a yeast iron-transport mutant, and in Arabidopsis, a loss-of-function mutation shows dramatically decreased Fe uptake and requires supplemental Fe for growth.

Yellow Stripe1-Like (YSL) proteins transport metals that are complexed with organic molecules. The gene name derives from the phenotype of a maize (*Zea mays*) mutant identified by Nobel Laureate George Beadle; the mutant has yellow, chlorophyll-less stripes between the veins. The phenotype of the loss-of-function *yellow stripe1* mutant mimics that of iron deficiency, and the phenotype can be partially complemented by the addition of iron. The *YSL* gene family has eight members in Arabidopsis and 18 in rice (*Oryza sativa*). Some members are expressed in roots and presumably contribute to metal uptake from soil, others are expressed in the cells surrounding leaf veins and contribute to metal transport from the xylem apoplast into the symplast, and others have been shown to be required for iron release from the chloroplast.

Broad-specificity metal cation/proton cotransporters or antiporters from the NRAMP (Natural Resistance Associated Macrophage Protein) family reside in the plasma membrane or vacuolar membranes. There are six NRAMP genes in Arabidopsis and seven in rice. In Arabidopsis, plasma membrane-localized NRAMP1 has a primary role in Mn uptake.

Our understanding of transport into plastids and mitochondria is incomplete, but some transporters have been identified, including PIC1 (plastid permease) and PAA (P-type ATPase), as has an organellar ferrireductase required for iron import into plastids. Given the importance of metalloproteins for photosynthetic and respiratory electron transport, characterization of these organellar transporters is an ongoing effort.

Chelators Contribute to Micronutrient Uptake, Transport, and Sequestration

The word “chelator” (pronounced kee-lator) is derived from the Greek word “chele,” meaning claw. Chelators bind metals such as Fe, Ni, and Zn in a pincher-like grasp using a pair of charged functional groups, usually carboxyl groups. The organic acids malate ($HOOCCH_2CHOHCOOH$) and citrate [$C_3H_5O(COOH)_3$] are commonly occurring metal chelators. Chelation increases metal nutrient bioavailability and uptake, maintains metals in soluble form, and shields cellular components from reactive metals during transport or storage. Chelators can be specific compounds or compounds that have other functions besides chelation; citrate and malate are examples of the latter, as are the amino acids histidine and peptide glutathione.

Other chelators function specifically or primarily in metal chelation. All plants can synthesize nicotianamine (NA), which is produced by the action of nicotianamine synthase from three molecules of *S*-adenosyl methionine. Sulfur is removed from *S*-adenosyl methionine during NA synthesis, so nicotianamines are non-sulfur-containing chelators. Nicotianamines are not secreted into the soil but are involved in chelating metals (including Fe^{3+}, Fe^{2+}, and Zn^{2+}) within plant cells and during cell-to-cell transport and transport across membranes. The critical role for NAs in metal homeostasis has been demonstrated by the phenotype of a quadruple nicotianamine synthase knockout mutant that is chlorotic and sterile as a consequence of numerous reproductive defects.

In grasses, NAs can be further modified to produce mugineic acid and derivatives that are collectively referred to as phytosiderophores (PSs). Siderophores, produced by a variety of organisms, are small molecules that are excreted into the extracellular environment where they bind Fe^{3+} or other metals to maintain the metal ion's solubility and, hence, availability for uptake. In soil, secreted PSs form stable complexes with metal ions, including Fe^{3+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}. The resulting metal complexes can be taken up into the roots through metal-PS transporters.

The interaction between chelators and metals is affected by pH. For example, at a slightly acidic pH, citrate effectively competes with nicotianamine, but at a neutral or slightly alkaline pH, nicotianamine is a much more effective chelator. Chelators must reach their target metals to bind them, so the activities of the transporters for unbound chelators also contribute to metal transport and homeostasis.

Phytochelatins and metallothioneins are sulfur-containing metal binding compounds, the sulfhydryl groups of which contribute to their metal ion binding affinity. Phytochelatins are Cys-rich peptides derived from glutathione that are produced enzymatically (rather than being encoded by a gene). The major role for phytochelatins may be protection from excess metals by promoting their sequestration in the vacuole. Metallothioneins are small ($<$10 kD) Cys-rich proteins that are encoded by genes, which makes it relatively easy to determine how many there are and how they compare with similar peptides from other organisms. Metallothioneins are diverse, and only some have been shown to participate in metal binding or detoxification.

A distinct and unrelated group of metal binding proteins called metallochaperones that are important in the delivery of Cu and possibly Ni to their target metalloproteins will be discussed further below.

IRON: ABUNDANT, IMPORTANT, AND LARGELY INSOLUBLE

Although iron is a relatively abundant element, it is usually present in very insoluble solid forms. Iron normally is in one of two oxidation states: Fe^{3+} (ferric ion) or Fe^{2+} (ferrous ion). Under neutral or alkaline pH or when oxygen is present, Fe^{3+} predominates, for example, as insoluble ferric oxide $Fe(OH)_3$. When life evolved, the atmosphere had little oxygen and the more soluble ferrous ion predominated, but $\sim$2.5 billion years ago with the advent of oxygenic photosynthesis, the "great oxidation event" led to the less soluble ferric form becoming more common. Iron mobilization requires redox chemistry to convert Fe(III) to Fe(II). Therefore, for most living organisms, including humans, iron acquisition is a significant challenge.

Biochemical Functions

Iron is the micronutrient required in the greatest abundance and it is involved in a large number of catalytic and redox reactions. Iron is needed in plastids and mitochondria, where it participates in photosynthetic or respiratory electron transport.

In cells, it is often found conjugated to proteins either within a heme group or as non-heme iron, which includes iron-sulfur clusters, di-iron centers, Fe-α-ketoglutarate centers, and mononuclear Fe.

Heme is an iron-containing tetrapyrrole prosthetic group in which the iron is held in the middle of the ring by conjugation to nitrogen. Side branches of the biosynthetic pathway that leads to heme production also give rise to siroheme, an iron-containing prosthetic group found in nitrite reductase and sulfate reductase, and chlorophyll, in which the tetrapyrrole group is conjugated to Mn. Heme is a particularly ancient compound that is found in all domains of life. Plants produce and use heme in their plastids, but it can also be used in the mitochondria, cytosol, and peroxisomes. It is an essential cofactor for the cytochromes that carry out redox reactions in electron transport chains and it is found in peroxidases and catalases.

Iron also can be found as iron-sulfur (Fe-S) clusters that are covalently attached as a prosthetic group to a polypeptide. The major classes are Fe_2S_2 and Fe_4S_4. Fe-S cluster-containing proteins occur in mitochondria, plastids, the cytosol, and the nucleus. Examples include the ferredoxins found in plastids and mitochondria, nitrite reductase and glutamine synthase (in plastids), photosystem I subunits PsaA, B, and C (in plastids), and xanthine dehydrogenase (in the cytosol).

Iron Uptake and Transport

Plants have two distinct strategies for iron uptake. Most plants use a reduction-based strategy known as Strategy I, in which the plasma membrane-bound ferric chelate reductase FRO2 (Ferric Reductase Oxidase2) reduces Fe(III) to Fe(II) outside of the plasma membrane. The solubility of ferric compounds is facilitated by acidification of the apoplast or soil through proton-extruding H^+-ATPases. Phenolic compounds, organic acids, and flavins excreted from roots keep the solubilized iron in solution by chelation. Reduced Fe(II) is transported across the plasma membrane mainly by IRT1, a ZIP-family metal transporter.

Some grasses of the family Poaceae, including barley (*Hordeum vulgare*), maize, sorghum (*Sorghum bicolor*), rice, and oat (*Avena sativa*), have evolved a different strategy for iron uptake, known as a chelation strategy or Strategy II, which involves the secretion of PSs into the rhizosphere, where they chelate Fe(III). The Fe(III)-PS complex is reimported into the plant through YSL transporters.

Once taken up, Fe has to be translocated to other tissues and this occurs in a chelated form of either Fe(II) or Fe(III). For

example, the chelator nicotianamine is required for transport of Fe(II) out of phloem and into sink organs. Recently, a tri-iron(III), tri-citrate complex was identified in tomato (*Solanum lycopersicum*) xylem, and other transported iron-citrate or iron-malate complexes have been described. Long-distance transport of iron occurs in the xylem and phloem. The phloem-specific transporter OPT3 loads Fe into phloem; through this activity, phloem-transported Fe can signal shoot Fe status to roots in order to regulate Fe uptake. FRD3 facilitates iron transport by exporting citrate into the apoplast, including the xylem apoplast where it is needed to chelate Fe and keep it in solution.

Where and how Fe is distributed within a cell depends on the cell type and relative amount of iron available. When it is abundant, excess Fe accumulates in protein complexes composed of ferritins, often in plastids; as much as 80% of a leaf's Fe may be found in non-green plastids. Fe(II) is oxidized to Fe(III) as it is imported into ferritin, and it forms a densely packed solid core within ferritin's protein shell. Ferritin's roles include iron storage and the protection of cells from oxidative damage resulting from uncomplexed Fe. Within mesophyll cells, Fe is mostly found in plastids and mitochondria. In seeds and cells in which iron is present to excess, it may be predominantly stored in vacuoles, which involves the action of the vacuolar iron transporter (VIT1).

Response to Iron Deficiency or Limitation

Plants respond to iron deficiency or limitation by increasing iron uptake, remobilizing it from storage, and by metabolic adjustments toward alternative, non-iron-dependent pathways. Increased uptake involves transcriptional and posttranscriptional controls. In Arabidopsis under iron-deficient conditions, the transcription factor FIT1 coordinately induces transcription of several genes involved in iron uptake or transport including *FRO2* (encoding ferric chelate reductase), *IRT1* (encoding a plasma membrane-localized Fe^{2+} transporter), and *AHA2* (encoding a proton pump that acidifies the soil to facilitate iron uptake). Another transcriptional network that contributes to iron homeostasis involves the transcription factor POPEYE, which is negatively regulated by an iron binding protein called BRUTUS. The expression of iron acquisition genes is also regulated by several hormones, nitric oxide, and the circadian clock. Reciprocally, Fe nutrient status feeds into the period of the circadian clock; these controls may help coordinate and optimize light-dependent photosynthetic processes and nutrient metabolism.

Activity of the uptake transporter IRT1 is regulated by proteolytic degradation and by its cycling between the plasma membrane and internal membranes. Interestingly, IRT1 subcellular distribution is affected by the availability of non-Fe metal IRT1 substrates, which suggests that transporter endocytosis helps to avoid the uptake of potentially toxic metals. Signal(s) of iron deficiency are not yet known, although several iron binding proteins (including BRUTUS and its orthologs) and metabolites that are affected by iron limitation have been proposed.

Iron deficiency affects cells in several ways. The classic sign of iron deficiency is interveinal chlorosis as a consequence of chlorophyll deficiency (one of the enzymes required for chlorophyll synthesis is a Fe-metalloprotein). Iron is also needed for energy production through electron transport chains. Several transcriptomic, proteomic, and metabolomic studies have shown that plants can switch to alternative metabolic pathways when Fe is limiting, such as relying on glycolysis or fermentation when the mitochondrial electron transport chain is affected. Another example of metabolic responses to iron limitation involves the family of genes encoding the enzyme superoxide dismutase (SOD). SOD requires a metal cofactor, and different genes encode isoforms that are specialized to use different metals. In plants, SOD can be found as MnSOD, FeSOD, and Cu/ZnSOD. Upon iron limitation in algae, MnSOD accumulates and functionally substitutes for FeSOD. Finally, cyanobacteria and many algae induce expression of flavodoxin (Fld) to substitute for ferredoxin (Fd) (which is involved in photosynthetic electron transport) when iron is limiting. The flavodoxin gene has been lost from terrestrial plants as has the ability to substitute Fld for Fd.

MICRONUTRIENT DEFICIENCIES ARE A GLOBAL HUMAN HEALTH CONCERN

Humans obtain micronutrients from dietary sources. Grains are not very good sources of iron and zinc, but they are the staple food for billions of people. The World Health Organization estimates that two billion people are not getting adequate amounts of these essential micronutrients for optimal health. Deficiencies cause a variety of health problems, including disease susceptibility, slow growth in children, and anemia in the case of iron deficiency.

Several approaches are being used to address micronutrient deficiencies, including oral supplements, adding supplements to prepared foods such as breakfast cereals, and applying Fe- or Zn-containing fertilizers to crop plants. Another approach is to breed plants that accumulate more Fe or Zn in their grains or other edible tissues. Screening plants to identify varieties with enhanced levels of micronutrients followed by the identification of genetic markers and breeding with preferred varieties is the approach taken by HarvestPlus, which has led to production of high-Fe and high-Zn beans as well as high-Zn rice and wheat. A targeted gene approach is also promising, with some success coming from increased production of ferritins or nicotianamine synthases (to better sequester nutrients in the grain) and increased uptake through enhanced production of phytosiderophores or expression of various transporters. As described below, similar strategies are being explored to increase the selenium content of foods because selenium deficiency is another serious human health concern. Interestingly, most plants do not require selenium, so it is not considered a plant micronutrient.

COPPER: CRITICAL FOR AEROBIC LIFE

Copper Proteins Have Diverse Functions

In Earth's early history when oxygen levels were low, iron was relatively more available than it is today, and copper was much less available than it is today. Therefore, the earliest life may have depended on Fe for reactions that require a metal cofactor. Following the advent of oxygenic photosynthesis, Cu became more bioavailable, and it became an important metal cofactor, mostly in aerobic processes such as oxidative phosphorylation

and reactive oxygen detoxification. Some organisms can substitute one enzyme or pathway for another if Fe or Cu is limiting for growth, suggesting some redundancy between the functions mediated by these metals.

Copper binding proteins are active throughout the cell, including in the electron transport chains of the mitochondria (cytochrome *c* oxidase) and chloroplast (plastocyanin), cytosol (Cu/ZnSOD; also found in the chloroplast stroma), endomembranes (including the ethylene receptor), and apoplast (laccase and other multicopper oxidases). Copper was confirmed as an essential micronutrient for plant growth in 1939.

Copper Transporters and Chaperones

Within cells, Cu is not found in free form. Like Fe, free Cu can drive the production of highly reactive free hydroxyl radicals (OH·) by way of the Fenton reaction. Furthermore, Cu binds more tightly to most proteins than do other metals, so if unguarded it can displace less competitive metals from metalloproteins. Copper is transported within cells in association with small proteins called copper chaperones (also known as metallochaperones), which were first identified in prokaryotes and yeast in the 1990s. These remarkable proteins take up Cu directly from transporter proteins through specific protein-protein interactions and then pass it directly to target Cu binding proteins again through specific protein-protein interactions. Different chaperones usually carry Cu to different target proteins.

An interesting glimpse into the evolution of copper chaperones comes from studies of the chaperone that delivers Cu to plastocyanin. Plastocyanin resides in the chloroplast lumen where it receives electrons from the cytochrome b_6f complex and transfers them to the oxidized special chlorophyll in photosystem I. Movement of copper into the chloroplast lumen involves transport across three membranes: the outer and inner chloroplast envelope and the thylakoid membrane. Loss of function of the inner membrane transporter PAA1 prevents Cu from reaching both Cu/ZnSOD, which resides in the stroma, and plastocyanin, which resides in the thylakoid lumen. By contrast, loss of function of the thylakoid membrane-localized transporter PAA2 only interferes with Cu delivery to plastocyanin. Recently, a chaperone that delivers Cu to PAA1 was identified. In many plants, it is encoded by a splice variant encoding just the first few exons of PAA1, meaning that the chaperone and the transporter it delivers Cu to share a protein sequence. Interestingly, this splice variant is only found in some plants; in other plant genomes, the chaperone and the transporter are encoded by separate genes.

Several transporter families are involved in movement of Cu across membranes. High-affinity COPT transporters (CTR in *Chlamydomonas reinhardtii*) function as trimers and are found in the plasma membrane and internal membranes and are responsible for Cu uptake into cells and reallocation; some *COPT* genes are upregulated in response to Cu deficiency. The HMAs have diverse functions, including xylem loading (HMA5 in rice), transport of Cu into chloroplasts (PAA1 and PAA2 are HMA family members) and into the endoplasmic reticulum (ER) (RAN1, which was identified by its altered sensitivity to ethylene, is a member of the HMA family and is required to transport Cu to the ER lumen-localized Cu binding domain of the ethylene receptor), and Cu efflux from the cytosol for transport or detoxification purposes. Finally, there is evidence for a role of ZIP and YSL transporters in Cu transport.

Copper Economy: Copper Sparing, Recycling, and Sequestration

Plants respond to Cu deficiency or limitation by increasing Cu uptake and, where possible, switching to non-Cu-requiring proteins. The SPL7 transcription factor contributes to both of these responses. Under Cu deficiency, SPL7 induces expression of the gene encoding a major plasma membrane Cu-influx transporter, COPT1. SPL7 also transcriptionally regulates the gene encoding miR398. When Cu is limiting, miR398 binds Cu/ZnSOD mRNA to prevent the production of this Cu-requiring enzyme, which is functionally substituted by FeSOD.

In Chlamydomonas but not land plants, the function of the Cu-protein plastocyanin can be fulfilled by cytochrome c_6, which uses Fe as a cofactor. Under Cu limitation, the plastocyanin apoprotein is degraded, and the gene encoding cytochrome c_6 (*CYC6*) is transcriptionally upregulated by the transcription factor CRR1, which is related to SPL7. Other studies show that when Chlamydomonas is grown heterotrophically, relying on an exogenously supplied source of fixed carbon rather than photosynthesis, Cu is reallocated to Cu binding proteins in the electron transport chain of the mitochondria at the expense of those in the plastid.

Some soils are heavily contaminated by Cu as a consequence of human activities including the formerly common practice of applying Cu-containing solutions to plants as antimicrobial treatments. Excess Cu contributes to oxidative stress damage, interferes with the uptake of other essential metals, and can lead to incorporation of Cu into the metal binding sites of other proteins, including chlorophyll; when Cu replaces Mg, the chlorophyll becomes nonfunctional. Plant responses to excess Cu include increased sequestration of Cu into the vacuole, downregulation of Cu uptake proteins, and upregulation of the Cu efflux transporter HMA5.

ZINC: DEFICIENCY COMMON IN PLANTS AND PEOPLE

Like Fe, Zn deficiency is common in humans as well as in plants. One survey reported that half of agricultural soils are Zn deficient. People whose diets are based on food crops grown in Zn-deficient soil are prone to Zn deficiency, which contributes to wide-ranging health problems. Worldwide, ~17% of people are at risk of Zn deficiency, predominantly children in developing countries. Zn deficiency raises the risk of growth stunting, infections, and even death; it is estimated that 400,000 children die each year as a consequence of Zn deficiency.

Zn is the second most used *d*-group metal element in biological systems; Arabidopsis has been estimated to express ~2400 proteins that interact with Zn. Unlike Fe or Cu, in biological conditions, Zn does not undergo a redox state change (i.e., gain or loss of electrons). However, it can compete effectively for metal binding sites in proteins, so excess Zn can still be toxic.

One of Zn's roles is as a structural stabilizer. Protein folds known as Zn-fingers are stabilized by Zn and occur in transcription factors, ribosomal proteins, and other nucleic acid binding proteins. Because ribosomes are very abundant in most cells, they represent the largest cellular pool of Zn. (Some prokaryotes can switch to non-Zn binding ribosomal proteins when Zn is limiting.) Other proteins that require Zn include the enzymes carbonic anhydrase, phospholipase C, and Cu/ZnSOD.

Zinc Uptake, Transporters, and Chelators

In soil, Zn is often present in insoluble forms, including carbonates, phosphates, and hydroxides. Zn insolubility is more pronounced in high pH soils, in soils treated with phosphate-containing fertilizers, and in waterlogged soils. Plants can enhance Zn's solubility and availability by secreting protons or organic chelators, including phytosiderophores in grass species.

ZIP transporters including IRT1 are thought to be the primary route for Zn uptake into the cytoplasm; by altering Zn and Fe levels, their competition for the same influx transporters can be shown. Some members of the *ZIP* gene family are upregulated by Zn deficiency, and some are expressed at significantly higher levels in Zn-hyperaccumulating plants (see below).

Three members of the HMA family have been implicated in Zn efflux from the cytoplasm, based on elevated expression or gene amplification in Zn-hyperaccumulating plants, the increased sensitivity to Zn of loss-of-function mutants, or functional studies in plants or yeast. Other transporters implicated in Zn transport include another type of transporter PCR2 (plant cadmium resistance), metal tolerance proteins, YSLs, and NRAMPs. Collectively, these and other unidentified transporters maintain Zn homeostasis through uptake, vacuolar sequestration, long-distance transport through xylem and phloem, and remobilization from senescing leaves and into seeds.

In the cytosol and during transport, excess Zn is chelated by various small molecules and proteins, including phytochelatins and nicotianamine, to prevent it from interacting with off-target proteins. ZIF1, a membrane-localized transporter, affects Zn transport indirectly by transporting nicotianamine into the vacuole, where it contributes to Zn sequestration. A related transporter ZIFL from rice is proposed to transport phytosiderophores from root to soil to enhance metal uptake.

Responses to Zinc Deficiency

Several genes encoding Zn transporters and NA synthase have conserved transcription factor binding sites in their promoters. Two related transcription factors have been identified that mediate the transcriptional responses to Zn deficiency, and loss-of-function mutants of these transcription factors fail to induce Zn uptake in response to Zn deficiency and so grow poorly on low-Zn medium. The signal that activates the Zn deficiency transcriptional response has not been identified yet, but one model suggests that these transcription factors themselves are Zn sensors. In this model, when Zn levels are sufficient, Zn binds the transcription factors and prevents them from functioning as activators, but when Zn levels are insufficient, the transcription factors become activated and induce gene expression. Alternatively, the transcription factors may be activated by an additional unknown factor.

MANGANESE: CENTRAL TO THE OXYGEN-EVOLVING REACTION

Manganese is very abundant in the earth's crust and in seawater. It primarily is taken up by plants as Mn^{2+} but is also present in soils as Mn^{3+} and Mn^{4+}, which must be reduced prior to uptake. Mn availability in soil is pH dependent. Mn^{2+} is more abundant in acidic soils; hence, plants take up Mn more readily in acidic soils. Mn deficiency is common, particularly in alkaline soils. Mn availability also is affected by soil microbes and root exudates including H^+ and low molecular weight organic acids.

Like most other metal micronutrients, Mn is toxic at high levels, which is particularly a problem in acidic soils. Elevated Mn^{2+} in soils can interfere with the uptake of other cations. Furthermore, when Mn^{2+} accumulates in excess, it can be oxidized in the apoplast to Mn^{3+}, which can damage lipids and proteins by oxidizing them. Therefore, the Mn transport machinery must ensure enough but not too much Mn accumulation. The sensitivity of plants to Mn is quite variable. Rice is relatively tolerant (able to accumulate Mn to more than 5 mg/g dry weight) compared with other crops, and some other species can accumulate Mn to even higher levels without showing toxicity symptoms; many Mn-hyperaccumulating species originated in New Caledonia and eastern Australia.

Biological Roles of Manganese

Take a deep breath! The oxygen you just inhaled was produced by the water-oxidizing, O_2-evolving complex of photosystem II (PSII) in the reaction: $2H_2O + 4\,h\nu \rightarrow 4e^- + O_2 + 4H^+$. This reaction requires a conserved Mn cluster (Mn_4CaO_5) in the oxygen-evolving complex of PSII. Remarkably, the Mn cluster appears to be structurally and functionally identical in all oxygen-evolving photosynthetic organisms, meaning that it has been essentially unchanged over three billion years. Photons excite P680 to generate $P680^+$, which is sufficiently oxidizing to pull electrons out of water bound to the Mn cluster. More about how it works and the proteins involved are described in *Teaching Tools in Plant Biology 32: The Light Reactions of Photosynthesis*.

Mn's other indispensable role is in MnSOD, a relatively abundant enzyme in mitochondria. Mn also is an activator or cofactor for more than 30 other enzymes in plants including Mn-catalase, pyruvate carboxylase, and various Golgi-localized glycosyltransferases, although in many of these enzymes Mg can substitute when Mn is limiting.

Manganese Transporters

Mn is found in most cellular compartments where it is either required for function or stored and so Mn transporters are found on many different membranes. NRAMP1 is plasma membrane localized, and uptake of Mn is defective in *nramp1-1* mutants from Arabidopsis, whereas NRAMP3 and 4 are vacuolar localized

and involved in transport from the vacuole to the cytosol. In rice, Nramp3 expression at the node is important for distribution of Mn; when Mn is in low abundance, Nramp3 translocates it from xylem into phloem for transport to young tissues, but when Mn is abundant, Nramp3 is degraded and Mn is transported preferentially into older tissues. YSL2 and YSL6 are also implicated in Mn transport in rice; in high-Mn conditions, loss-of-function *ysl6* mutants show Mn toxicity symptoms and an accumulation of Mn in the apoplast (where it can be detrimental). Some members of the ZIP family are able to complement a Mn transporter-deficient yeast line, and some mutants show abnormal responses to low or high Mn conditions. There is also evidence for movement of Mn through Ca-permeable channels and calcium/cation antiporters. Members of the MTP family also have been implicated in Mn transport. For example, *mtp8* mutants in rice show decreased tolerance to the relatively high Mn levels normally tolerated by rice. Finally, some members of the ECA (ER-type calcium ATPases) family of P-type ATPases may be involved in Mn transport into endomembranes.

MOLYBDENUM

Molybdenum is the heaviest of the essential micronutrients and the least abundant in soil. Like many other metals, its form in soil and consequently its bioavailability is pH dependent. Plants take it up as molybdate MoO_4^{2-}, which is structurally similar to sulfate (SO_4^{2-}) and so it is transported via SULTR transporters: specifically, high-affinity transport through MOT1 (SULTR5;2) and MOT2 (SULTR5;1) and lower-affinity, nonspecific transport through other SULTR transporters.

Molybdate ion is not biologically active but instead is functional in one of two cofactors. In most cells, Mo functions as a structure called the molybdenum cofactor (Moco) in which the Mo is bound to a pterin molecule. The genes involved in pterin biosynthesis are conserved from bacteria to plants to humans, and mutations that interfere with its production are lethal. Mo deficiency symptoms are pleiotropic because of its contribution to several enzymes in plants, including nitrate reductase, sulfite oxidase, aldehyde oxidase, and xanthine dehydrogenase.

Legumes and other plants that form nitrogen-fixing symbioses with bacteria depend on a different and structurally dissimilar iron-containing Mo cofactor known as FeMoco. This cofactor is essential for activity of the bacterial enzyme nitrogenase, which breaks the triple bond in N_2 to reduce it to NH_3.

NICKEL: NECESSARY BUT RARELY LIMITING

Nickel Requirement, Uptake, Transport, and Tolerance

The abundance of Ni in most soils combined with the very low amounts required in most plants mean that in natural or agricultural systems Ni deficiency is rare. Because of nickel's prevalence, it was not until the 1980s that it was conclusively demonstrated to be an essential micronutrient. In 1975, nickel was identified as an activator of the enzyme urease (which breaks down urea to CO_2 and NH_3), and in 1983, Ni-deficient plants were shown to accumulate cytotoxic levels of urea. Finally, in 1987, Ni-deficient barley seeds (produced by growing their parents, grandparents, and great grandparents on Ni-deficient growth medium) were shown to be unable to germinate, proving Ni to be essential. Currently, urease is the only known Ni-activated enzyme in plants, although others are suspected. Additional Ni enzymes have been identified in bacteria, including a Ni-dependent glyoxalase I, which in other organisms is a Zn-dependent enzyme.

Ni is taken up into cells through ZIP transporters, and there is evidence to suggest that Ni may compete with Fe for uptake. Ni also is chelated by nicotianamine, and the Ni-NA complex may compete with Fe-NA complexes for transport through YSL transporters. Because Ni toxicity may derive from this competition, a high shoot ratio of Fe to Ni is correlated with the ability to tolerate elevated Ni.

About 1% of the earth's soils are classified as serpentine, characterized by a low ratio of Ca to Mg, low abundance of macronutrients, and high abundance of metals including Ni. Several hundred plant species have been identified in these regions that are able to tolerate high Ni. Studies of these plants are revealing the mechanisms for metal tolerance, which include the strategies of exclusion and hyperaccumulation. As described below, Ni hyperaccumulators usually store Ni in the shoot in vacuoles or the apoplast and produce elevated levels of glutathione and metal chelators, including nicotianamine and the amino acid histidine.

METAL TOLERANCE AND METAL HYPERACCUMULATION

Some plants have evolved mechanisms to avoid the toxic effects of metals through exclusion, detoxification, or hyperaccumulation. Some of these species are considered metal tolerant, others are metal hyperaccumulators, and others are both. By definition, hyperaccumulation means the plants concentrate the element to levels 50 to 100 times that of the surrounding vegetation or to between 100 and 10,000 mg/kg dry weight depending on the element.

The ability to tolerate or hyperaccumulate metals has evolved independently many times. In some cases, this ability allows plants to exploit less crowded niches and avoid competition from other plants. Most metal-tolerant or metal-hyperaccumulating plants have been found in soils that are rich in naturally occurring or anthropogenic Ni or soils that are rich in Cd, Se, Zn, Cu, or other metals. Metal-hyperaccumulating species are overrepresented in the Brassicaceae and Euphorbiaceae families. *Arabidopsis halleri* and *Noccaea caerulescens* (formerly *Thlaspi caerulescens*) are widely used models in which to explore the genetic basis of hyperaccumulation because of their genetic similarity to *A. thaliana*.

Metal-tolerant plants can survive on soils that have elevated levels of metals, but they do not necessary take up the metals; some metal tolerant plants are particularly good at excluding metals. By definition, metal excluders exclude metals from the shoot, but in some cases they appear to accumulate it in the roots, although it is unclear whether this is in the symplast or apoplast. Other plants are able to minimize damage from the metals they take up; this strategy involves increased levels of chelators such as phytochelatins, nicotianamine, or histidine, or glutathione, which also scavenges free radicals. Sequestration

of the metal in the apoplast or vacuole is another common strategy of metal tolerance.

By contrast, metal hyperaccumulators have evolved mechanisms through which they concentrate the metals at levels much higher than in the surrounding soil; the adaptive advantage of the accumulated metals appears to be in defense against pathogens and herbivores. Hyperaccumulation involves changes in the activities of metal transporters. Hyperaccumulators sequester metals in their shoots and compared with other plants can show enhanced uptake (which can involve the participation of soil microbes as well as enhanced root exudation), enhanced mobility within the root symplasm, enhanced transport into the apoplasm and xylem stream, effective sequestration in the leaf cell vacuole or cell wall, and effective chelation of the metals or formation of metal complexes. As an example, by quantitative trait locus mapping of a population derived from *A. halleri* and *A. thaliana*, metal hyperaccumulation was found to correlate with elevated expression (by gene triplication and enhanced transcription) of the HMA4 metal transporter, which transports metals into the xylem for transfer to the shoot. Hyperaccumulators also often produce elevated of nicotianamine, histidine, and organic acids.

Phytoremediation and Metal Farming

Hyperaccumulators can contribute to phytoremediation efforts, in which contaminated soils or aquatic systems are made less toxic through the action of plants or algae. The metal-containing shoots and leaves can be burned, condensing the metal in ash. Naturally occurring metal hyperaccumulators that are being explored for phytoremediation include *T. caerulescence*, which accumulates lead, zinc, cadmium, and nickel, *Alyssum* species that accumulate nickel and cobalt, and *Pteris vittata* that accumulates arsenic. Some trees, including willow and poplar, are not truly hyperaccumulators, but they can extract some metals from soils, and their larger size contributes to their efficiency. Phytoremediation efforts can be enhanced by adding chelators to the soil or by increasing the uptake of the metals through breeding or genetic engineering approaches.

Sometimes the extracted metal is treated as a hazardous waste, but it is also possible to make use of the metals for other purposes, in which case the process is referred to as agromining or phytomining. In some cases, it can be cheaper to use plants to pull metals from the soil than to use conventional mining and extraction processes, but the economic feasibility also depends on the market value of the metal extracted. Soils that are naturally or industrially contaminated with metals and otherwise would lie fallow are good candidates for phytomining. Strategies to increase a plant's phytomining efficiency are similar to biofortification strategies in that both processes involve the uptake of metals from soils and their sequestration or concentration in aboveground tissues.

TOXIC METALS AND METALLOIDS

Because many plant metal transporters are not very specific, plants can take up elements that have little or no biological value as well as toxic elements.

Toxic metals can end up as part of the human food supply, particularly in regions where they are naturally abundant in soils or where they have been deposited by human activities such as mining or manufacturing. Uptake by plants is generally correlated with metal concentration in the soil. Discussed here as examples of harmful metal and metalloid elements are arsenic and cadmium, which are particular concerns for human health, and aluminum, which is one of the major limitations to plant growth in tropical and subtropical soils.

Arsenic Is Toxic to Plants and Humans and Affects Millions

Arsenic and cadmium have the dubious distinction of being on the World Health Organization's list of "ten chemicals of major public health concern." Arsenic is the twentieth most abundant element in the earth's crust, a frequent contaminant of groundwater, and can be assimilated into plants destined for human consumption. In regions of the world with high levels of arsenic in the soil and groundwater, particularly Southeast Asia, arsenic has been implicated in as many as 20% of human deaths. Arsenic is a carcinogen and its effects may not be evident for many years after its ingestion. It can also cause development defects and nervous system damage and contribute to chronic illnesses such as diabetes and heart disease. Human exposure to arsenic comes from contaminated water as well as from crops irrigated with contaminated water. Arsenic contamination of rice is a major concern because it is a staple grain in many of the countries that have naturally high levels of arsenic in the soil, and it is particularly prone to arsenic accumulation.

Arsenic sits below phosphorus in the periodic table, meaning that the two elements have similar properties. The toxicity of As rests in part on this similarity; when taken up into a cell, it can replace P in some cellular processes. In the terrestrial environment, inorganic As is most commonly found as arsenate [As(V)] or arsenite [As(III)]. Arsenate is more abundant in aerobic soils, it structurally resembles phosphate, and it is transported into cells nonspecifically through phosphate transporters as a charged anion. Arsenite is more abundant in anaerobic soils such as flooded rice paddies, is usually uncharged and enters cells through aquaporin channels and silicon transporters, and can affect proteins by reacting with sulfhydryl groups. Rice has a highly expressed pathway for silicon uptake, which is one of the reasons that it tends to accumulate arsenic.

Many strategies are being investigated to ameliorate As effects and lower its accumulation in the rice grain. Adding competing P or Si species to soil competes with As uptake, and lowering the activity of phosphate transporters can decrease As uptake. Within cells, As can be detoxified by binding to glutathione or phytochelatins and/or sequestered in the vacuole as As(III)-phytochelatin complexes. Tonoplast-localized transporters specific for metal-phytochelatin compounds have been identified that contribute to As as well as Cd tolerance. There is considerable natural variation in As accumulation in plants, some of which points to potential mitigation strategies; as an example, natural variation in As reductase activity has been correlated with tolerance to arsenate. Naturally occurring As-tolerant plants such as the fern *P. vittata* are being studied as

well. Interestingly, an As efflux protein thought to sequester As into the vacuole in ferns is not found in angiosperm genomes, suggesting another possible strategy for As sequestration.

Cadmium Is an Extremely Toxic Heavy Metal

Cadmium is extremely toxic to plants and animals and a major public health concern. Cd enters air, soil, and water mainly as a consequence of human activities and then moves into plants and humans. An infamous outbreak of Cd poisoning in Japan in the mid-20th century raised awareness of this environmental pollutant. Industrial emissions and the residue of the Ni-Cd batteries that were widely used in the 1980s and 1990s are major contributors to Cd exposure, although tobacco smoking is a significant source of exposure for some humans. However, for many people in Asia, the rice in their diet is the most important source of Cd exposure.

Although most plants restrict Cd entry through both symplastic and apoplastic pathways, Cd can enter root cells through Ca channels and other transporters. Cellular Cd can be detoxified through interactions with phytochelatins, glutathione, and metallothioneins and sequestration into the vacuole. HMA metal pumps contribute to the movement of Cd into the xylem stream and subsequently the shoots. Free Cd^{2+} in cells can bind sulfhydryl groups in proteins, displace other metals from metalloproteins, and generate damaging reactive oxygen species. Cd^{2+} may also mimic some effects of Ca^{2+}, such as inducing stomatal closure. Cd is not entirely without biological benefit; in diatoms, Cd can substitute for Zn in carbonic anhydrase and actually stimulate growth in Zn-limiting conditions.

Efforts to breed low-Cd rice have been encouraging. As examples, rice varieties with decreased expression of LCT1 (a Cd efflux transporter), HMA2, or NRAMP5 or elevated expression of HMA3 all show decreased Cd accumulation in the rice grain even when grown on Cd-contaminated soils.

Aluminum, a Damaging Element in Acidic Soils

Aluminum is the most abundant metal in the earth's crust. In acidic soils, which include the majority of tropical and subtropical soils, aluminum toxicity is the major limitation of plant growth. In neutral soils, Al forms stable complexes that are not taken up by plants, but at low pH, Al^{3+} is released from the complexes and taken up into the roots. Al^{3+} is highly toxic to roots and causes a dramatic growth arrest, which leads to inefficient water and nutrient uptake. The mechanisms of toxicity include increasing cell wall rigidity, interfering with Ca^{2+} homeostasis and nutrient uptake, and production of harmful reactive oxygen species that can induce programmed cell death.

Aluminum tolerance is genetically determined and ranges widely between species and varieties. Many Al-tolerant plants use an Al exclusion strategy, through an Al-induced increase in extrusion of organic acids such as malate, citrate, and sometimes oxalate. These organic acids complex with Al^{3+}, prevent it from being taken up into root cells, and prevent its binding to the cell wall. Al tolerance through exclusion has been correlated with increased copy number or expression level of genes encoding root cell malate exporters, and these also prove effective in transgenic approaches.

Al can also be managed once it enters the cell by complex formation with organic acids or sequestration into the vacuole. An Al-specific transporter Nrat1 has been reported, raising the question as to why plants would selectively take up a nonessential element. One hypothesis is that once taken up into the plant Al can be sequestered into the vacuole and/or transported into shoot tissues, keeping it away from sensitive root cells. This shoot accumulation strategy can be seen in several Al-accumulating plants such as tea (*Camellia sinensis*), buckwheat (*Fagopyrum* spp), and *Melastoma malabathricum*. These plants are able to tolerate elevated levels of Al by chelating it, storing it in vacuoles or plastids, or secreting it into the cell walls. An interesting response to aluminum accumulation can be observed in the garden plant *Hydrangea macrophylla*. As most gardeners know, hydrangea sepal color is affected by soil acidity: lower pH shifts the color toward blue. Increasing soil acidity also increases Al uptake by the plant, and the blue color comes from a metalloanthocyanin complex between Al^{3+} and the anthocyanin delphinidin-3-glucoside.

Transcriptional responses to Al support a model in which Arabidopsis primarily uses an exclusion strategy and rice an internal tolerance strategy. In some species, supplying the plants with Mg or increasing Mg uptake by way of increased expression of a Mg transporter is sufficient to alleviate Al toxicity effects. The mechanism of this may be direct competition, as Mg^{2+} and Al^{3+} have similar hydration ratios, but the mechanism may be indirect, as adding Mg to a plant also causes an increase in organic acid secretion.

OTHER MICRONUTRIENTS

Boron Is an Essential Micronutrient

Boron was identified in plant ash in the 19th century and was shown to enhance plant growth in the early 1900s. In 1923, Warrington demonstrated that B is an essential micronutrient for plant growth. The best characterized physiological function for B in plants is to cross-link pectin polysaccharides in cell walls. Accordingly, one of the symptoms of B deficiency is an abnormality in cell walls and growth defects; similar effects are seen in plants deficient in B transporter activity. This primary defect can lead to other abnormalities that arise from the decreased structural integrity of cells, including abnormalities in root growth and pollen tube defects.

Boron has additional functions in other organisms. Animal cells that have lost B transporter activity are not viable, and B is involved in quorum sensing in some bacteria. The ability of B to form bridges between *cis*-dioxyl-containing molecules could extend to other molecules, and it has been suggested that B deficiency may alter membrane structure. As an example, in N_2-fixing cyanobacteria, B deficiency lowers the efficiency by which O_2 is excluded from N_2-fixing heterocysts, which lowers the efficiency of N_2 fixation. B is also required for the establishment of symbiosis between *Rhizobium* and plant.

Boron's diverse roles in other organisms lend support to the idea that additional roles for B beyond pectin cross-linking may be

found in plants. Recently, it was found that B-deficient plants show altered expression patterns of several transcription factors and altered hormone responses, and there is a suggestion that B may confer some protection against Al toxicity. Although B's functions in plants are still being unraveled, it is well established that in many conditions adding B to fields can increase yields, so B is now frequently a component of fertilizers.

Recent work has highlighted the how B transporters contribute to B uptake and transport and how these transporters are regulated. Inorganic B is usually found as the uncharged species H_3BO_3 at acidic pH, including in the apoplast, and as the anionic species $B(OH)_4^-$ in alkaline conditions. Boron influx into cells primarily occurs in the uncharged form through NIP aquaporin channels, and its efflux primarily occurs through the BOR1 transporter. BOR1 is expressed in root pericycle cells and facilitates the import of borate into the xylem stream for transport to the shoot; loss-of-function *bor1* mutant shoots are B deficient and develop abnormally.

Like many micronutrients, B in excess is toxic. The activities of influx and efflux transporters are high when B is deficient and rapidly downregulated when it is resupplied. BOR1 appears to be endocytosed and degraded upon B resupply, whereas NIP1 is downregulated at the transcriptional level. A recent study showed that wheat breeders have matched B tolerance, determined in part by variant alleles of a B transporter, to their growing environment; in regions of high soil B, varieties carrying a B transporter that is more highly expressed and so confers greater B tolerance have been selected relative to poorly expressed and nonfunctional alleles.

Silicon Is Essential for Some Plants and Beneficial for Many

Silicon is an essential micronutrient for diatoms and *Equisetum* spp (horsetail) but does not appear to be essential for other plants. Nevertheless, Si has a measurable positive impact on the fitness of many plants under stressful conditions. Some authors call Si a micronutrient and others refer to it as a nonessential nutrient.

After oxygen, silicon is the second most abundant element in the earth's crust, and it is present to a large extent as solid, insoluble silicon dioxide minerals, including sand. In biological systems, Si is found as silicic acid [$Si(OH)_4$], it is never charged, and it does not participate in organic chemistry. Silicic acid is a product of rock weathering. Plants of the families Poaceae, Equisataceae, and Cyperaceae accumulate Si to the highest levels. Silica can be deposited throughout the plant within or between cells in hard, rock-like deposits called phytoliths ("plant rocks"). Paleobotanists can use the shape and abundance of phytoliths deposited in soils as indicators of distributions and abundances of ancient plant taxa.

Silicic acid transporters were characterized in rice mutants and named Lsi (low silicon). Si transport is similar to B transport, with influx of the uncharged molecule occurring through a NIP aquaporin (Lsi1) and efflux through a transporter pump Lsi2. As described above, arsenic moves into rice plants through the Si transporter Lsi. Arsenic uptake decreases when Si is added to soil, possibly as a result of lower expression levels of Lsi1 and Lsi2.

Silicon may confer protection against various types of abiotic stress, including metals, salinity, and water stress, as well as against some bacterial and fungal pathogens. Although its protective methods are generally poorly understood, there is speculation that Si may contribute to a protective barrier to the environment. Because Si is not metabolized and largely transported passively with the transpiration stream, Si-based structures and defenses have been suggested to be metabolically cheap compared with carbohydrate or secondary metabolism-based defenses. Hard, insoluble phytoliths are effective deterrents of herbivory and can be a significant source of wear on herbivore teeth or mandibles, and a coevolution between phytolith-containing plants and the origin of long or continuously growing teeth in herbivores has been proposed.

Chlorine Is an Essential Micronutrient That Functions Mainly as an Anion

Chlorine is an abundant element usually found in soil as the anion chloride (Cl^-). It is very mobile in soil and can be leached away where rainfall is heavy. Nevertheless, deficiency is rare, and care has to be taken to avoid exposing plants to too much Cl, especially because it is often added to soil as KCl or $CaCl_2$, or $MgCl_2$.

Chloride's major characteristic is that it is an abundant, nonmetabolized anion that participates in membrane charge dynamics, cell signaling, and cell turgor. There are many types of Cl transporters, some of which are specific for Cl and others that preferentially transport nitrate (NO_3^-) and are often described as anion transporters. Cl transporters are located on the plasma membrane as well as internal membranes including the tonoplast membrane. They are best characterized for their contributions to guard cell turgor changes. When turgid guard cells (which permit transpiration through open stomata) sense the hormone abscisic acid or other stimuli, anion channels and potassium channels open. Cl^- and K^+ flow out of the cell (down their concentration gradients) through their respective channels and the resulting loss of osmolytes and turgor causes the stomata to close.

Like K^+ but unlike metal micronutrients, Cl^- is highly mobile in the plant and readily transported to supply high-priority tissues (see *Teaching Tools in Plant Biology 29: Plant Nutrition 1: Membrane Transport and Energetics, Potassium Nutrition and Sodium Toxicity*). Salinity is detrimental to most plants as a consequence of both excess Na and excess Cl. Although the consequences of Na excess are better characterized, excess Cl is also harmful, and a plant's ability to tolerate salinity has been correlated with the expression levels of both Cl and Na transporters.

Cl is important in pH regulation and osmoregulation by acting as a counterion for cations that accumulate in the cytosol. Cl also has specific roles in the regulation of several enzymes, and it is needed for proper function of the oxygen-evolving complex of PSII, although under laboratory conditions it sometimes can be substituted by other anions. Finally, Cl is sometimes incorporated into chlorinated organic molecules that primarily have roles in defense.

Selenium Is an Essential Micronutrient for Animals

Se is generally considered beneficial for plants, but it is essential for animals, some bacteria, and some green algae. Se deficiency

contributes to several human health problems, including immune, thyroid, and reproductive disorders, cancer, and death; in the early part of the 20th century, many deaths in Keshan province in China were found to be caused by a severe Se deficiency, which is now known as Keshan disease. Se deficiency is endemic in regions where soil Se levels are low, which include large parts of China, Africa, and northern Europe. In these regions, Se can be supplemented to the diet directly, by adding Se to soils, or by growing crop plants that are more effective at Se uptake and incorporation into the grain.

The human genome has 25 genes encoding selenoproteins, which incorporate the amino acid selenocysteine (SeCys). SeCys is sometimes described as the 21st amino acid. It is incorporated into selenoproteins during translation via a tRNA charged with SeCys that recognizes a UGA stop codon in conjunction with a particular mRNA secondary structure. Selenoproteins are involved in diverse processes, including the production of thyroid hormones and immune functions.

There is no evidence that plants require Se, although they can benefit from it due to antioxidant and possibly allelopathic or defensive effects. Depending on the soil pH and other factors, plants assimilate selenium mainly as selenate (SeO_4^{2-}) through sulfate transporters, or to a lesser extent as selenite (SeO_3^{2-}) through phosphate transporters. Selenate resembles sulfate and can be assimilated into organic compounds including SeCys through the sulfur assimilatory pathway. Se biofortification strategies are most effective when the Se as provided in organic form. Broccoli and garlic naturally accumulate Se and are good candidates for biofortification efforts.

Excessive Se can be toxic to plants and animals. Toxicity is thought to come from the fact that excess Se can inappropriately become integrated into proteins and can induce oxidative stress. Nevertheless, some plants adapted to seleniferous soils can naturally accumulate high levels Se. Studies of Se hyperaccumulators and Se uptake and assimilation pathways highlight the close association between S and Se metabolism and also underpin both biofortification and phytoremediation efforts.

SUMMARY AND ONGOING RESEARCH

Micronutrients make up only a small portion of a plant's mass, but their contributions to its metabolic processes are vital. Many micronutrients help to catalyze reactions that would otherwise be impossible in the cellular environment, others contribute to the stabilization of macromolecules and cells, and another serves primarily as a charged ion.

The study of plant micronutrients focuses as much on how plants avoid poisoning themselves by taking up too much of these essential but hazardous elements as it does on how they ensure adequate supply. This dichotomy requires a delicate set of responses to balance the availability of the nutrient and the need of the plant. When nutrients are deficient, uptake transporters are activated, and in some cases small molecules are secreted into the rhizosphere to facilitate nutrient uptake. When nutrients are very scarce, metabolic processes can be shunted toward pathways that do not require the limiting nutrient, and the nutrient can be recycled from a less critical metalloprotein or process in favor of a more critical one. On the other extreme, plants can minimize their uptake of nutrients by shutting off or internalizing transporters. When supply exceeds demand, nutrients can be stored in vacuoles, exported into the apoplasm, or detoxified by association with small molecules, peptides, or proteins. A few plants have adapted to high-metal environments, and some even actively accumulate the metal for defensive purposes. The low specificity of many metal transporters confounds the plant's efforts to maintain each nutrient within its optimal range in the cell and leads to complex interactions between metal nutrients, including competition for uptake of a scarce element when a more abundant element is present, and the unintended consequences of increasing expression of a transporter to try to control metal uptake or distribution. Because their ability to control metal uptake is imprecise, plants depend heavily on the protective functions of chelators and other metal binding compounds.

Just as plants have to balance their need for essential micronutrients with their avoidance of toxicity, so do people. Humans are heterotrophs whose nutrient needs are met by the consumption of complex materials. Transporters lining the digestive tract confer some selectivity toward the nutrients assimilated into the body, but human micronutrient transporters are also imprecise. As an example, when dietary iron is limiting, the activity of iron uptake transporters is increased, which also leads to increased nonspecific uptake of other metal elements. Therefore, when humans eat an iron-deficient diet, they are more vulnerable to the effects of toxic metals like Cd or As in the food they eat.

Clearly, the challenge of ensuring human micronutrient sufficiency and security rests firmly on the micronutrient sufficiency and security of plants. Although plants' fundamental requirement for many of the micronutrients has been known for several decades, the processes controlling micronutrient homeostasis have been discovered only recently and continue to be identified. Already this knowledge is being applied toward the development of iron- and zinc-rich biofortified foods and low-cadmium rice, as well as the development of agronomic practices that can decrease the accumulation of arsenic or cadmium in rice grains. Ensuring adequate nutrition for all people by eliminating "hidden hunger" (micronutrient deficiency) is one of the United Nations' Millennium Development Goals (2000-2015) and Sustainable Development Goals (2015-2030) that plant science has a key role in addressing.

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RECOMMENDED READING

(This is a representative list of sources to help the reader access a huge body of literature. We apologize in advance to those whose work is not included.)

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